Review Article

New Genetic Insight of Orphan Progressive Renal Disorder, Fabry: A Review

ABSTRACT – Fabry is the rare X-linked genetic disorder caused due to mutation in Alpha – Galactosidase encoding GLA gene mutation in chromosome number 22. Fabry has wide diversification in prevalence due to clinical heterozygosity. The biomarker is the potential tool for evaluation as an indicator of the normal or altered gene. Advances in the research of biomarkers over the years have made significant development like urine-derived cells, oxidative stress, DNA methylation are currently in practice. Screening methodology like Enzyme Replacement Therapy (ERT)are in use for several years, Chaperone therapy and mRNA-based therapy, and Second generation therapies are under preclinical trials.

KEYWORDS- Rare inherited disease, Genomic Variants Fabry, Biomarker, Diagnostics, Treatment.

INTRODUCTION-

Fabry disease is a rare renal disorder caused by mutation of the X-linked GLA gene which results in the deficiency of alpha-galactosidase.[1] The cells which are deficient in the alpha-galactosidase enzyme cannot metabolize glycosphingolipid mainly Globotriaosylceramide (GB_3). Accumulation of GB_3 can lead to various symptoms and for the progression of the cascade (series of progressive events) rather than deficiency of enzymes so it can be used as a diagnostic tool for the detection of Fabry disease.[2] GB_3 cannot gain its original metabolized form Alpha-D-Galactosyl residue which is the end product of the reaction and gets clustered in higher accumulation of Globotriaosylceramide in lysosomes of cells and tissues. It belongs to renal lipidosis and may virtually accumulate into different organs which causes multisystem disease**.**[3]

Fabry prevalence is associated with a wide range in white male population approximately 1:17000 to 1:117000, in which early manifestation mutation is seen in 1:22000

to 1:40,000 males, later onset manifestation is seen in about 1:6000 to 1:40,000 females and 1:1000 to 1:3000 males.[3]

European database designed by Fabry Outcome Survey to check on the efficiency, potential, and safety of Enzyme Replacement Therapy with algalsidase alpha with the major point of consideration to monitor cardiac events in Anderson Fabry Disease.[4]

In India, the estimated data for the prevalence of Fabry disease is 1/40000 to 1/117000 is of live birth and includes both classical and non-classical phenotypes.[5]

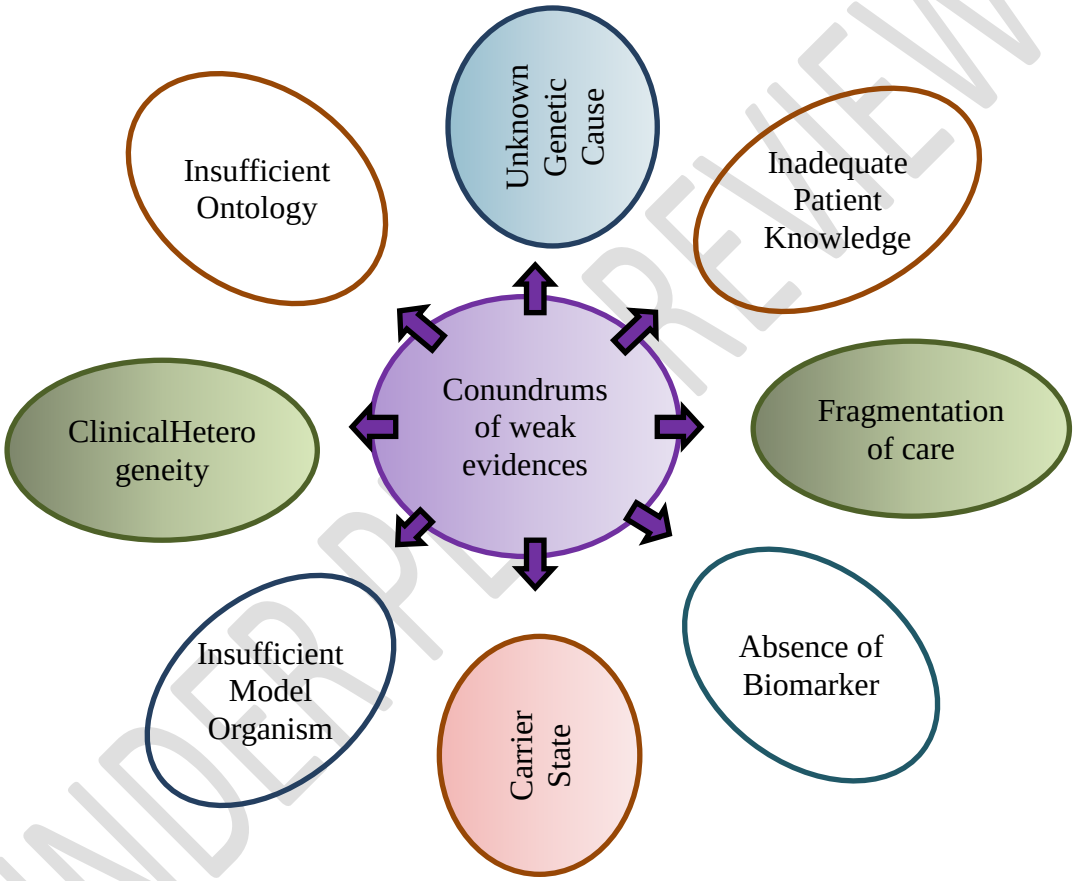


Fig. 1. Conundrums of evidence

There are two major forms of Fabry based on enzymatic activity-

1. **Classical form-**
   - Lesser amount enzymatic activity.[6]
   - Early manifestation[5]
2. **Attenuated disease/Nonclassical-**
   - A high amount of enzymatic activity.[7]
   - Later manifestation[5]

The rarity of Fabry made it difficult to understand the mechanism. Age and onset are variable, Person with unknown family history is diagnosed in later life, the reason for later onset detection is the slower progression of detectable alpha-galactosidase and causes less damage to the organs. Glomerular mesangial illness, vacuolated epithelial cells, lipid inclusions are the signs of renal abnormality. The early detection of proteinuria is the apparent sign of abnormal renal dysfunction that leads to renal failure in many individuals before the treatment methodology was developed like Enzyme replacement therapy.[6]

TABLE-1 CHARACTERIZATION OF CLASSICAL AND NON-CLASSICAL FABRY

Classical	Non-classical
Early symptoms such as anhydrosis and neuropathic pain result in severe phenotype in Hemizygous males.	Asymptomatic to the involvement of some indispensable organs in the female heterozygote.
Mostly null mutants, No residual enzyme activity at early onset.	A high amount of enzyme activity on later onset.
Event rate high Hazard ratio- • Men-3.17 to 10.00 • Women-1.54 to 5.40	Event rate low
Women develop more complications; major organ complications diagnosed early at the age of 20 to 30 years	Non-classical Fabry develops Less complication at their 50s.
Men show lower GFR, higher left ventricular mass, and elevated plasma	Comparatively high GFR, low ventricular mass, and plasma with less globotriaosylsphingosine

GENETIC ARCHITECTURE OF FABRY

There may be huge clinical variations among the same family mutation which creates difficulty in its diagnosis and men can be diagnosed only if the residual enzyme activity is found to be <35% of the mean. Females' heterozygote shows high enzyme activity due to mosaic (random inactivation) in later stage and which shows the involvement of few organs only.8Gla variant with unclear significance is also present and termed as a fringe allele.[9]

The deficiency of alpha-galactosidase (GLA) lysosomal hydrolase enzyme (EC 3.2.1.22) causes the accumulation of GB_3(alpha-linked Galactosyl moiety).[3] Prenatal diagnosis and heterozygous carrier identification, candidate identification for enzyme replacement therapy can be made easier with the help of mutation heterogeneity provides molecular pedigree analysis and stability properties.[10]The period involved from the visibility of the first symptom to its proper diagnostics is approximately 15 years sometimes it may vary from individual to individual and severity of the disease.[7] Alpha-galactosidase enzyme has approx. 1318 nucleotide in the X-Chromosome q22.1 which encodes 429 amino acid long protein chain. In this sequence, 1-31 act as signal peptides, and 32-429 form the main protein. An amino acid from 203 to 207 makes substrate binding region, amino acid 139, 192 & 215 are glycosylation region and amino acid from 170 to 231 makes active region. This region is very important for the normal function of protein and mutation in the region as shown in Table 3, and Figure 2 showing loss of function in protein (UniProt P06280).

GLA gene mutation is somewhat limited to the uniqueness of the family, there are currently more than 900 Gene variations that have been identified which results in its limitation to the genotype-phenotype positive correlation within the families.[11,12]

Aetiology of GLA Gene and Genomic Variants

A vast variety of polymorphisms (Figure 2 and Table 3) have been reported in GLA variants. Some intronic mutations have been reported as a pathogenic variant of the GLA gene and the mode of mutation comprises nonsense, splice mutation, rearrangements, deletion, and duplication in a wider range.[13]

The molecular analysis of classical and nonclassical variants revealed that CpG (5'C-Phosphate-G-3'=Cytosine and Guanine are separated by only one phosphate group) dinucleotide alteration in codon 227 is the epicenter (Mutational hotspot) for most of the mutations in unrelated Fabry alleles, except N215S, R227Q, and R227X mutation[4], viz.-

- **Classical mutation**-N34S,C56G,W340X,W162R,R22Q,R227X,D264V,D266V,S297F,D313Y,G328A,E398X,IVS2+2,IVS5,delta2,3,773 delta 2,delta 5,1123 delta 53,1016 delta 11.[4]
- **Asymptomatic**-1208 delta 3, is the frameshift mutation due to deletion of arginine 404 in a polypeptide of the residual enzyme.[4]
- **Nonclassical**-N215Sshows cardiac and renal manifestation.[4]

Novel Genomic Variants of Fabry Disease

The **c.448delG** is the de novo mutation, found as a result of a deletion in exon 3 of the GLA gene. Some of the symptoms include vertigo, acroparesthesia, cornea verticilata, and abdominal main.[14]

1. In the population of Argentina, two nonclassical missense mutations were found-
    - **p.(Cys174Gly)-**This mutation causes unfolding by disturbing thermal stability and as result protein aggregates lead to organ damage.[15]
    - **p.(Arg363His)-** In this case heat shock protein (Hsp 70) in normal condition binds to Arginine (positively charged amino acid) but in mutant form Histidine 363 is unable to bind properly with Hsp 70 causes instability in protein and the transportation to lysosome does not take place.[15]
2. **Alpha GAL A** Intron splicing mutation**al c.801 C 1G > A-** It is an insertion mutation that causes Shifting of the frame by addition of 36 nucleotide termination sequence TGA in the Exon 5 placing 12 nucleotides downstream causes formation of 270 amino acid residue of truncated GLA protein that declines the mRNA stability with the nonsense-mediated decay approach**.**[16]
3. **Genomic variants in India-**The genotypic analysis of 54 patients from 37 families were done in India and the data was taken from 10 territory referral centers which revealed 33 GLA gene varieties in 49 FD patients scattered among 7 exons in which 11 novel and 22 unknown variants were found with symptoms related to cardiovascular, ocular and cardiac dysfunction.[17] (Table-2)

TABLE.2 MUTATIONS IN EXON 5 [17]:-

S.No.	Mutation type	Mutation
1.	Missense mutation	GLA:c657C>G:pIle219Met
2.	Nonsense mutation	GLA:c658C>T:pArg220Term
3.	Duplication mutation	GLA:c683dupA
4.	Small deletion	GLA:c782delG:p.Gly261Valfs*8

CLINICAL FEATURE

Fabry manifestation has been reported both in males and females, males with severe renal dysfunction and female with heterozygote in early-stage shows renal insufficiency and

random inactivation in advance stage besides, non-classical symptoms involving target organs.[8]

Threshold

The appropriate threshold for the detection of Fabry considers 30-35% alpha-galactosidase enzyme activity as the cut-off for diagnosis and the frequent measurement of enzyme activity done in dry blood spots or peripheral leucocytes.[8,18]

The major value for pathogenicity in the form of threshold is still unknown as it varies depending upon the individual to individual and also upon the organ involved.[18]

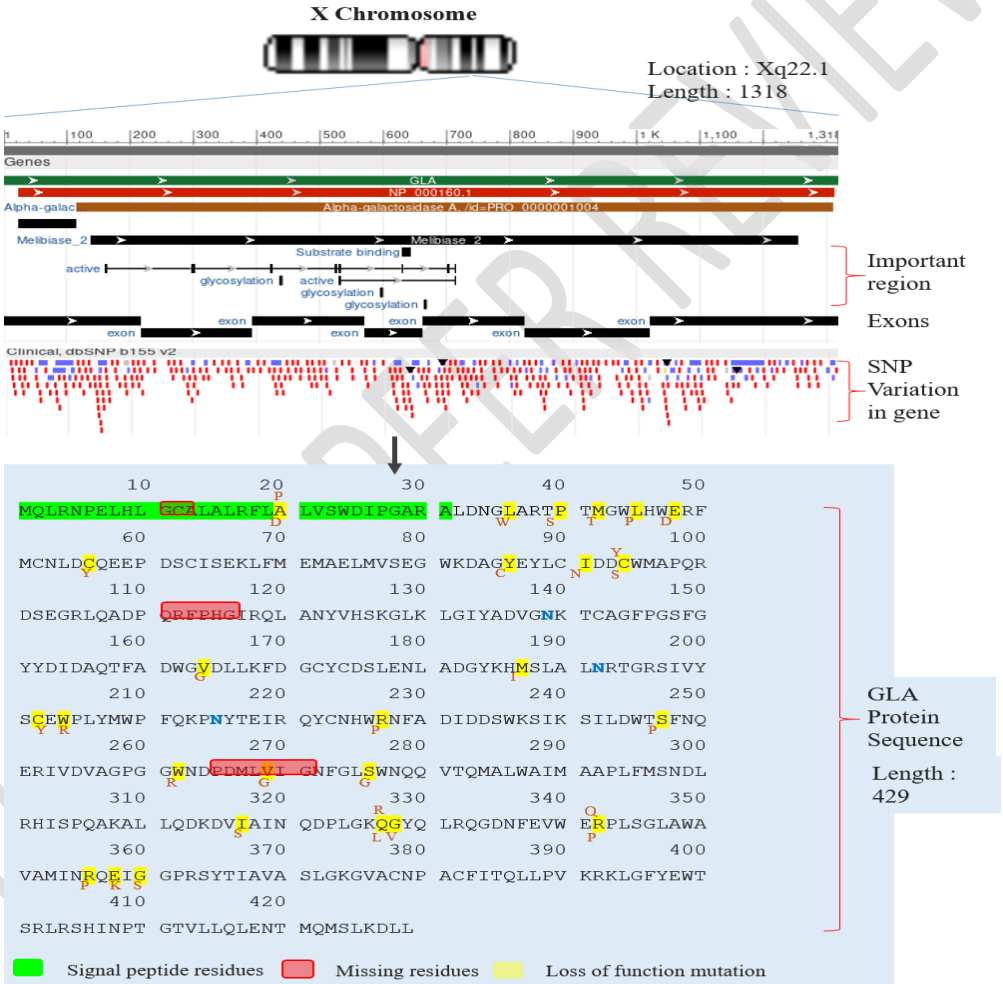


Figure 2 – GLA: Gene to protein sequence and its variations.
Gene Image were captured from NCBI (https://www.ncbi.nlm.nih.gov/nuccore/NM_000169.3?report=graph) and the protein sequence from uniprot (p06280)

Associated Risk-

Individuals' risk for complications depends not only on the alpha-galactosidase but also on other factors like epigenetic, environmental, and genetic factors which are considered to be the risk factors for the prevalence of Fabry disease.[8]

The MRI (Magnetic Resonance Image) of the brain shows the presence of an ischemic cerebral lesion, due to several mutations detected by genetic polymorphism (Table-4).[19, 20]

Factor V Leiden mutation and alpha-galactosidase A activity which was seen subsequently in the mouse model confirmed the enhanced thromboembolism along with up-regulation of vascular thrombi level which was not seen in the mouse model alone.[8]

Symptoms

Renal disease is considered to be the most potent convict for mortality and morbidity among individuals with Fabry disease.[22]

Table. 3- Showing mutation causing loss of function of protein

Residue No.	Mutation (from-to)	dbSNP
20	A-->D/P	rs89312134, rs104894847
36	L-->W	rs869312138
40	P-->S	rs104894831
42	M-->T	rs398123201
45	L-->P	
48	E-->D	rs869312254
56	C-->Y	rs869312258
86	Y-->C	rs869312140
91	I-->T	rs869312141
91	I-->N	rs869312141
94	C-->S	
164	C-->Y	rs113173389
187	M-->I	rs869312146
202	C-->Y	rs869312344
204	w-->R	rs869312148
227	R-->P	rs164894840
247	S-->P	rs869312393
262	W-->R	rs869312154
269	V--G	rs28935488
276	S-->G	rs869312432
317	I-->S	
327	Q-->L/R	rs869312160
328	G-->V	rs104894832
342	R-->P	
342	R-->Q	rs28935493
356	R-->P	rs869313163
358	E-->K	rs797044774
360	G-->S	

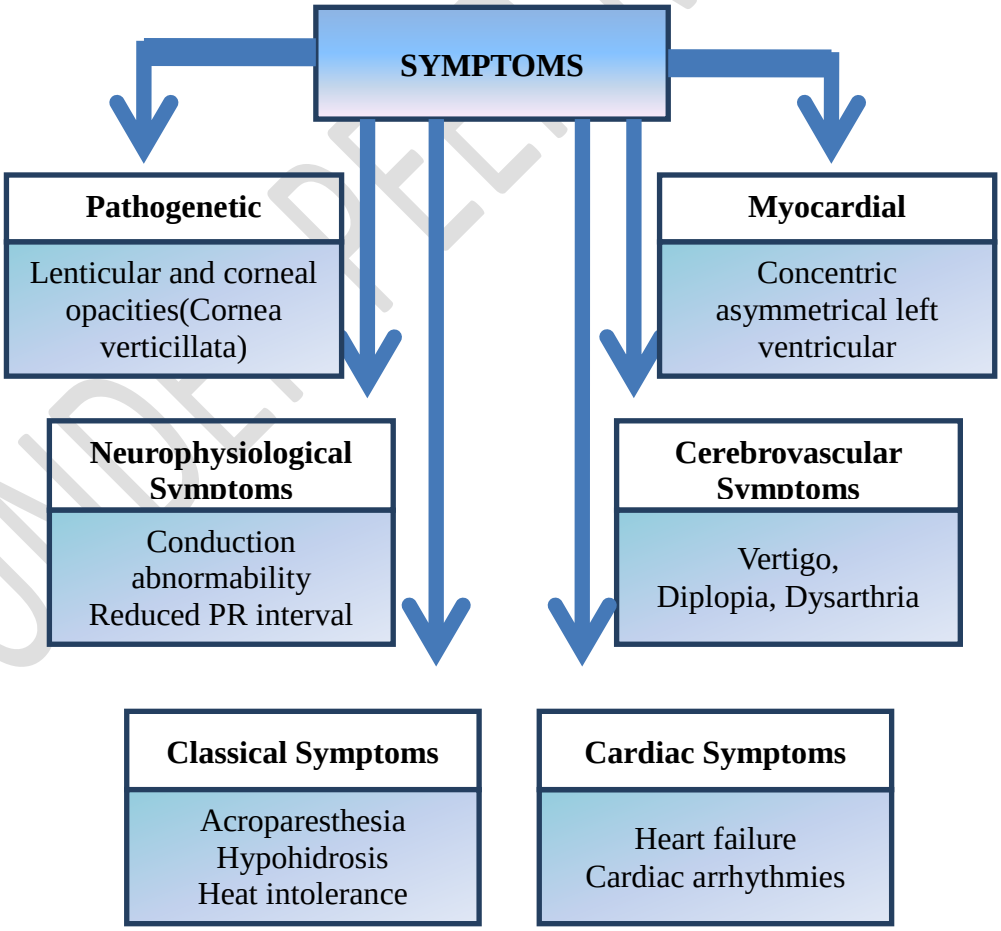
As per the diagnosis cascade and screening of relatives for phenocopy identification in patients with inherited cardiomyopathies regardless of the age of the patient, it becomes evident that chromosome inactivation mostly did not manifest any effect in the female carrier but due to random inactivation some discrete symptoms were observed in affected females.[21] Renal disease is considered to be the most potent convict for mortality and morbidity among individuals with Fabry disease.[22] There is enormous variability within clinical courses and symptoms (Figure-3) of affected People suffering from Fabry disease and

the basis of morbidity could be determined with the help of positive family history, Severity which leads to morbidity condition diagnostics showed involvement of renal, cerebrovascular and cardiac involvement.

TABLE 4. - GENETIC POLYMORPHISM IN MRI OF FABRY PATIENT

Factors	Genetic polymorphism	
Interleukin-6	G174C	
Endothelial nitric oxide synthase	G894T	
Factor V leiden	Factor V G161A	
Protein Z	A13G	G79A

The affected male shows symptoms like proteinuria, microhematuria, and lipiduria, angiokeratomas, edema, abdominal pain. Acroparesthesia is more often seen in childhood. The main factor for elevated risk is premature stroke and transient ischemic attack, dementia.[2] Pathogenetic, myocardial, neurophysiological, cerebrovascular, classical-like



many symptoms were observed in the FD patients which further acts as diagnostic criteria.

Figure 3- Clinical Manifestation

LITERATURE SEARCH METHOD

Present Review Article has been prepared after a critical review of various kinds of literature articles outsourced from various databases such as PubMed, Shodhganga, Google Scholar, Research Gate, Science direct, WHO(World Health Organization) records with the help of different keywords like Fabry disease, Genomic variants, Correlation with other diseases Biomarker, treatment methodologies, etc.

TOOLS FOR DIAGNOSIS AND RESEARCH PATHOMECHANISM

There are many diagnostic tools which are being used as potent diagnostics tools for determination of Fabry disease such as-

- Globotriaosylceramide (GB3) accumulation.
- Primary Urine derived cell cultures
- Oxidative stress biomarkers

1. GB_3 Accumulation

To check or monitor the efficacy of therapy and the progression of the disease is being monitored with the help of measurement of GB3 accululaion.[24]

GB3 accumulation is the potent biomarker because of its direct use as it releases secondary mediators for glomerular Fabry nephropathy and causes disease pathogenesis and progression through fibrosis and inflammation stimulation.[24] Invitro analysis of prime urine-derived cells showed elevation in the expression level of COL4, FN1, HES1, and TGFβ1.[25]

2. Primary urine-derived cells-

Urine cells are non-invasive cells of a patient with Fabry disease decreases Alphagalactosidase enzyme activity and that mimics the cellular in vivo model and dysregulation of lysosomal protein, so the study was done based on proteomics, qRT-PCR, western blot, immunoassay, this could be the prospects for the diagnostic purpose as both show Globotriaosylceramide accumulation (lysosomal storage material).[26]

3. Oxidative stress biomarkers –

Several mutations are found related to oxidative stress revealed the involvement of smooth muscle cells, cardiac myocytes, and all types of renal cells such as podocytes, tubular and glomerular cells. Oxidative stress is responsible for organ damage is a hypothesis considered by several authors.[27]

Occurrence of pro-oxidant state induced (Table-5) by Lysosomal Gb3 in Fabry patient had been demonstrated by Biancini, *et.al*.,(2012)[28]. A positive correlation was found as the level of advanced oxidation product increases the reduction thiol group and ferric reducing power[27] results in a reduction of proteins responsible for oxidative damage.[28] The involvement of mitochondrial DNA haplogroups for modulation of oxidative stress in patients suffering from Fabry had been resulted as before the Enzyme replacement event.[29] Elevated Lipid peroxidation showed irreversible effect after Enzyme replacement therapy as per hypothesis.[30]

TABLE. 5 - OXIDATIVE STRESS ACTIVATION[28]

S.No.	Result of oxidative stress activation	
	Increase level	**Decrease level**
1.	Erythrocytic Superoxide dismutase/catalase ratio	Anti oxidatant scavangers
2.	Malondialdehyde	Reduced glutathione
3.	Protein carbonyl group product	Glutathione peroxidase

Cardiovascular renal remodeling pathophysiology suggested oxidation stress signaling cascade and activation of oxidative stress and p22phox expression found substantially elevated as per Ravarotto, *et.al.,*.in his studies.[31] Table-6 shows the diagnostic approaches and Table-7 shows the emerging potential tools in the study for diagnosis purposes.

TABLE.6- CURRENT DIAGNOSTIC APPROACH

S. NO.	**DIAGNOSTIC APPROACH**	**CHARACTERISTICS**
1.	Serum creatinine and	Increase in GFR-Early detection tool.[32] A decline in GFR-Later stage.[33]

	GFR (Glomerulus Filtration Rate)	Two different strategies based on serum creatinine equation:- **Adult**-Chronic Kidney Disease Epidemiology Collaboration(CKD-EPI)[34] **Children**-Swartz Formula.[35]
2.	Albuminuria	A high concentration of Albumin was observed in patients with Fabry disease.[36]
3.	Cystatin –C	Cystatin-cis the potent inhibitor act as a protease inhibitor found in all nucleated cells and is useful for the diagnosis of early-onset that is classical renal dysfunction diagnosis. It can decrease the GFR to a little extent in both males and females.[37]
4.	Urine microscopy	**Phase-contrast microscope**-Tubular epithelial cells were seen. **Polarized microscope** -Urine sediments observation showed Maltese cross bodies encapsulated in the mulberry cells.[36]
5.	Renal biopsy	Renal biopsy(histological study)is the most trustable tool. **Stereomicroscopy-** For more sensitive histological renal structures stereomicroscope is used. **Electron Microscopy**-Zebra bodies (small osmophilic structures) were observed which were seen encased in a coated membrane.[36]
6.	Proteinuria	Amiloride has an important function *in vitro* to reduce podocyte (renal cells) motility and it causes proteinuria in mice.[37]

TABLE.7- POTENTIAL DIAGNOSTIC TOOLS

S.No.	Analysis	Observation
1.	**Transcriptomics (mi RNA analysis)**	mi RNA analysis shows **mi R 29** and **mi R 200** detected from the expression of urine mi RNA can cause renal fibrosis before the visibility of signs of albuminuria.[38,39]
2.	**Epigenomics**	Fabry patient's nephropathy from urine sample determination by comparing with control revealed the

		presence of a greater number of post-translational modifications such as GB3 methylated isoforms found in urine and plasma of the patient.[40]
3.	**Proteomics**	Proteinuria cannot be treated as such even after treatment and cause loss of GFR (<60mL/min/1.73m^2).[41] The study of proteomics is quite easy in patients with Fabry disease because of its noninvasiveness, and no limit on the number of samples to be used.[42]
4.	**Metabolomics**	Male individuals have a higher amount of metabolites in urine samples as gender influences Urine level in pediatric patients.[43]

Potential Biomarker

- **Bikunin**- Bikunin is the Urine Protease inhibitor(serine protease).Bikunin was found elevated in the Fabrypatients compared to the individuals with other renal impairment. The elevated bikunin along with proteinuria is used as a novel biomarker[44] (Figure 4).
- **Urine Podocyte-** As the glomerular impairment progresses in Fabry patients, the podocytes enter into urine and resulting in an elevated concentration of urine podocytes. The latter condition is known as podocytinuria (Figure 4).[45]
- **Pelvic cyst-** Renal impairment correlates with the pelvic cyst reported by regular monitoring with ultrasonography. It is not the only pathognomic or significant tool for Fabry diagnosis but their presence can recruit further diagnosis for Fabry (Figure 4).[46]
- **Tubular Protein- Type IV collagen** and **Transferrin**protein leakage is observed are shows correlation With GFR and their presence in urine can be considered as the novelty of the diagnostic approach (Figure 4).[47]

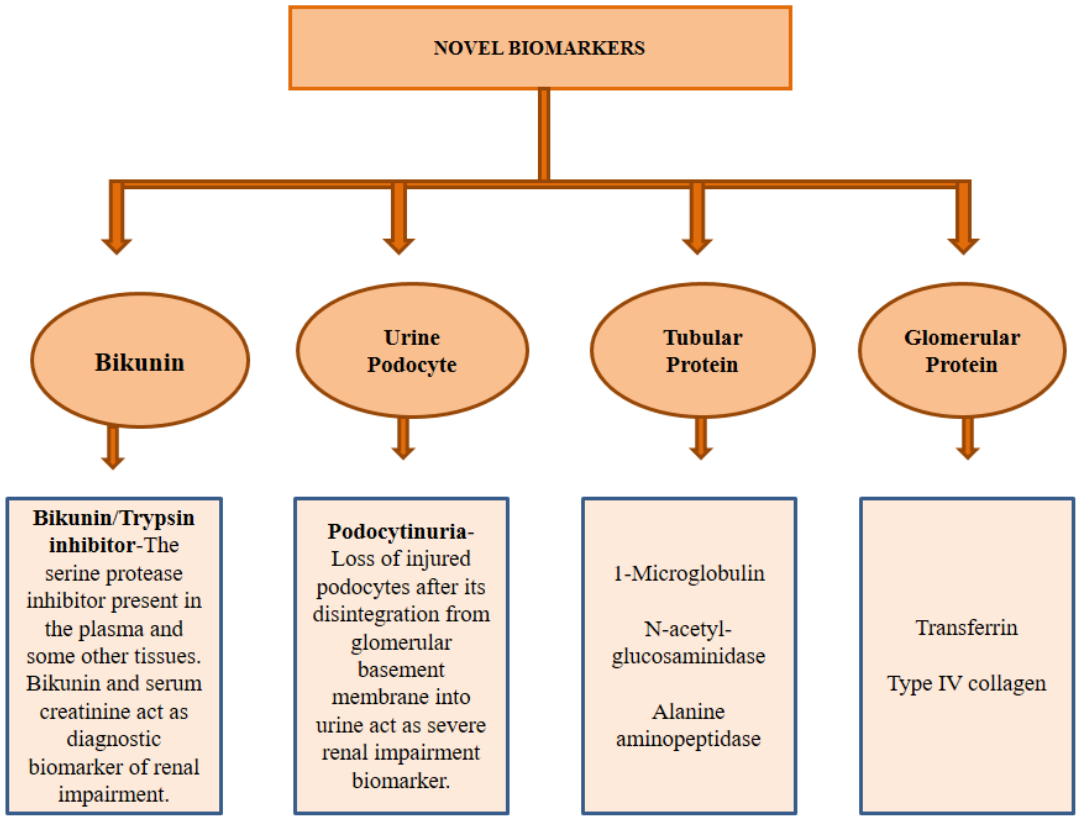


Figure 4 - Potential Marker for Diagnosis

- **Glomerular protein-** Alpha 1 Microglobulin and N-Acetyl-beta-Glucosaminidase and Alanine aminopeptidase are the glomerular proteins and their dysfunction can be indicated as the sign of Fabry renal impairment (Figure 4).[47]
- **Differential DNA Methylation**. Diffe- ential DNA Methylation has the immense potential for the characterization of rare diseases to detect renal anagenesis, glomerular disease, proteinuria, and membranous nephropathy (Figure 4).[49]

TREATMENT

1. Systemic mRNA (Messenger RNA) therapy

From the preclinical studies, it was revealed that intravenous administration of human GLA encoding systemic mRNA helps to promote the production of the alpha-galactosidase enzyme in mice at its first administration and in humans. It was found thermostable and capable of substrate reduction (accumulated GB3 reduction) as an alpha-galactosidase enzyme in Fabry patients who lack wild-type alpha-galactosidase enzyme. This approach can be used for other lysosomal storage disorders.[51]

2. Enzyme replacement therapy

Enzyme placement therapy(ERT) has been in practice since2001, which implies the introduction of recombinant GLA into the host (Fabry patient)once in two weeks intravenously. It can revert the abnormalities and metabolic dysfunctionalities.[52] ERT is a costly procedure and requires a lot of skill. Mostly effective in the treatment of people with cardiac and renal Fabry disease at classical onset however its non-classical/later-onset effect is not known.[52] Regular administration is required as these enzyme does not have a significant half-life. [53]

The two major enzymes are used for enzyme replacement therapy-

1. Agalsidase -alfa "Replagal" (Shire Human Genetic Therapies, Cambridge, A)
   -0.2 mg/kg administration in each infusion.
2. Agalsidase-beta "Fabrazyme", (Genzyme Corporation, Cambridge, MA)
   - 1 mg/kg administration in each infusion.[54]

The FDA(Food and drug administration the USA) has approved only the use of Fabrazyme for ERT.[54]

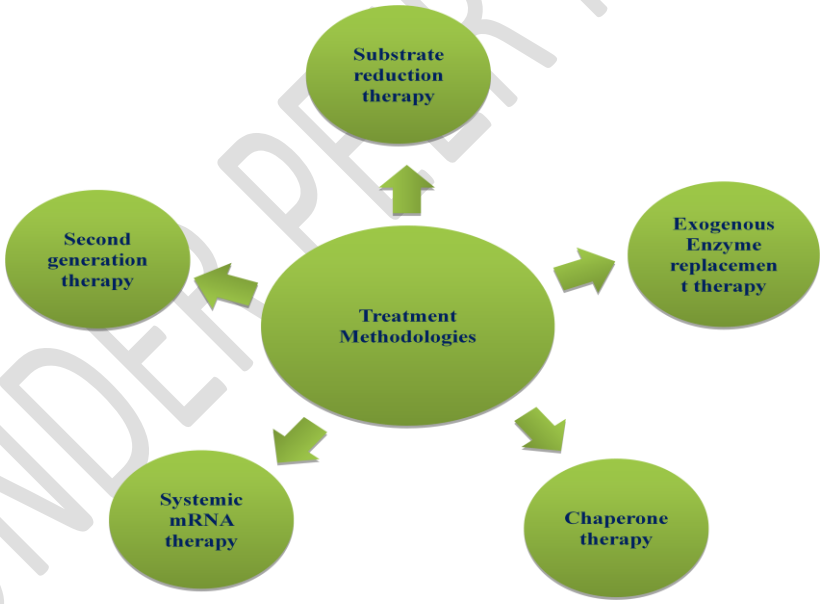


FIG.5 Methodologies Of Fabry Treatment

3. Chaperone therapy

Frustaci et al. Performed the first clinical trial as pharmacological chaperon therapy. (using galactose).[55] In Europe and Canada the approval was given to the pharmacological **migalastat**(Galafold™; Amicus Therapeutics, Cranbury, NJ, USA) for treatment of Fabry disease caused due to amenable mutation.[56]

During the study of misfolding mutation in the enzyme, to enhance the intracellular activity of mutant enzyme the competitive inhibitor application (results in the proper folding of mutant enzyme, protein maturation as well as transportation to the lysosomal) approach is applied to treat Fabry disease strictly with misfolding mutant enzyme.[57]

1-deoxygalactonojirimycin(DGJ) is the competitive inhibitor of the potent alpha-galactosidase enzyme that binds to the active site of the enzyme and causes its proper folding as well as intracellular targeting to the lysosome for the degradation of accumulated GB3 in mammals. It has been proved during the study of transgenic mice with the similar alpha-galactosidase enzyme(misfolding mutant)by oral administration.[58] **S**eventy-eight misfolding mutations are treated with this approach some of them are assayed with patient's cells - A20P, N34S, R49L, F113L, Y207C [59] andR49C, M51K, I91T.[60]

4. Substrate reduction therapy-

SRT maintenance therapy requires less dependency on enzyme infusion consequently it shows excellence in improvement in the quality of life as it circumvents ERT (Enzyme replacement therapy.[61]

In-vivo study in a mouse model with early neonatal death indistinguishably showed GB3 accumulation in kidney and vasculature as due to lack of an alpha-galactosidase enzyme in Fabry patients. Lymphoblasts were transformed from Fabry disease patients with EtDO-P4 showed a significant reduction of glucocerebroside and globotriaosylceramide.[61] (Sanne J. van der Veen, et.al.,). In vivo assay of two mouse models with early neonatal death had been studied- 1. conduritol _ epoxide(CBE)-induced mouse model of neuronopathic GD. 2. genetic 4L; C model. GENZ-682452 and GZ/SAR402671 were the two compounds under clinical development for the treatment of Fabry disease as both the model mouse showed enhancement in survivorship and cause most neurogenerative disorders that cause accumulation of some compounds on basis significant studies revealed the effectiveness over the treatment with SRT.[61]

5. Second-generation therapy

1) Pegunigalsidase-alfa - Cells of Tobacco plant produces Pegunigalsidase-alfa**(PRX-102)** which was then modified with fusogen (PEG) polyethylene glycol for maintenance of stability and to tolerate reduction from clearance was confirmed by Experiment on Human plasma. The murine model showed an extra reduction in the GB3 level as compared to an alpha-galactosidase enzyme with the help of PRX 102 in plasma as well as renal biopsy of Fabry patients.[62]

2) Moss-aGal - R- αGAL's plant-derived form is moss aGal and has immense potential of enzyme activity during the renal biopsy, it has the property that it can easily take up by endothelial cells. Moss aGAL has higher enzymatic activity than alpha-galactosidase as revealed from the studies performed on the FD mice model.[63] Moss aGAL is much more effective for clearing accumulated GB3 than alpha-galactosidase as examined by the mouse model. In a single infusion, moss aGAL has a half-life of 14 minutes in plasma as detected by pharmacokinetics, less than normal alpha-galactosidase enzyme,[61] (Fig. 5).

CONCLUSION

Clinical heterogeneity of Fabry Disease endorses the individualized diagnostic approach discovery with transcriptomics, epigenomics, metabolomics based on the patient's age, gender, type of mutation, and the onset of manifestation. The diagnostic tools utilize oxidative stress biomarkers, elevated GB3, some novel biomarkers like bikunin, tubular and glomerular cellular concentration, urine podocytes, cystatin C, and hold potential promise for Fabry diagnosis and will further expand in foreseeable with several mutations in GLA gene. The classical ERT(Enzyme Replacement Therapy), Chaperone therapy and recently mRNA-based therapy, substrate reduction therapy, second-generation therapy like Pegunigalsidase-alfa, Moss-aGal have the most significant clinical benefits. The conundrums of rarity summarize the need for progressive research and development.

REFERENCES

1. Brady RO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L. “Enzymatic defect in Fabry's disease. Ceramidetrihexosidase deficiency”. *N Engl J Med*. 1967;276(21):1163-1167. doi:10.1056/NEJM196705252762101
2. Sweeley CC, Klionsky B. “Fabry’s Disease: Classification as a sphingolipidosis and partial characterization of a novel glycolipid”. *J Biol Chem*. 1963;238:3148-3150.
3. Eng CM, Ashley GA, Burgert TS, Enriquez AL, D'Souza M, Desnick RJ. Fabry disease: thirty-five mutations in the alpha-galactosidase A gene in patients with classic and variant phenotypes. *Mol Med*. 1997;3(3):174-182.
4. Eng CM, Resnick-Silverman LA, Niehaus DJ, Astrin KH, Desnick RJ. “Nature and frequency of mutations in the alpha-galactosidase A gene that causes Fabry disease”. *Am J Hum Genet*. 1993;53(6):1186-97.
5. Germain, D.P. “Fabry disease”. *Orphanet J Rare Dis* **5,** 30 (2010). https://doi.org/10.1186/1750-1172-5-30
6. Banton, Mary H., Raphael Schiffmann, Sharda G. Sabnis, Gary J. Murray, Jane M. Quirk, Gheona Altarescu, and Lev Goldfarb et al. 2002. "Natural History Of Fabry Renal Disease". *Medicine* 81 (2): 122-138. doi:10.1097/00005792-200203000-00003.
7. Desnick RJ, Allen KY, Desnick SJ, Raman MK, Bernlohr RW, Krivit W. “Fabry's disease: enzymatic diagnosis of hemizygotes and heterozygotes. Alpha-galactosidase activities in plasma, serum, urine, and leukocytes”. *J Lab Clin Med*. 1973;81(2):157-171.
8. Schiffmann R, Fuller M, Clarke LA, Aerts JM. “Is it Fabry disease?” *Genet Med.* 2016;18(12):1181-1185. doi:10.1038/gim.2016.55
9. Linhart A, Kampmann C, Zamorano JL, et al. “Cardiac manifestations of Anderson-Fabry disease: results from the international Fabry outcome survey”. *Eur Heart J*. 2007;28(10):1228-1235. doi:10.1093/eurheartj/ehm153
10. Bernstein HS, Bishop DF, Astrin KH, Kornreich R, Eng CM, Sakuraba H, Desnick RJ. “Fabry disease: six gene rearrangements and an exonic point mutation in the alpha-galactosidase gene”. *J Clin Invest.* 1989;83(4):1390-9. doi: 10.1172/JCI114027.

11. "The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1,092 human genomes". *Nature* **491.**2012;50-56 https://doi.org/10.1038/nature11632
12. Laney DA, Fernhoff PM. "Diagnosis of Fabry disease via analysis of family history". *J Genet Couns*. 2008;17(1):79-83. doi:10.1007/s10897-007-9128-x
13. Iemolo, F., Pizzo, F., Albeggiani, G. *et al.* "*De novo* mutation in a male patient with Fabry disease: a case report". *BMC Res Notes* **7,** 11 (2014). https://doi.org/10.1186/1756-0500-7-11
14. Calabrese, Esteban & Botta, Guillermo & Rosenfeld, Dra. (2021). "New mutation in Fabry disease: c.448delG, first phenotypic description". Molecular genetics and metabolism reports. 27. 100708. 10.1016/j.ymgmr.2021.100708.
15. Serebrinsky, G et al. "Late-onset variants in Fabry disease: Results in high-risk population screenings in Argentina." *Molecular genetics and metabolism report* 2015, vol. 4 19-24. doi:10.1016/j.ymgmr.2015.05.006
16. Li P, Zhang L, Zhao N, Xiong Q, Zhou Y-A, Wu C, and Xiao. "A Novel _-Galactosidase A SplicingMutation Predisposes to Fabry Disease". Front. Genet. 2019;10:60.
17. 18. Nampoothiri S, Yesodharan D, Bhattacherjee A, Ahamed H, Puri RD; et .al. 2020"Fabry disease in India: A multicenter study of the clinical and mutation spectrum in 54 patients." JIMD Reports.2020;56:82–94.
18. Leinenkugel P, Michel S, Conzelmann E, Sandhoff K. "Quantitative correlation between the residual activity of beta-hexosaminidase A and arylsulfatase A and the severity of the resulting lysosomal storage disease". *Hum Genet*. 1992;88(5):513-523. doi:10.1007/BF00219337
19. Shen YC, He ZC, Cai RL, Pan JZ, Wang XM, Li J. Zhonghua Xue Ye Xue Za Zhi. "Effect of alpha-galactosidase A deficiency on FV Leiden fibrin deposition and thrombosis in mice"2009;30(3):162-165.
20. Lenders M, Karabul N, Dunning T, et al. "Thromboembolic events in Fabry disease and the impact of factor V Leiden."*Neurology.*2015;84:1009–1016
21. Rasmus Bo Hasselbalc, Per Lav Madsen, Henning Bundgaard and JulianeTheilade."Presymptomatic diagnosis of Fabry's disease: a case report"; Journal of Medical Case Reports".2016,10:330

22. ThadhniR, Wolfe, WestML, *et al*: "Patients with Fabry disease on dialysis in the United States". *Kidney Int* .2002**,** 61:249–255

23. Frank Breunig, Frank Weidemann, Meinrad Beer, Andreas Eggert,VeraKrane,MatthiasSpindler,Jo RN Sandstede,Jo.RGStrotmann,ChristophWanner."Fabry disease: Diagnosis and treatment." *Kidney International.2003; Vol. 63, Supplement 84 , pp. S181–S185*

24. Nowak, A., Mechtler, T., Kasper, D. C. &Desnick, R. J. "Correlation of Lyso-Gb3 levels in dried blood spots and sera from patients with classic and Later-Onset Fabry disease."*Molecular Genetics and Metabolism.2017;* 121, 320–324.

25. Sanchez-Niño, M. D. *et al*. "Lyso-Gb3 activates Notch1 in human podocytes."*Hum. Mol. Genet*. 2015;ddv291, https://doi.org/10.1093/hmg/ddv29.

26. Slaats, G.G., Braun, F., Hoehne, M. *et al.* " Urine-derived cells: a promising diagnostic tool in Fabry disease patients." *Science Report* .2018;**8,** 11042 .https://doi.org/10.1038/s41598-018-29240-w.

27. Simoncini C, Torri S, Montano V, et al. "Oxidative stress biomarkers in Fabry disease: is there a room for them?". *J Neurol*. 2020;267(12):3741-3752. doi:10.1007/s00415-020-10044-w

28. Biancini GB, Vanzin CS, Rodrigues DB, Deon M, Ribas GS, Barschak AG et. al**.;** "Globotriaosylceramide is correlated with oxidative stress and inflammation in Fabry patients treated with enzyme replacement therapy."BiochimBiophysActa.2012;1822(2):226–232.

29. Simoncini C, Chico L, Concolino D, Sestito S, Fancellu L, Boadu W et. al; "Mitochondrial DNA haplogroups may influence Fabry disease phenotype".NeurosciLett.2016; 629:58–61.

30. Biancini GB, Jacques CE, Hammerschmidt T, de Souza HM, Donida B, Deon M et.al.; "Biomolecules damage and redox status abnormalities in Fabry patients before and during enzyme replacement therapy."ClinChimActa.2016;461:41–46.

31. Ravarotto V, Carraro G, Pagnin E, BertoldiG, Simioni F, Maiolino G .et.al.; "Oxidative stress and the altered reaction to it in Fabry disease: A possible target for cardiovascular-renal remodeling?".2018,PLoS One 13(9):e0204618

32. Riccio, E.; Sabbatini, M.; Bruzzese, D.; AnnicchiaricoPetruzzelli, L.; Pellegrino, A.; Spinelli, L.; Esposito, R.;Imbriaco, M.; Feriozzi, S.; Pisani, A. "Glomerular hyperfiltration: An early marker of nephropathy in Fabry disease". Nephron .2019, 141, 10–17.

33. Ramaswami, U.; Najafian, B.; Schieppati, A.; Mauer, M.; Bichet, D.G." Assessment of renal pathology and dysfunction in pediatric patients with Fabry disease."Clin. J. Am. Soc. Nephrol. 2010, 5, 365–370.
34. Levey, A.S.; Stevens, L.A.; Schmid, C.H.; Zhang, Y.; Castro, A.F.; Feldman, H.I.; Kusek, J.W.; Eggers, P.; Van Lente, F.; Greene, T.; et al. "A new equation to estimate glomerular filtration rate". Ann. Intern. Med.2009, 150,604–612
35. Schwartz, G.J.; Muñoz, A.; Schneider, M.F.; Mak, R.H.; Kaskel, F.; Warady, B.A.; Furth, S.L. "New equations to estimate GFR in children with CKD." J. Am. Soc. Nephrol.2009, 20, 629–637
36. Tina Levstek ,BojanVujkovac,and Katarina TrebusakPodkrajsek."Biomarkers of Fabry Nephropathy: Review and Future Perspective."Genes,2020, 11, 1091
37. B. Zhang, S. Xie, W. Shi, and Y. Yang, "Amiloride off-target effect inhibits podocyteurokinase receptor expression and reduces proteinuria," *Nephrology Dialysis Transplantation .2012*, vol. 27, pp. 1746–1755.
38. Jaurretche, S.; Perez, G.R.; Venera, G. "High Lyso-Gb3 plasma levels associated with decreased miR-29 and miR-200 urinary excretion in a young non-albuminuric male patient with classic Fabry disease." Case Rep.Nephrol.2019, 4980942.
39. Jaurretche, S.; Venera, G.; Antongiovanni, N.; Perretta, F.; Perez, G.R. "Urinary excretion of microRNAs in young Fabry disease patients with mild or absent nephropathy". Open J. Nephrol.2018, 8, 71–83.
40. About, M.; Boutin, M.; Lavoie, P.; Auray-Blais, C. "Tandem mass spectrometry multiplex analysis of methylated and non-methylated urinary Gb3 isoforms in Fabry disease patients." Clin. Chim. Acta.2016, 452,191–198.
41. Schi_mann, R.; Hughes, D.A.; Linthorst, G.E.; Ortiz, A.; Svarstad, E.; Warnock, D.G.; West, M.L.; Wanner, C.Screening, diagnosis, and management of patients with Fabry disease: Conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. Kidney Int.2017, 91, 284–293
42. Schi_mann, R.; Waldek, S.; Benigni, A.; Auray-Blais, C. "Biomarkers of Fabry disease nephropathy". Clin. J. Am. Soc. Nephrol. 2010, 5, 360–364.
43. Moore, D.F.; Krokhin, O.V.; Beavis, R.C.; Ries, M.; Robinson, C.; Goldin, E.; Brady, R.O.; Wilkins, J.A.; Schi_mann, R. "Proteomics of specific treatment-related alterations in Fabry disease: A strategy to identify biological abnormalities."Proc. Natl. Acad. Sci. USA.2007, 104, 2873–2878.

44. Lepedda AJ, Fancellu L, Zinellu E, et al. “Urine bikunin as a marker of renal impairment in Fabry's disease”. *Biomed Res Int*. 2013;2013:205948. doi:10.1155/2013/205948
45. Fall, B.; Scott, C.R.; Mauer, M.; Shankland, S.; Pippin, J.; Je_erson, J.A.; Wallace, E.; Warnock, D.; Najafian, B. “Urinary podocyte loss is increased in patients with Fabry disease and correlates with clinical severity of Fabry nephropathy”.PLoS ONE .2016, 11, e0168346
46. .Eleonora Riccio, Massimo Sabbatini, Ivana Capuano, Antonio Pisani “Early biomarker of fabry nephropathy.” Nephron .2019;143:274–281
47. Aguiar P, Azevedo O, Pinto R, Marino J, Baker R, Cardoso C, et al. “New biomarkers defining a novel early stage of Fabry nephropathy: A diagnostic test study.”Mol Genet Metab.2017; Jun; 121(2): 162–9
48. Katie Kerr1, Helen McAneney, Cheryl Flanagan, Alexander P. Maxwell, and Amy Jayne McKnight.”Differential methylation as a diagnostic biomarker of rare renal diseases: a systematic review. “Kerr et al. BMC Nephrology.2019**;**20:320
49. Fabry disease presents as an autoimmune rheumatic disease. Hannah J, Sangle S, D'Cruz D. 81. *Rheumatol Adv Pract*. 2018;2(Suppl 1):rky034.044. Published 2018 Sep 20. doi:10.1093/rap/rky034.044
50. Mehta A, Ginsberg L, Investigators FOS. “Natural history of the cerebrovascular complications of Fabry disease”. ActaPadiatrica.2005;94(Suppl 447):24–7.
51. Zhu X, Yin L, Theisen M, et al. “Systemic mRNA Therapy for the Treatment of Fabry Disease: Preclinical Studies in Wild-Type Mice, Fabry Mouse Model, and Wild-Type Non-human Primates”. *Am J Hum Genet*. 2019;104(4):625-637. doi:10.1016/j.ajhg.2019.02.003
52. Lidove O, Joly D, Barbey F, *et al*“ Clinical results of enzyme replacement therapy in Fabry disease: a comprehensive review of the literature.Int J Clin Pract.2007; 61: 293-302.”
53. Pierre-Louis B, Kumar A, Frishman WH. “Fabry disease: cardiac manifestations and therapeutic options.”Cardiol Rev.2009; 17: 31-5.
54. Eng CM, Griffon N, Wilcox WR, *et al. “*Safety and efficacy of recombinant human alpha-galactosidase A--replacement therapy in Fabry's disease.” N Engl J Med.2001; 345: 9-16.

55. Frustaci, A., Chimenti, C., Ricci, R., Natale, L., Russo, M.A., Pieroni, M., Eng, C.M. and Desnick, R.J."Improvement in cardiac function in the cardiac variant of Fabry's disease with galactoseinfusiontherapy." N. Engl. J. Med.2001; 345, 25–32.
56. KDIGO, 2012 "Clinical practice guideline for the evaluation and management of chronic kidney disease", Kidney Int.2013; Suppl 3,1–150
57. Fan, J.-Q., Ishii, S., Asano, N. and Suzuki, Y. "Accelerated transport and maturation of lysosomal,-galactosidase A in Fabry lymphoblasts by an enzyme inhibitor". Nat. Med. 1999; 5, 112–115.
58. Satoshi ISHII. "Pharmacological chaperone therapy for Fabry disease. The Japan Academy".2012;doi: 10.2183/pjab.88.18
59. Benjamin, E.R., Flanagan, J.J., Schilling, A., Chang,H.H., Agarwal, L., Katz, E., Wu, X., Pine, C.,Wustman, B., Desnick, R.J., Lockhart, D.J. andValenzano, K.J. "The pharmacological chaperone1-deoxygalactonojirimycin increases alpha-galactosidase A levels in Fabry patient cell lines." J.Inherit. Metab. Dis. 2009;32, 424–440
60. Shin, S.H., Kluepfel-Stahl, S., Cooney, A.M.,Kaneski, C.R., Quirk, J.M., Schiffmann, R.,Brady, R.O., and Murray, G.J. "Prediction of the response of mutated,-galactosidase A toa pharmacological chaperone. Pharmacogenet." Genomics .2008; 18, 773–780
61. Sanne J. van der Veen, Carla E. M. Hollak, André B. P. van Kuilenburg, Mirjam Langeveld. "Developments in the treatment of Fabry disease." J Inherit Metab Dis. 2020;43:908–921.
62. Schiffmann R, Goker-Alpan O, Holida M, et al.; " Pegunigalsidasealfa, a novel PEGylated enzyme replacement therapy for Fabry disease, provides sustained plasma concentrationsandfavorable pharmacodynamics: a 1-year phase ½ clinical trial". J Inherit Metab Dis.2019;3:534-544.
63. Shen JS, Busch A, Day TS, et al. Mannose receptor-mediated delivery of moss-made alpha-galactosidase and efficiently correct enzyme deficiency in Fabry mice. J Inherit Metab Dis.2016;2:293-303.