Advances in Diagnosis and Treatment of Fabry Disease: A Review of Clinical Manifestations and Therapeutic Interventions

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Abstract
Atherosclerosis is a disease with a complex pathogenesis, consisting of the interrelationships of many different elements. In light of the increasing spread of cardiovascular diseases, the precursor of which is atherosclerosis, the study of the intricacies of its pathogenesis remains an important research task. For its achievement, it is necessary to choose the right model. To date, the most common are models of small animals, in particular mice. However, extensive work is being carried out towards the development of cellular models that would allow moving away from the use of animals as model objects, as well as bypassing the problems of translating the results. In this review, we collected data on the current advances in the field of cellular models of atherosclerosis.

Keywords: Cellular models; In Vitro models; Atherosclerosis.

Introduction
Fabry disease, initially described in the late 19th century as "Diffuse angiokeratoma," has since been recognized as an X-linked multisystem lipid storage disorder caused by mutations in the GLA gene, responsible for encoding the lysosomal enzyme α-galactosidase A (α-Gal A). This genetic defect leads to the accumulation of glycolipids, primarily globotriaosylceramide (Gb3), and its deacylated form glo-botriaosylsphingosine (lyso-Gb3), in lysosomes throughout the body (Fabry J, 1898; Azevedo et al., 2020; Cybulla et al., 2022; Mehta et al., 2006; Sweeley and Klionsky, 1963).

With over 1000 genetic variants identified in the GLA gene to date, Fabry disease presents a wide spectrum of clinical manifestations, ranging from the classic phenotype with severely reduced α-Gal A activity to the late phenotype characterized by milder enzyme deficiency and later onset of organ complications (Bernardes et al., 2020; Mehta and Hughes, 2002). Affected individuals experience progressive cellular dysfunction, microvascular damage, and metabolic disturbances, ultimately leading to irreversible fibrosis in vital organs such as the heart, kidneys, blood vessels, and nervous

Significance
Atherosclerosis models, from 2D cultures to 3D tissue-engineered vessels, offer cost-effective avenues for drug screening and personalized medicine, advancing therapeutic discovery.

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Clinical manifestations develop and offer hope for improved outcomes and quality of life with therapeutic approaches for Fabry disease. These promising developments are making significant strides towards advancing therapy, enzyme replacement, and biomarker discovery, enabling clinicians to better track disease progression and monitoring and treatment assessment is a key area of research. Additionally, the identification of reliable biomarkers for disease and improvement in treatment outcomes is of the highest significance. In order to make an early diagnosis, it is advisable to conduct a screening of risk groups and diagnose the disease as early as possible. Once diagnosis is established, the patient requires an interdisciplinary approach and must be closely monitored by specialists and receive pathogenetic therapy.

To establish the diagnosis of FD in men, measurement of the enzyme activity of α-Gal A and lyso-Gb3 from a dried drop of blood is used. In most men, enzyme activity decreases to less than 30% of normal, while in some men there is no activity at all. The diagnosis is confirmed by molecular genetic testing, detecting the causative mutation (van der Tol et al., 2014; Desnick et al., 1989). In women, molecular genetic testing is mandatory to establish a diagnosis because α-Gal A activity in leukocytes may be normal despite low levels in other organs. At present, lyso-Gb3 isolated from blood plasma is the optimal biomarker for diagnosing FD.
An important step in diagnosing FD is the genealogical method. By analyzing a patient’s genealogy based on the mode of inheritance of the disease, several additional patients in the family may be identified. Therefore, all patients with a suspected or established diagnosis require medical genetic consulting to determine the risk group and to conduct an examination (Stroh, 2011). Patients due to their inability to accurately mimic the structure of blood vessels and human plaques. Therefore, single-cell models are

**Therapy**

Until 2001, there was no specific therapy available for FD; the disease could only be treated symptomatically. This determined an unfavorable prognosis, especially in classic variants of the disease with multisystem manifestations (Oder et al., 2016). The use of enzyme replacement therapy (ERT), first approved in 2001, has led to significant improvements in morbidity and early mortality. Biotechnologically produced α-Gal A is administered intravenously at two-week intervals. Two medicines are available: agalsidase alfa, derived from human cells, and agalsidase beta, derived from Chinese hamster ovary cells. However, ERT has not been shown to prevent further progression when started late in the disease (Germain et al., 2015; Weidemann et al., 2013).

The International FD Outcome Study (FOS) was initiated in 2001 to collect long-term clinical and safety data for individuals with confirmed FD who either received α-Gal A treatment or do not receive ERT. This database has contributed to the study of many aspects of FD and the consequences of ERT, including renal and cardiac outcomes (Nakamura et al., 2023; Mehta et al., 2009; Beck et al., 2022; Schwarting et al., 2006; Linhart et al., 2007; Mehta et al., 2004). The results of this retrospective comparison of FOS data confirm the long-term efficacy of α-Gal A in the treatment of FD. The results show that α-Gal A treatment slowed the decline in kidney function and slowed or stabilized the progression of cardiomyopathy.

**Materials and methods**

A retrospective analysis of the medical records of 12 patients from 8 unrelated families living in the Moscow region with an established diagnosis of Fabry disease and observed from 2015 to 2022 was carried out. All the patients underwent enzyme replacement therapy.

The average arterial pressure values, the presence of heart rhythm disturbances, as well as data on the therapy (pharmacological medicines, dosage, duration) were analyzed. Echocardiographic data including left ventricular myocardial mass index (LVMMI) and left ventricular thickness were also analyzed. Echocardiography data were obtained on ARTIDA ultrasound device (Toshiba, Japan) in B- and M-modes, with a sectoral sensor (2.7-3.5 MHz).

The levels of α-Gal A enzymatic activity, Lyso-Gb3 concentrations, as well as the results of DNA diagnostics: pathogenic variants in the GLA gene, were analyzed. Raw data of the patients are presented in Table 1.

**Limitations**

The retrospective analysis conducted in this article serves as a valuable tool to examine the medical records of patients with Fabry disease over a specific period. By looking back at data collected from past cases, researchers can gain insights into the disease progression, treatment outcomes, and genetic variations among patients. However, it is important to acknowledge the inherent limitations associated with retrospective studies that may impact the generalizability of the findings.

One significant limitation of retrospective analysis is the reliance on existing data collected for clinical purposes, which may lead to incomplete or missing information in the records. In this study, the small sample size of 12 patients from 8 unrelated families in the Moscow region could limit the ability to draw broad conclusions about Fabry disease outcomes and treatment efficacy across different populations. The limited sample size may also introduce potential selection bias, as the patients included in the study may not be representative of the broader Fabry disease population. Despite these limitations, retrospective analyses play a crucial role in generating hypotheses, exploring trends, and providing preliminary insights that can guide future prospective studies. By thoroughly documenting the clinical characteristics, genetic mutations, treatment regimens, and outcomes of patients with Fabry disease, researchers can identify patterns and associations that inform further research directions.

To overcome the limitations of small sample sizes and potential selection bias in retrospective studies, researchers can consider collaborating with multiple centers or institutions to increase the diversity and representativeness of the patient population. Additionally, conducting multicenter studies, prospective cohort studies, or randomized controlled trials can provide more robust evidence to validate the findings from retrospective analyses and enhance the generalizability of the results.

In conclusion, while retrospective analyses offer valuable insights into disease characteristics and treatment outcomes, researchers should interpret the findings with caution due to inherent limitations. Collaborative efforts, larger sample sizes, and complementary study designs can help mitigate biases and strengthen the validity of research findings in the field of Fabry disease.
Table 1. Raw data of the patients. AP is arterial pressure; LVMMI is left ventricular myocardial mass index; LVH is left ventricular hypertrophy; ARBs are angiotensin receptor blockers; ACEI is angiotensin-converting enzyme inhibitor; AFib is atrial fibrillation; α-Gal A is α-galactosidase A (enzyme activity: for patients No. 1, 2, 3, 4, 5, 7, 10, 11 the norm is 0.8-15 µM/l/h, for patients No. 6 and 8 the norm is 48.6-150.3 nM/mg/h, for patient No. 12 the norm is >1.89 µM/l/h); Lyso-Gb3 is globotriaosylphosphoglycerol (normal 0.05-3.0 ng/ml); ERT is enzyme replacement therapy.

<table>
<thead>
<tr>
<th>№ of the patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
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<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>F</td>
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<tr>
<td>Age, years</td>
<td>51</td>
<td>17</td>
<td>31</td>
<td>42</td>
<td>34</td>
<td>45</td>
<td>48</td>
<td>37</td>
<td>67</td>
<td>44</td>
<td>36</td>
<td>70</td>
</tr>
<tr>
<td>Average initial blood pressure, (AP) mm Hg</td>
<td>129/81</td>
<td>131/83</td>
<td>125/79</td>
<td>129/84</td>
<td>127/83</td>
<td>124/70</td>
<td>136/86</td>
<td>132/81</td>
<td>124/77</td>
<td>127/79</td>
<td>123/84</td>
<td>130/100</td>
</tr>
<tr>
<td>Therapy</td>
<td>ARB</td>
<td>ARB</td>
<td>-</td>
<td>ARB</td>
<td>ARB</td>
<td>-</td>
<td>-</td>
<td>ACEI</td>
<td>ACEI</td>
<td>ARB</td>
<td>-</td>
<td>ACEI</td>
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<tr>
<td>LVMMI at the beginning of the study, g/m²</td>
<td>66</td>
<td>49</td>
<td>9</td>
<td>52</td>
<td>55</td>
<td>54</td>
<td>57</td>
<td>58</td>
<td>50</td>
<td>56</td>
<td>53</td>
<td>55</td>
</tr>
<tr>
<td>LVH, cm</td>
<td>1.9</td>
<td>1.7</td>
<td>1.8</td>
<td>1.9</td>
<td>1.8</td>
<td>1.9</td>
<td>2.0</td>
<td>1.7</td>
<td>1.9</td>
<td>1.8</td>
<td>1.9</td>
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<tr>
<td>Rhythm disorders</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AFib</td>
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<td>-</td>
<td>AFib</td>
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<td>AFib</td>
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<td>AFib</td>
</tr>
<tr>
<td>α-Gal A activity, µM/l/h</td>
<td>0.49</td>
<td>1.76</td>
<td>0.35</td>
<td>0.72</td>
<td>0.02</td>
<td>5.1</td>
<td>0.04</td>
<td>7.4</td>
<td>-</td>
<td>0.75</td>
<td>0.72</td>
<td>3.33 (normal)</td>
</tr>
<tr>
<td>Lyso-Gb3, ng/ml</td>
<td>118.36</td>
<td>16.82</td>
<td>64.3</td>
<td>34.98</td>
<td>44.73</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>66.63</td>
<td>38.98</td>
<td>10.39</td>
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<tr>
<td>ERT, years</td>
<td>5 (1.5)</td>
<td>4</td>
<td>7 (4)</td>
<td>5 (1)</td>
<td>4</td>
<td>5 (12)</td>
<td>6 (0.5)</td>
<td>10</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Outcome</td>
<td>Death</td>
<td>Under supervision</td>
<td>Death</td>
<td>Under supervision</td>
<td>Death</td>
<td>Under supervision</td>
<td>Under supervision</td>
<td>Under supervision</td>
<td>Under supervision</td>
<td>Under supervision</td>
<td>Under supervision</td>
<td>Under supervision</td>
</tr>
</tbody>
</table>
Figure 1. Algorithm for early detection of FD
Results and discussion

According to world statistics, signs or symptoms in patients with FD because of the heart were recorded in 60% of men and 50% of women, the average age of occurrence of the disease was 29 and 34 years, respectively (Mehta et al., 2009). Cardiac symptoms were the main manifestations of FD in 13% of men and 10% of women (Eng et al., 2007). In our study there were 12 people: 8 men and 4 women, the ratio of men to women in the study supports the conclusion that the incidence of the disease is higher in men than in women.

According to world statistics, left ventricular hypertrophy (LVH) is found in 43% of men and 26% of women, occurs earlier and progresses faster in men than in women (average age of the occurrence: 39 years versus 50 years) (Lenders and Brand, 2021; Kampmann et al., 2008). In our study, the average initial left ventricular myocardial mass index (LVMMI) of the subjects was 54.1 g/m2, and 9 patients were diagnosed with left ventricular hypertrophy (LVH) at the beginning of the study. From this group, 7 people get cardioprotective therapy in the form of ACEIs/ARBs. Atrial Gb3 deposition and subsequent fibrosis, together with LVH, diastolic dysfunction and atrial dilatation, are the proposed mechanisms for the development of atrial fibrillation, which was reported in 3% of patients with FD in retrospective studies (Shah et al., 2005; Chimenti et al., 2010; O’Mahony et al., 2011). It was also shown that 3.9% of patients had persistent atrial fibrillation and 13.3% had paroxysmal atrial fibrillation. In the late-onset cardiac-predominant phenotype, atrial fibrillation was found in 7.6% of men and in 2.4% of women, and atrial flutter in 2.5% and 0.8%, respectively. The average age for diagnosis of atrial fibrillation was 67 years in men and 77 years in women (Azevedo et al., 2020a; Azevedo et al., 2020b). From the cohort which we considered, signs of cardiac arrhythmia in the form of atrial fibrillation were detected in two patients (16.6% of the sample).

In all patients considered in this study, a mutation in gene GLA was confirmed: in 5 patients, mutation c.614C>G (p.P205R) was detected in exon 4 in a hemizygous state; in 2, nucleotide variant c.1231G>C (p.G411R) was detected in a hemizygous state; in 2, nucleotide variant c.1134T>A (p.Cys378Ter) in exon 7 in a hemizygous state, and in 1 patient a previously undescribed mutation c.237delA (G80AfS*41) in exon 2 in a hemizygous state, leading to a shift in the gene reading frame and the formation of a premature stop codon. In 9 patients, a decrease in the activity of the α-Gal A enzyme was confirmed (in the remaining 2 patients, the study was not conducted) and in 7 patients, excessive accumulation of the Lyso-Gb3 protein was confirmed, which correlates with the observed clinical picture (in the remaining patients, the study was not conducted).

According to the literature, cardiac events (defined as myocardial infarction, arrhythmia, angina pectoris, congestive heart failure (CHF), or major cardiac procedures such as pacemaker placement, stent placement, and valve replacement) were reported in 69.9% of men and 81.6% of women. Cardiac events were the first clinical event in 21.4% of men and 16.9% of women. Ultimately, heart diseases are the leading cause of death in patients with FD (40% in men and 41.7% in women) (Waldek et al., 2009). The annual incidence of cardiac death was 0.52 per 100 people, and the only independent predictor was left ventricular mass (Patel et al., 2015). Cardiac death has been reported to occur at an average age of 55.5 years in men and 66.0 years in women (Waldek et al., 2009). In our group, death occurred in 3 patients from cardiac pathology, the rest are under supervision.

Advancements in the management of Fabry disease (FD) present exciting opportunities for further investigation and scholarly discourse. Areas of interest include optimizing combination therapies to enhance treatment efficacy and long-term outcomes. Personalized medicine approaches, incorporating genetic sequencing and biomarker discovery, offer a promising avenue for tailoring treatments to individual patient profiles and genotypes (Izhar et al., 2023).

Exploring alternative delivery methods for gene therapy and enzyme replacement therapies could improve treatment effectiveness, reduce side effects, and enhance patient adherence. Furthermore, establishing comprehensive protocols for long-term monitoring and follow-up of FD patients is crucial in understanding disease progression, treatment response, and optimizing patient care (Palaiodimou et al., 2023).

In addition, patient-centered outcomes research focusing on the impact of FD on quality of life, psychosocial well-being, and daily functioning can inform holistic care strategies that prioritize patient preferences and experiences. By fostering interdisciplinary collaboration and encouraging dialogue in these research areas, the field of FD management can continue to evolve, driving innovation, advancing scientific knowledge, and ultimately improving outcomes for individuals affected by this rare genetic disorder (Engle et al., 2021).

Conclusion

On the basis of the literature review results, we examined clinical manifestations, diagnosis and treatment of FD, as well as statistics on the detection of the disease among risk groups and manifestations of various syndromes, including cardiac ones. We analyzed retrospective data on cardiac manifestations in patients with FD. Screening for FD can be carried out by various specialists, since FD is a multidisciplinary disease. In cardiac pathology, to increase the detection of FD, we propose the following algorithm: at the visit to a therapist or cardiologist for selective screening for FD, patients
must be selected based on a combination of the following criteria: age under 40 years, diagnosis of HCM or fact of LVH≥1.5 cm, the absence of persistent arterial hypertension, absence of CKD more than stage 3A, the presence of aortic valve defects. Such patients require consultation with a cardiac surgeon, geneticist, as well as a referral for the diagnosis of FD: determination of the level of the enzyme α-Gal A and the bi-omarker Lyso-Gb3 from a dried drop of blood. If a decrease in enzyme activity and an increase in the level of Lyso-Gb3 is detected, then subsequent molecular genetic diagnostics are carried out. A patient with a confirmed diagnosis of FD is registered with the Medical Genetics Center of MONIKI and with high-qualified specialists. The patient is prescribed pathogenetic therapy (ERT).

Therefore, by conducting targeted selective screening of risk groups, the detection of FD among the population will increase, which will lead to early treatment of classic and late phenotypes. To implement this algorithm, we propose the following algorithm for distribution among cardiologists and therapists (Figure 1).

Perspectives
In looking towards the future of Fabry disease research and therapeutic strategies, several promising avenues emerge. One significant focus lies in the realm of precision medicine, where advancements in genetic sequencing technologies offer the potential for personalized treatment approaches. By identifying specific genetic mutations and understanding their impact on disease progression, researchers aim to tailor interventions to individual patients, optimizing outcomes (Li et al., 2022). Another area of intense exploration involves gene therapy, a cutting-edge field that holds promise for providing long-term solutions to genetic disorders like Fabry disease. Through the delivery of corrected genes to replace or repair faulty ones, gene therapy opens up new possibilities for curing the disease at its root cause. Ongoing research into gene therapy approaches, including the use of viral vectors and gene editing technologies, seeks to expand treatment options for individuals affected by Fabry disease (Dommm et al., 2021). Meanwhile, the development of enzyme replacement therapies (ERTs) continues to progress, with efforts aimed at enhancing their efficacy, dosing regimens, and delivery mechanisms. Innovations in next-generation ERTs that target tissues more effectively, extend half-lives, and reduce immunogenicity hold the potential to improve patient outcomes and quality of life (Puhl and Ekins, 2022).

In parallel, small molecule chaperones are being explored as potential therapeutic agents for Fabry disease. These chaperones stabilize mutant enzymes, enhancing their activity and potentially alleviating disease symptoms. Further research into novel chaperone therapies and their performance in preclinical and clinical settings could offer alternative treatment avenues for individuals with Fabry disease (Weidemann et al., 2022). Additionally, the investigation of combinatorial therapies that combine various treatment modalities—such as ERTs, chaperone therapies, and gene therapy—may yield synergistic effects and enhanced outcomes for Fabry disease patients. Understanding the interactions and mechanisms of action between these diverse treatment approaches is crucial for optimizing their combined efficacy (Li et al., 2022).

Moreover, the discovery of reliable biomarkers to monitor disease progression, treatment response, and clinical outcomes represents a critical area of investigation in Fabry disease research. Identifying biomarkers from different biological sources, such as blood, urine, and tissues, can enhance disease monitoring, prognosis, and treatment decision-making (Levstek et al., 2020).

In conclusion, collaborative research efforts, interdisciplinary partnerships, and translational studies that bridge basic science with clinical practice are essential for advancing knowledge and improving care for individuals affected by Fabry disease. By prioritizing precision medicine, exploring innovative therapeutic avenues, and leveraging emerging technologies, the field is poised to make significant strides in understanding and effectively treating this rare genetic disorder (Lenders et al., 2021).

Author contribution
M.A.P., J.Y.K. conceptualized, M.A.S., M.D.S., V.V.D. wrote, drafted; A.B.Z., V.S.T., A.V.P., M.A.P., J.Y.K., E.M.P., V.I.G., V.N.S., A.N.O. wrote, reviewed, edited, prepared the graph of the article. All authors have read and agreed to the published version of the manuscript.

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Competing financial interests
The authors have no conflict of interest.

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