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ARTICLE

Prospective characterization of early symptom onset and progression in young pediatric patients with variants in the *GLA* gene across 5 years: Longitudinal data from the Fabry MOPPet Study

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ABSTRACT

Purpose: This prospective, longitudinal study was designed to determine the natural history of Fabry disease (FD) in early pediatric patients across the disease spectrum.
Methods: In this observational study of children under 5 years of age with variants in the *GLA* gene, prospective phenotypic and urinary biomarker data were collected annually over 5 years.
Results: The study population included 40 participants (35 male, 5 female) with *GLA* variants including 15 with classic pathogenic variants (CFD), 6 with nonclassic pathogenic variants (NFD), and 19 with a variant of uncertain significance. The most common first symptoms reported were in participants with CFD and included gastrointestinal symptoms (13/15), heat intolerance (13/15), reduced sweating after previously sweating normally (6/15), and neuropathic pain/uncomfortable feet/hands (3/15). Mapping symptom onset and progression reveals a consistent pattern of frequency and severity occurring in the first years of life and beginning at an average age of 23.4 months (range 11-32 months) in males with CFD. Participants with nonclassic pathogenic variants and variant of uncertain significance did not exhibit consistency in symptom onset or progression during the study period.
Conclusion: This study highlights the onset and pattern of progression of the earliest Fabry-related symptoms in children with CFD.

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Introduction

Fabry disease (FD) (OMIM 301500) is a progressive X-linked lysosomal storage disorder caused by pathogenic variants in the *GLA* gene, leading to decreased or deficient levels of the enzyme α-galactosidase A (α-gal A) (EC 3.2.1.22).[1] An α-gal A deficiency results in the abnormal accumulation of globotriaosylceramide (GL3 or Gb_3), globotriaosylsphingosine (lyso-Gb_3), and related glycosphingolipids in the lysosomes of many cell types.[1-3] These accumulations initiate a cascade of events, beginning with the metabolic dysfunction on the cellular level and progressing to cell death, inflammatory events, and progressive major organ dysfunction.[2,3]

FD affects both males and females, but symptom onset and phenotypic progression varies between individuals.[3,4] "Classic" or early-onset FD is defined as childhood onset of symptoms and α-gal A enzyme levels less than 1% to 5% of normal levels (in males) with the most predictable path of disease progression to end organ damage.[3] "Nonclassic" or later-onset FD is defined as no onset in childhood and α-gal A enzyme levels greater than 5% of normal levels (in males).[3] Without early diagnosis and timely treatment, the negative impact on quality of life and health is significant, leading to decreased life expectancy across the entire disease spectrum.[3,4]

Treatment addressing the underlying FD pathology (enzyme replacement therapy [ERT] and chaperone therapy [CT]) has positively affected the natural history and progression of FD in many patients.[5-9] Twenty-year data on ERT reports fewer "severe events" (end organ damage, strokes, and death) after 12 months on therapy.[5-7] Data on the long-term use of chaperone therapy also found a decreased rate of Fabry-associated clinical events.[8] However, recent studies suggest that if chronic neuropathic pain begins before primary therapy initiation, it may be refractory to treatment.[5] Additionally, therapies have not been able to reverse fibrosis after it occurs at any age.[3,5-9] Additional data indicate that beginning treatment in childhood allows for a more complete removal of stored material in the podocytes of the kidney that do not clear when treatment is begun in adulthood.[9] Lyso-Gb_3 can also be reduced to undetectable levels in the blood when young children with classic FD begin treatment in the first decade of life.[10,11]

Systematic literature review indicates that gastrointestinal (GI) symptoms, neuropathic pain, slow growth, heat/cold intolerance, exercise intolerance, vertigo, T-wave inversion on electrocardiogram, angiokeratoma, and other symptoms may arise in early childhood (<5 years), in both males and females.[12,13] However, the optimal timing of primary therapy initiation to improve quality of life and halt the insidious disease progression is still undefined.[3,9,12-15] To address these uncertainties, a longitudinal, prospective study tracking the earliest manifestations of FD during childhood is essential. This study was designed to determine the natural history of classic and nonclassic FD in early pediatric patients diagnosed prenatally or at birth across the disease spectrum to assist clinicians in caring for this population.

Materials and Methods

Participants between 2014 and 2019

A total of 40 participants aged 49 months or younger from 34 families in 9 different states who were diagnosed prenatally or in early childhood with variants in the *GLA* gene were recruited for the study. Participants were identified through existing patient populations at the Emory Lysosomal Storage Disease Center, Children's Mercy Kansas City, the University of Iowa, the University of Missouri, and Washington University. Recruitment flyers were also distributed by United States based Fabry advocacy groups (Fabry Support and Information Group and the National Fabry Disease Foundation).

Procedures

After an appropriate consent process, all participants underwent baseline assessments, including urine collection, parental completion of validated patient outcome surveys, and a Fabry specific review of symptoms. When participants turned 6 years of age, a formal assent was obtained before they continued in the study. Medical record releases were utilized to obtain formal copies of lab results, physical exams, review of systems, and any records from routine medical visits or hospitalizations. The children were also offered enrollment (or confirmed to be enrolled) in the Sanofi FD registry to expand pediatric data available in that longitudinal data set and provide access to validated questionnaires. All variants in *GLA* were categorized as "classic," "nonclassic," or "variants of uncertain significance (VUS) based on literature categorization in ClinVar,[16] Sakuraba's Fabry-database,[17] Fabry-Gen-Phen,[18] and/or the FD genotype-phenotype database (dbFGP).[19] All assessments were repeated annually for at least 4 years.

The outcome surveys used in this study included the ROME III questionnaire,[20] a validated questionnaire focused on pediatric functional GI disorders and additional GI symptoms, and the Face, Legs, Activity, Cry, Consolability scale,[21] a measurement scale used to assess pain for children between the ages of 2 months and 7 years. Although the ROME III questionnaire is validated for children ages 4 years of age and older, it was used as an exploratory endpoint for younger children.

When age-appropriate, the children self-completed the validated Pediatric Quality of Life Inventories[22] that measured fatigue, pain, and quality of life. These scales were accessed through the Sanofi FD registry.

Urinary testing included random urine creatinine and urinary Fabry biomarkers, including Gb_3, lyso-Gb_3, and 7

lyso-Gb_3-related analog biomarkers. Biomarkers were analyzed in all participants using an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) high-sensitivity methodology.[23-28]

Statistical analysis

Given the size of the study population, data analysis was mostly descriptive. In appropriate cases, categorical variables were compared between groups of patients using the Fisher exact 2-tailed test. Continuous variables were expressed as means and standard deviations and compared between patient groups. The occurrence and onset of key clinical manifestations of FD were evaluated by genotype, biological sex, and age in months. To best describe the relationship between age of symptom onset and biomarker levels, a Fagan nomogram was used, applying Bayesian theory to show the pre- and post-probabilities and the likelihood ratio for patients with FD who exhibit an initial symptom before age 36 months and have specific total urinary lyso-Gb_3 levels. Statistical significance was considered for $P < .05$.

Ethics review

This research was approved by the institutional review board under Emory Institutional Review Board protocol review number IRB00072967.

Results

The baseline study population included 40 participants enrolled between the ages of 1 month and 49 months representing 34 different families living in 9 states (Table 1). Fourteen of the participants were diagnosed via family history and 26 through newborn screening. Fifteen participants had classic *GLA* pathogenic variants, 6 participants had nonclassic *GLA* pathogenic variants, and 19 participants had a VUS in *GLA* (17 with p.A143T; 2 with p.I198T). Five of the participants were female, and 35 were male. Data were available for at least 2 data points for 34 of the 40 participants (15 classic, 3 nonclassic, and 16 VUS). Data were available for at least 4 time points for 24 of the 40 participants. Three of the classic participants began primary therapy with ERT during the course of the study, and any data collected after that point is notated as post-ERT or removed from any "untreated" natural history analysis.

Symptoms

The timing, presence/absence, and type of presenting symptoms related to FD in the study patients varied mostly based on the category of predicted phenotype classic, nonclassic, or VUS (Table 2, Supplemental Table 1).

Classic FD

Initial symptom presentation data were available for all participants (15/15) with classic FD (Table 2, Supplemental Table 1). The participants reported their initial symptoms at an average of 22.9 months (males: 22.3 months, range 10-32 months; females; 24.2 months, range 8- 40 months). The most common first symptoms reported included GI (13/15), heat intolerance/hypohidrosis (13/15), reduced sweating after previously sweating normally (6/15), and neuropathic pain/uncomfortable or "hot" feet/hands (3/15) (Supplemental Table 1). Most participants experienced more than 1 symptom at time of onset (12/15; 80%). All 15 participants had initial symptoms associated with FD before 48 months of age. Based on this small data set, the likelihood of a male with classic FD developing at least 1 initial symptom of FD is 50% (5/10) at or before age 2 years, and 100% before age 3. For females with classic FD, the likelihood of developing at least 1 symptom of FD was 40% by age 2 years, 80% by age 3 years, and 100% by age 4 years. Additionally, participants with classic FD were reported to have severe abdominal pain lasting >2 hours at an average of 64.7 months (range 48-85 months).

Most participants with classic FD experienced a consistent pattern of symptom progression. From time of onset, GI symptoms progressed from chronic bloating to diarrhea and/or alternating constipation to abdominal pain. Sweating and heat intolerance progressed from flushing/average sweating to hypohidrosis to anhidrosis. In participants over 4 years of age, signs of neuropathy progressed from "hot feet" to "burning feet" to "burning chronic" pain (Figure 1A-C). Self-reported phrases used by participants 5 years or older on the Pediatric Quality of Life Inventories to describe this pain include: "burning," "minecraft guys digging their pickaxes into my feet," and "when my feet burn it feels like knives in my feet." Notably, 3 male participants with classic FD aged 4 years and older who have not yet begun ERT are the only participants who reported episodes of severe/intense abdominal pain lasting more than 2 hours, leading them to stop all activities.

Enzymatic activity in plasma or leukocytes was available in 6 participants with classic FD (5 males and 1 female). Males with classic FD and leukocyte alpha-galactosidase A levels below 1 nmol/hr/mg, experienced at least 1 FD sign or symptom before the age of 36 months. However, because of the limited sample size, the *P* value did not reach significance.

All participants with classic FD submitted urine samples for biomarker testing at several points during the course of the study. Twelve participants (8 male and 4 female) with classic FD submitted urine samples that coincided with reported initial symptom onset.

All male participants with classic FD had abnormal total urinary lyso-Gb_3 levels and total urinary Gb_3 level unless treated with ERT (see Figure 2A-D). Participant 01-029's first urinary lyso-Gb3 level was abnormal but also collected

Table 1 Baseline Demographics of Enrolled Participants

ID	Sex	Race/Ethnicity	Variant	Classification	Alpha Gal Levels nmol/hr/mg (Leukocyte)[a]	Method of Diagnosis	Baseline Age (Months)
01-001	**male**	White/Non-Hispanic	c.1024C>T / p.R342*	Classic	0.9	FamHX	11
01-008	**female**	White/Non-hispanic	c.777delA/ p.G261fs*8	Classic	20.3	FamHX	49
01-009	**male**	White/Non-hispanic	c.777delA/ p.G261fs*8	Classic	0.2	FamHX	21
01-011	**female**	White/Non-hispanic	c.982G>C / p.G328R	Classic	n/a	FamHX	21
01-012	**male**	White/Non-hispanic	c.1042insG/p.A348Gfs*27	Classic	n/a	FamHX	7
01-014	**male**	White/Non-hispanic	c.999+2T>C/IVS6+*2T>C*	Classic	0	FamHX	31
01-021	**female**	White/Non-hispanic	c.776C>G /p.P259R	Classic	n/a	FamHX	8
01-025	**female**	White/Non-hispanic	c.777delA/ p.G261fs*8	Classic	n/a	FamHX	25
01-029	**male**	White/Non-hispanic	c.1024C>T /p.R342*	Classic	n/a	FamHX	1
01-033	**male**	White/Non-hispanic	c.151C>T /p.R49C	Classic	n/a	FamHX	21
01-034	**male**	White/Non-hispanic	c.777delA/ p.G261fs*8	Classic	n/a	FamHX	10
01-035	**female**	White/Non-hispanic	c.777delA/ p.G261fs*8	Classic	n/a	FamHX	3
01-036	**male**	White/Non-hispanic	c.982G>A /p.G328R	Classic	n/a	FamHX	22
01-051	**male**	White/Non-hispanic	c.679C>T /p.R227*	Classic	1.1	FamHX	28
03-006	**male**	White/Non-hispanic	c.359 T>C; c.361 G>A /p.L120P; A121T	Classic	0.7	NBS	25
01-023	**male**	White/Non-hispanic	c.781G>A / p.G261S	NonClassic	n/a	FamHX	17
01-030	**male**	White/Non-hispanic	c.870G>A /p.M290I	NonClassic	n/a	NBS	16
01-031	**male**	White/Non-hispanic	c.870G>A / p.M290I	NonClassic	n/a	NBS	36
02-008	**male**	White/Non-hispanic	c.369+5G>T/IVS2+5G>T	NonClassic	3.6	NBS	28
03-003	**male**	White/Non-hispanic	c.335G>A /p.R112H	NonClassic	0.7	NBS	25
04-001	**male**	White/Non-hispanic	c.806 T>A / p.V269E	NonClassic	0.8	NBS	17
01-018	**male**	White/Non-hispanic	c.427G > A / p.A143T	VUS	n/a	NBS	21
01-004	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	n/a	NBS	5
01-005	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	n/a	FamHX	6
01-019	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	n/a	NBS	5
01-027	**male**	White/Non-hispanic	c.427G > A / p.A143T	VUS	n/a	NBS	22
02-001	**male**	White/Non-hispanic	c.427G > A / p.A143T	VUS	n/a	NBS	16
02-003	**male**	White/Non-hispanic	c.427G > A / p.A143T	VUS	11.6	NBS	29

(continued)

Table 1 Continued

ID	Sex	Race/Ethnicity	Variant	Classification	Alpha Gal Levels nmol/hr/mg (Leukocyte)[a]	Method of Diagnosis	Baseline Age (Months)
02-006	**male**	White/Non-hispanic	c.427G > A / p.A143T	VUS	12.2	NBS	25
02-010	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	0.007 U/L	NBS	26
02-012	**male**	White/Non-hispanic	c.593T>C / p.I198T	VUS	n/a	NBS	18
03-001	**male**	White/Non-hispanic	c.593T>C / p.I198T	VUS	8.5	NBS	26
03-004	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	n/a	NBS	2
03-007	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	n/a	NBS	4
03-008	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	12.8	NBS	3
04-002	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	2.9	NBS	7
04-004	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	n/a	NBS	n/a
04-005	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	12.3	NBS	25
04-007	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	15.8	NBS	40
04-010	**male**	White/Non-hispanic	c.427G > A /p.A143T	VUS	n/a	NBS	n/a

[a]Normal range of enzyme assay depended on laboratory completing analysis, each of these levels were considered "abnormal."

on the 26th day of life in a period with a very different normal range and not included in the figure for reasons of scale. Changes of these biomarker levels were not significantly correlated with disease progression before primary FD therapy. All female participants with classic FD (5/5) had elevated total urinary Gb_3 levels, but only 3 out of 5 had abnormal total urinary lyso-Gb_3 levels at some time point during their participation in the study. The youngest age at which elevated total urinary lyso-Gb_3 levels in the females were observed was at 25 months of age.

Focusing on the urinary lyso-Gb_3 analogs,[24] all male participants with classic FD had elevations of all 7 analogs at every time point before beginning therapy (Supplemental Table 2). Over the course of the study, all females had 5 to 7 abnormal analogs included elevated −12, +14, +16, +34, and +50 analogs. Furthermore, all participants exhibited at least 1 elevation of the 16+ analog, which has previously been identified as the strongest indicator of FD.[24]

Reviewing urinary biomarker levels available at the time of initial symptom onset, 10 participants (8 males and 2 females) had abnormal total urinary Gb_3 and lyso-Gb_3 levels (Supplemental Table 1). The additional 2 females also had elevations in total urinary Gb_3, but their levels of total urinary lyso-Gb_3 were undetectable.

The total urinary lyso-Gb_3 levels in males with classic FD at the time of initial symptom onset ranged from 17 to 106 pmol/mmol creatinine with a mean of 62.3 and a median of 62 (Figure 2A-D). All 7 analogs were also abnormal in all untreated classic males. The total urinary Gb_3 levels in males with classic FD at the time of initial symptom onset ranged from 512 to 2381 with a mean of 1086 and a median of 987. At the time of initial symptom onset, only 2 females (01-008 at 7 pmol/mmol creatinine and 01-025 at 8 pmol/mmol creatinine) had abnormal total urinary lyso-Gb_3 levels.

The level of total urinary biomarkers in patients with classic FD did not statistically significantly correlate with the onset of FD-specific symptoms or any specific symptom (such as heat intolerance, abdominal pain, or hypohidrosis onset). However, the levels did distinguish the classic phenotype participants from the participants with VUS and most nonclassic participants (Table 3, Supplemental Table 2). Supplemental Figure 1 shows the use of Bayesian theory to adjust the chance for a male with classic FD to have symptoms before age 36 months based on their urinary lyso-Gb_3 levels (Supplemental Figure 1).

Individual participants with classic FD exhibit relatively stable longitudinal total urinary lyso-Gb_3 and Gb_3 levels that do not consistently correlate with age or symptom progression. However, there is an exception: all male participants with available biomarkers who were treated with ERT experienced a significant decrease in urinary total lyso-Gb_3 and Gb_3 with 1 participant even normalizing their levels. None of the females with FD in our study reported beginning primary therapy during this study period.

Table 2 Earliest reports of symptom(s) by age

Fabry-Related Signed and Symptoms	Earliest Report of Symptom In This Population (In Months)[a]	Earliest Report In Literature (In Years)[12,25]
Bloating	10 months in males 8 in females	Not reported
Diarrhea	12 months in males 41 months in females	1 year in males; 1 year in females
Constipation	11 months in males 41 months in females	1 year in males; 6.4 years in females
Abdominal pain	26 months in males 25 months in females	1 year in males; 1.7 years in females
At least one episode of severe or intense abdominal pain 2+ hours/stop everything	48 months in males 75 months in females	Not reported
Burning pain in feet	28 months in males; 44 months in females	2 years (information on biological sex unavailable)
Chronic pain	No participants reported with chronic burning pain hands/feet > 3 days/week	3.0 in males; 1.8 in females
"hot feet"	8 months in males; 44 months in females	Not reported
Hypohidrosis	17 months in males; 60 months in females	1.0 in males; 1.9 in females
Anhidrosis	46 months in males; Not reported in females	3.2 in males
Heat intolerance	10 months in males; 8 months in females	1 year in males; 1 year in females
Cold intolerance	24 months in males; Not reported in females	1 years in males; 1.7 years in females
Angiokeratomas	97 months in males; Not reported in females	5.5 years in males; 4.4 years in females

[a]All earliest reported symptoms occurred in participants with classic FD.

Although posttreatment data are not the primary focus of this natural history study, longitudinal data were available for the 3 males with classic FD who began treatment during this study. All 3 participants initiated ERT after experiencing neuropathic pain, anhidrosis, and GI pain at age 26, 67, and 84 months. All 3 reported a reduction in neuropathic pain in their extremities and GI pain by their next study time point. Additionally, they observed minor improvements in sweating after 1 year of therapy. Notably, there was no symptom progression nor development of angiokeratomas. Despite improvements in frequency and severity, all 3 continue to have intermittent neuropathic pain, GI symptoms, and decreased sweating. All male participants with available biomarkers who were treated with ERT experienced a significant decrease in urinary total lyso-Gb_3 and Gb_3 in the year after beginning treatment that was maintained over the additional 2 to 4 years of available data.

Nonclassic FD

Six male participants were represented in the nonclassic FD group. All participants had a pathogenic variant in the *GLA* gene classified as nonclassic (see Table 1).

Three participants developed symptoms that may or may not be related to FD. Participant 01-023 reported isolated heat intolerance at 64 months, which did not worsen or progress by 88 months of age. Participant 01-031 reported isolated abdominal pain at 85 months described as a "nervous belly at times" that did not worsen at his next time point at 96 months of age. His biological sibling 01-030 did not report any issues with abdominal pain or other FD related symptoms up to 66 months of age. Finally, Participant 04-001, reported onset of decreased sweating beginning at 17 months of age, but no evaluable data were available beyond 17 months of age to determine if additional symptoms occurred. The remaining participants with nonclassic variants did not report any symptoms that could be associated with FD during their participation in the study. However, 2 of those participants had visits at the third time point of the study (24 and 29 months) and did not participate for the full 48 months.

Alpha-galactosidase A enzymatic activity in plasma or leukocytes was available in 3 participants with nonclassic FD. The levels did not correlate with the development of clinical manifestations. Two of the participants with enzyme levels of 0.7 and 3.6 nmols/hr/mg did not report any signs or symptoms of FD. Participants 04-01 with an enzyme level of 0.8 nmols/hr/mg did report decreased sweating at 17 months of age.

Across all time points, the levels of total urinary lyso-Gb_3 and urinary Gb_3 biomarkers were elevated in only 1 patient

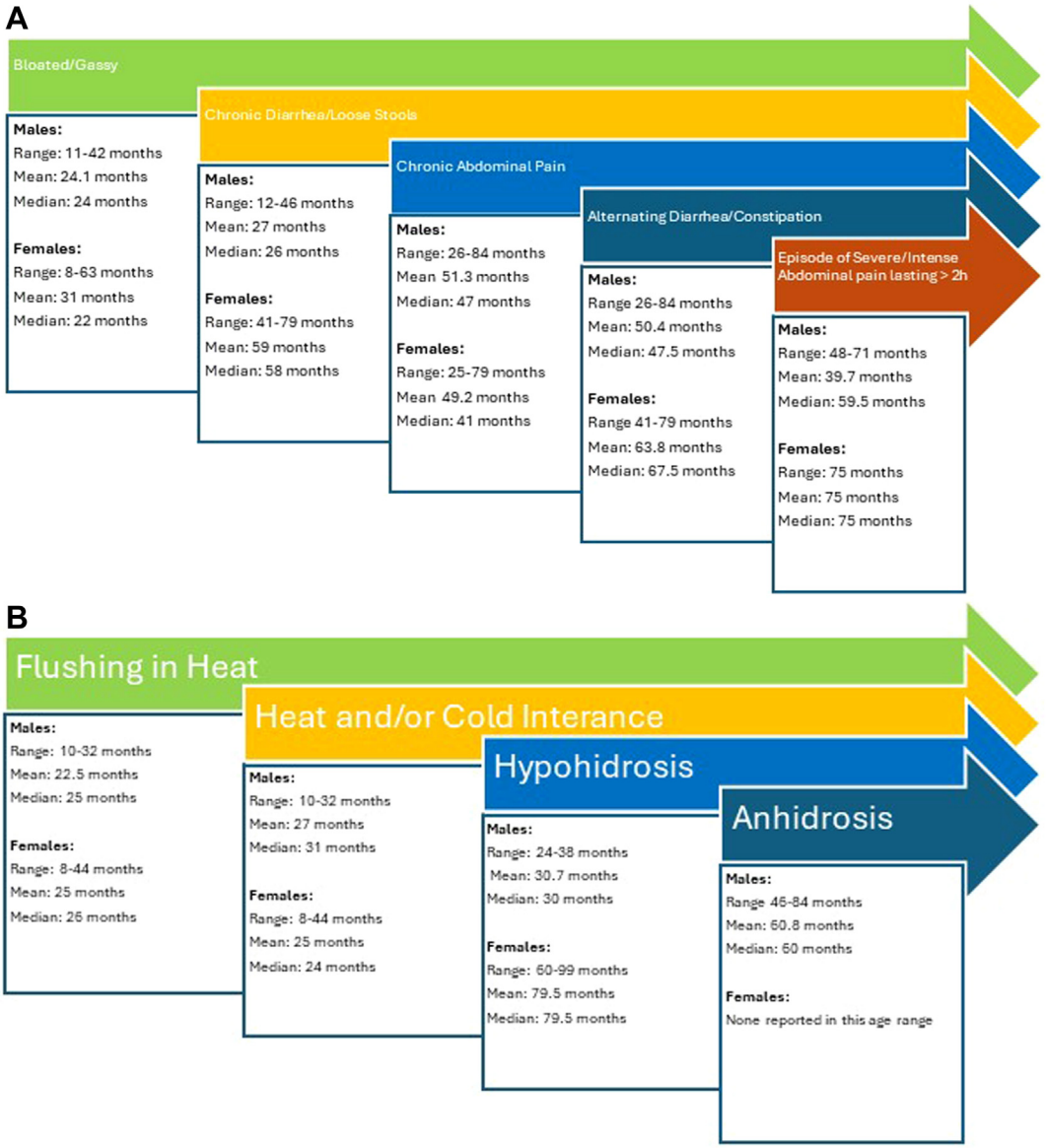

Figure 1 Progression of symptoms in children with classic Fabry disease by organ system. A. Progression of gastrointestinal symptoms in children with classic Fabry disease not on enzyme replacement therapy. B. Progression of heat intolerance/hypohidrosis in children with classic Fabry disease not on enzyme replacement therapy. C. Progression of neuropathy in children with classic Fabry disease not on enzyme replacement therapy.

(04-01) classified as having nonclassic FD (Table 3, Supplemental Table 2). Except for 04-01, longitudinal data on total urinary Gb_3 for the participants with nonclassic FD were normal (Supplemental Figure 2). All participants with nonclassic FD and biomarker data had at least one urinary lyso-Gb_3 analog elevated during one time point in the study. Only 1 participant (04-01) had abnormal levels of lyso-Gb_3 and seven related analogs. For all other participants with nonclassic FD with available biomarker data, there was a range of 1-7 abnormal analogs, with a mean of 3.3, and a median of 2. The urinary lyso-Gb_3 analog (−12) was abnormal in all participants at all time points.

The levels of total urinary lyso-Gb_3 and urinary Gb_3 at the time of symptom onset were available in all 3 of the participants with nonclassic FD reporting symptoms (Supplemental Table 1). Among these, 2 participants had normal total urinary lyso-Gb_3 and urinary Gb_3 levels at their first reported symptoms. One participant had undetectable levels of total urinary lyso-Gb_3 and normal levels of urinary Gb_3. Participant 01-23 had normal total urinary lyso-Gb_3 but had elevations of 5 analogs (−2, −12, −28, +14, +16). Participants 01-31 normal total urinary lyso-Gb_3, but had elevations of 2 analogs (−12, −28). Participant 04-01 had high levels of total urinary lyso-Gb_3 at 72 pmol/mmol creatinine and total urinary Gb_3 at 4653 μg/mmol creatinine at the time of first reported symptom at 12 months of age. Of note, he also had all 7 analogs elevated in a pattern similar to the classic

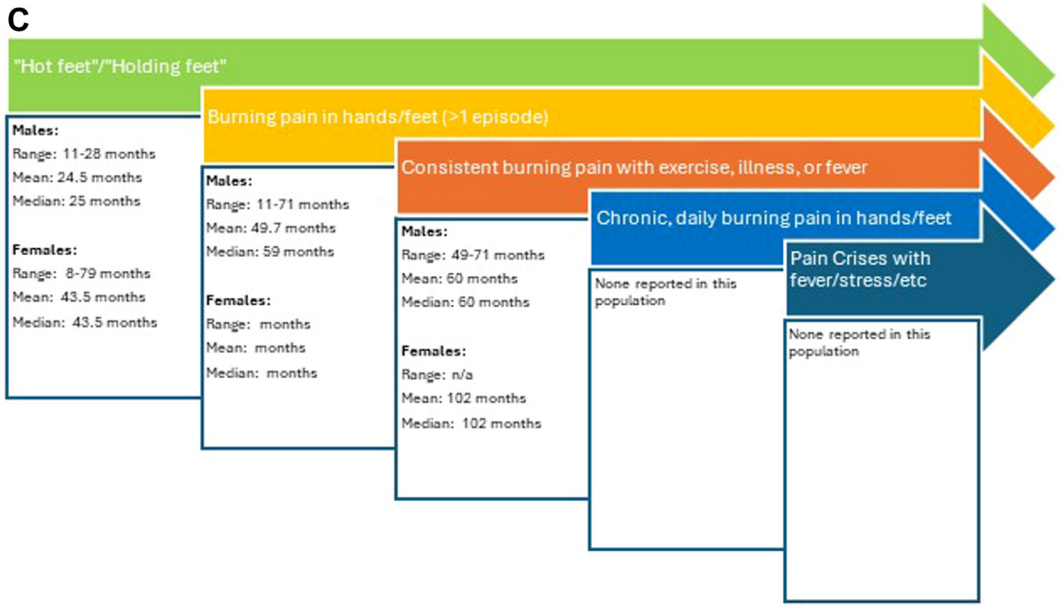

Figure 1 Continued.

males with FD (Supplemental Table 1, Supplemental Figure 2).

The levels of biomarkers in all participants with non-classic FD did not trend upward over the course of the study (Supplemental Figure 2). None of the patients with non-classic FD in our study reported beginning primary therapy during this period of study.

VUS in the *GLA* gene

Nineteen male participants diagnosed through newborn screening (Table 1) represented this group. Most of the participants (10/19; 52.6%) did not exhibit any symptoms that could be associated with FD during the study. Five of the participants with the p.A143T variant reported having isolated symptoms associated with FD ranging from hot or painful feet to GI issues (Supplemental Table 1). Participants 03-001 (p.I198T) developed isolated abdominal pain at 31 months of age. No additional follow-up was available for this participant after the 31-month timepoint.

Longitudinal data on the participants with symptoms did not mirror the progressive pattern of symptoms onset seen in the participants with classic FD. Individuals with the same p.A143T pathogenic variants exhibited a variability of nonspecific symptoms without a detectable pattern of severity, frequency, and timing of onset.

Data on biomarkers were available for 12 out of 19 participants with a VUS. None of the participants had elevated total Lyso-Gb_3 levels at any time point (Table 3, Supplemental Table 2). Ten of the participants had at least 1 elevated level of a lyso-Gb_3 analog during the study. In 8 of the participants with at least 1 elevated level at any time point, the elevated analog was lyso-Gb_3 (+50). All available time points in all participants had a range of 1 to 6 abnormal analogs (mean of 1.12, median of 1). In the 4 participants with 4+ Lyso-Gb_3 analogs, all had abnormal −12 and +16 analogs, 3 had abnormal +50 or +34, and 2 had abnormal +14 (Table 3, Supplemental Table 2).

Participant 03-001 (p.I198T) was the only participant with a VUS to have elevated levels of total urine Gb_3 of 68, normal levels of total urinary lyso-Gb_3, and 6 elevated lyso-Gb_3 analogs at the time point closest to the onset of a symptom. Participant 01-018 had slightly elevated total urinary Gb_3 levels at the time of a symptom onset but all normal urinary total lyso-Gb_3 levels with all normal analogs. Two participants with p.I198T had at least 4 elevated urine lyso-Gb_3 analogs (04-02 with 4 and 03-001 with 6). Among the elevated analogs in lyso-Gb_3, 3 were common: −12, +16, and +34. In contrast, among 3 of the 10 participants with the p.A143T variant in *GLA* (01-019, 03-004, and 03-007), only 1 time point showed 4 elevated analogs, with overlaps in all 3 participants occurring only in 2 analogs: −12 and +16.

Discussion

The MOPPet study is the first prospective longitudinal natural history study focused on children diagnosed with FD before age 4 across the disease spectrum. The objective of the study is to provide useful knowledge on the early natural history of FD for clinicians and families.

The longitudinal findings support prior research identifying initial symptoms of classic FD as heat intolerance, decreased sweating, and GI issues.[12,13,29] The study's prospective and longitudinal design provides new insights into

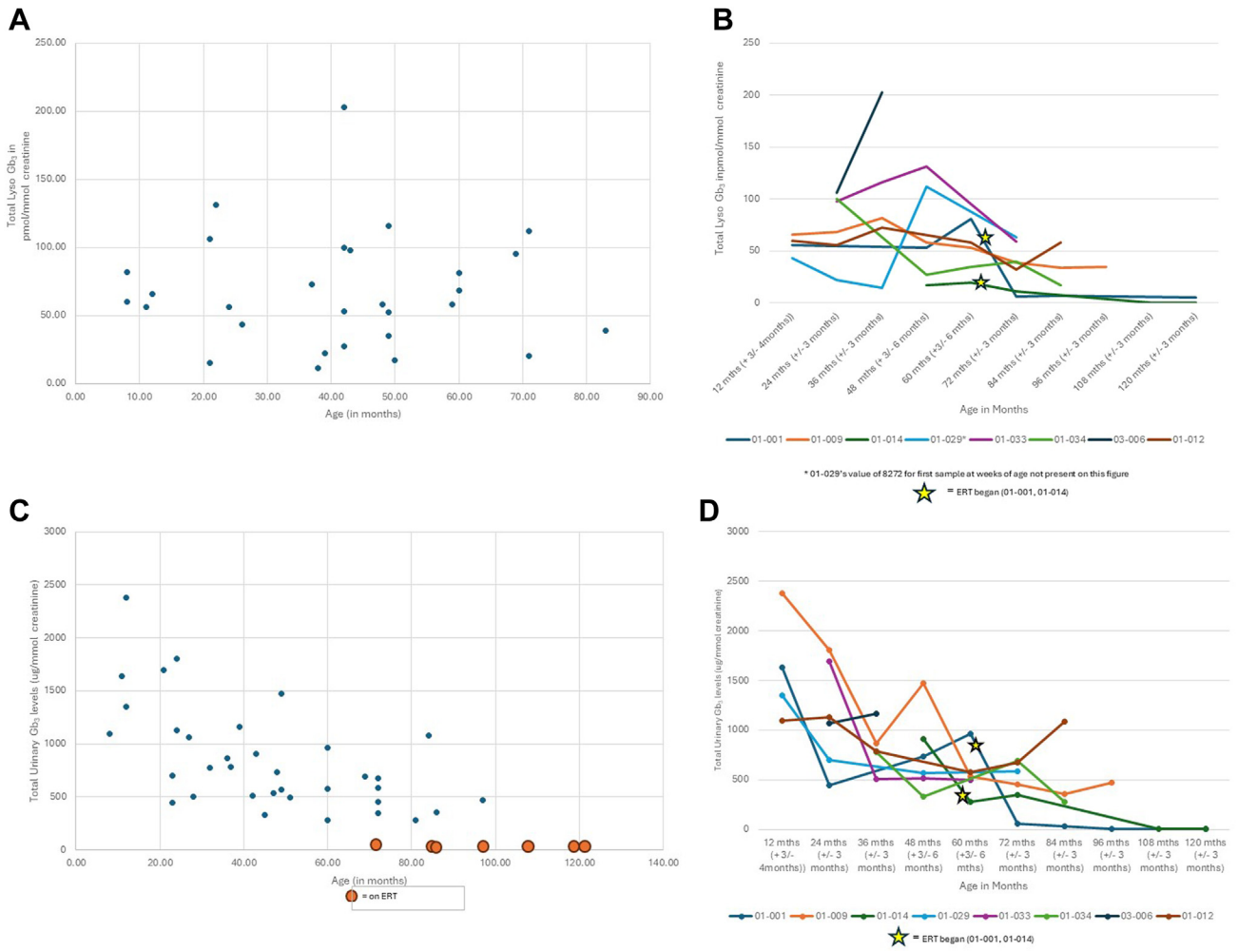

Figure 2 **Urinary biomarkers in males with classic Fabry disease not on enzyme replacement therapy.** A. Total urinary lysoGb$_3$ levels by age in males with classic Fabry disease collected during the study. B. Longitudinal total urinary lysoGb$_3$ levels by age in males with classic Fabry disease. C. Total urinary Gb$_3$ levels by age in males with classic Fabry disease. D. Longitudinal total urinary Gb$_3$ levels by age in males with classic Fabry disease.

Table 3 Patterns of average elevated urinary biomarker patterns by variant classification and sex[c]

Category	Total Gb_b	Total Lyso-Gb_3	Lyso-Gb_3 (-28)	Lyso-Gb_3 (-12)	Lyso-Gb_3 (-2)	Lyso-Gb_3 (+14)	Lyso-Gb_3 (+16)	Lyso-Gb_3 (+34)	Lyso-Gb_3 (+50)
Classic Males (untreated)	↑↑↑	↑↑↑	↑↑↑	↑↑↑ [a]	↑↑↑	↑↑↑	↑↑↑ [a]	↑↑↑ [a]	↑↑↑
Classic Males (treated)	↑	↑	↑	↑- ↑↑	↑↑	↑↑	↑↑	↑	↑ [a,b]
Classic Females	↑	↑	↑	↑↑	↑↑	↑↑	↑↑ [a]	↑	↑↑
Nonclassic Males	Normal	Normal	↑	↑↑ [a]	↑	↑	↑ [a]	↑ [a]	↑
VUS (p.I198T) Males	↑	Normal	↑↑	↑↑↑	↑↑	↑	↑	↑	↑
VUS (p.A143T) Males	Normal	Normal	Normal	↑	Normal	↑	Normal	Normal	↑
	↑↑↑ = >76 ↑↑=66-75 ↑=26-65 Normal <25	↑↑↑ = >66 ↑↑=11-65 ↑=0-10 Normal <0	↑↑↑ = > 66 ↑↑=11-65 ↑=0-10 Normal <0	↑↑↑ = > 66 ↑↑=11-65 ↑=0-10 Normal <0	↑↑↑ = >66 ↑↑=11-65 ↑=0-10 Normal <0	↑↑↑ >65 ↑↑=11-65 ↑=0-10 Normal <0	↑↑↑ = >400 ↑↑=100-400 ↑=27-100 Normal <26	↑↑↑ = >350 ↑↑=300-304 ↑=23-300 Normal <22	↑↑↑ =>400 ↑↑=170-399 ↑=2-169 Normal <2

[a]Most discriminate biomarker(s) for this population.
[b]Normalized over increased time of treatment.
[c]Gb_b levels are measured in μg/mmol creatinine; Lyso-Gb_3 levels are measured in mol/mmol creatinine.

symptom timing, clustering, and progression in young children with classic FD, without recall bias. Specifically, it reveals a systemic worsening of symptoms across multiple organ systems with age. Moreover, children with classic FD typically exhibit a combination of symptoms (decreased sweating, heat intolerance, and GI symptoms) rather than isolated manifestations. Notably, all classic participants displayed symptoms before 40 months of age with an average onset of 27.3 months (range: 11-45 months), preceding the appearance of neuropathic pain or angiokeratomas.[13]

Early signs of FD, such as bloating or heat intolerance, are nonspecific and might be missed without posing FD-specific questions to the parents. Collaboration among health care providers, caregivers, and patients is crucial for recognizing and understanding the subtle features of FD. Given that these symptoms often require parental observation and reporting, educating parents and regularly assessing for such symptoms is essential for informed decision making about care plans. Developing guidelines with parental training, a parent-reported questionnaire, and provider education would standardize procedures for managing pediatric patients with FD. Recognizing that the overall progression of FD is more informative than individual symptoms can aid in determining when to initiate treatment.

All males with classic FD displayed elevated levels of total urinary Gb_3 and lyso-Gb_3 from birth, suggesting the potential use of these noninvasive biomarkers to identify patients likely to develop early-onset symptoms before age 3, especially in the cases of VUS or uncertain variants. However, this pattern of symptom onset and urinary total Gb_3 and lyso-Gb_3 does not hold true in the female patients. Although 4 out of the 5 female participants with classic FD had elevated total urinary Gb_3 levels, they were considerably lower than those in male participants, and only 2 female participants showed elevated total urinary lyso-Gb_3 levels.

Additionally, 1 male participant with classic FD (01-029) whose urine lyso-Gb_3 was collected on the 26th day of life had very elevated levels of total urinary lyso-Gb_3 levels at 8272 pmol/mmol creatinine. By 22 months, this level had decreased to 22 pmol/mmol creatinine. Per Barr et al, it is typical for infants without FD to have urine Gb_3 elevations as high as 669.9 during the first 30 days of life.[23] Gb_3/creatinine ratios in newborns without FD increase during the first 29 days of life, peak at 30 days, and then gradually decrease to normal adult levels by 6 months of age in male infants and nearly adult levels by 3 months in females.[16] Despite the established normal levels for infants under 6 months, this pattern warrants further investigation, especially considering its potential application to infants diagnosed through newborn screening.

Longitudinal data on male participants with classic FD did not show a gradual increase in urinary total Gb_3 and lyso-Gb_3 levels associated with symptom progression or age. This corresponds with prior research indicating plasma lyso-Gb_3 levels in untreated adults with FD general remain stable over time; however, it does not investigate any increases or trends that may occur in untreated patients after age ~5 years of age.[24,30,31] It does suggest that monitoring urinary lyso-Gb_3 levels may not be a reliable marker for initiating treatment in the pediatric FD population before age 5 years.[30] Nevertheless, the potential effects of starting therapy during childhood should still be considered, balancing the benefits of treatment with potential impacts on quality of life, school attendance, pain management, and emotional well-being. Ultimately, the decision to begin therapy should prioritize the preferences of the parents while aligning with physician recommendations to optimize outcomes.

Although not the study's primary focus, insights can be gleaned from the classic FD participants who began ERT during the study. Despite experiencing subjective improvements in symptom severity and frequency, along with a significant reduction in urinary lyso-Gb_3 levels, these patients still experienced decreased sweating, intermittent GI symptoms, and occasional neuropathic pain described as "stabbing pain" similar to a "knife" or "Minecraft pickaxe in the foot." This aligns with findings from the Fabry Registry and Fabry Outcome Survey indicating that initiating ERT earlier may alleviate symptoms to some extent.[21,31] Given the persisting symptoms and their impact on quality of life, particularly pain and GI discomfort, systematic clinical trials investigating the initiation of therapy in classical patients before the onset of burning pain may be more effective. Timing therapy initiation with early signs of neurological impact, such as anhidrosis or hypohidrosis, could potentially mitigate development of ongoing neuropathic pain and GI issues.

Interestingly, although participant 04-001 has a variant classified as nonclassic FD (pathogenic *GLA* variant 806 T>A / p.V269E), he had elevated urinary total lyso-Gb_3/Gb_3 levels and a consistent pattern of symptom onset and progression unlike the other participants in this category. The symptom onset and elevated urinary biomarkers challenges the current classification of this variant as nonclassic. Further investigation is warranted to ascertain whether this participant is an outlier, further studies into the current "nonpediatric" definition for nonclassic variants in *GLA* is necessary, or if their variant should be reclassified as a classic FD genotype.

A systematic pattern of early symptoms or elevations in urinary biomarkers in participants with p.A143T or other VUS was not observed. However, 1 participant (03-001 with p.I198T) had elevated urinary total lyso-Gb_3 or Gb_3 levels and symptoms possibly related to FD. These were not seen in our other participant with the same p.I198T variant. Either of these participants may be an outlier; however, given the frequency of polymorphisms in the general population that can further reduce alpha-galactosidase A effectiveness, further research is necessary to understand their combined impact on both pediatric and adult patients.

Although most participants with VUS in this study did not experience life-affecting symptoms associated with FD, it does not rule out the possibility of disease progression beyond the pediatric period. This may offer reassurance for health care providers and families that infants with p.A143T variants have a reduced likelihood of pediatric symptom onset in the first years of life.

Patterns in the Gb_3, lyso-Gb_3, and their analogs can differentiate between FD categories (classic, nonclassic, and VUS) based on biomarker profiles.[25,32] Males with classic FD typically exhibit significantly elevated or elevated levels of all urinary biomarkers. In fact, the striking nature of these biomarker profiles suggests that one of the participants currently categorized as nonclassic could be reclassified as classic. Treatment notably normalizes lyso-Gb_3 analog −50 levels, particularly in classic FD. Although other FD categories lack such a clear a distinction, male participants with undetectable levels of all markers are more likely to have a VUS and no early childhood symptom onset. These patterns may aid health care providers and families in interpreting newly detected variants in the *GLA* gene in children.

Limitations of this study include its small sample size, lack of racial/ethnic diversity, lack of age-matched controls, and further division of participants by age, genotype, and biological sex, hindering the attainment of clinical significance in most analyses. Prospective data on symptom onset before enrollment were unavailable for some participants, whereas others had only 1 time point available for symptoms and/or biomarkers. Biomarker data collection was limited to urine because of logistical challenge and parental resistance to blood collections and did not include renal specific biomarkers such as microalbumin or albumin to creatinine ratio. Likewise, enzyme activity was collected from medical records and not collected as part of the study in a standardized manner. Symptom data were collected through parental reports until age 5 years and supplemented by review of medical records.

This longitudinal prospective study underscores the importance of systematically monitoring children under 5 with classic FD and actively seeking early signs of the disease rather than waiting for later onset symptoms. The progression of symptoms in these young patients is consistent and predictable. Monitoring is essential for both male and female patients. For children under 5 with nonclassic FD variants or uncertain VUS, close collaboration with families is crucial to understand the impact on affected family members alongside systematic observations of the children. These data support the theory that it is not necessary to wait for each classic male patient to present with chronic pain or severe abdominal pain before starting primary therapy, especially because it appears these symptoms are refractory to therapy after they present.

Important next steps for supporting clinicians in providing personalized monitoring and management of patients in this population include the updating and validation of comprehensive guidelines for presymptomatic children with FD, clinical trials in children with classic FD with composite outcomes to determine the impact of primary therapy initiation before onset of neuropathic pain, and larger prospective longitudinal studies in a more racially and ethnically diverse population of children with FD with the goal of attaining clinical significance in the analyses and removing data biases.

Conclusions

Systematically monitoring children under the age of 5 years with FD in collaboration with their parents can identify early signs of FD that affect quality of life. The most reliable data comes from parental and health care provider training, which focuses on identifying key early symptoms, watching for the pattern of early symptom progression, and using validated parent/patient reported outcome scales focused on pain and GI symptoms under age 5, even if they are not yet validated for small children. This study provides small-scale evidence that initiation of primary therapy under age 5 based on symptoms and lab data may be beneficial in patients with classic FD before the development of chronic neuropathic pain. Likewise, patients with nonclassic FD under age 5 could be managed with a conservative approach of monitoring symptoms without expectation of starting primary therapy under age 5 for most patients. More prospective, longitudinal data are still needed to fully support these initial, preliminary findings. The hope is that these results will continue to help inform predictions about the future phenotype and prognosis, as well as guiding parental expectations related to medical issues, monitoring guidelines, and the optimal timing for treatment initiation. This is particularly important in de novo variant cases or family-specific variants that have not been well described.

Data Availability

All data and materials are available individually upon request to the corresponding author.

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Author Contributions

Conceptualization: D.A.L.; Data Curation: A.L.F., A.Q., C.A.-B., D.A.L., M.F.H., R.V.; Formal Analysis: A.Q., C.A.-B., D.A.L.; Investigation/Acquisition of Data: A.L.F., A.M.A., A.Q., B.A.H., C.A.-B., D.A.L., D.K.G., D.S.P., L.P.M., M.D.H., M.F.H., R.J.H., R.V.; Writing-original draft: D.A.L.; Writing-review and editing: A.L.F., A.M.A., A.Q., B.A.H., C.A.-B., D.A.L., D.K.G., D.S.P., L.P.M., M.D.H., M.F.H., R.J.H., R.V.

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Ethics Statement

This research was approved by the Emory University Institutional Review Board (IRB) under Emory IRB protocol review number IRB00072967. All institutions involved in human participant research received local IRB approval. Assent/informed consent was obtained from all participants as required by the IRB(s). The research included in this report was conducted in a manner consistent with the principles of research ethics, such as those described in the Declaration of Helsinki and/or the Belmont Report.

Conflict of Interest

D.A. Laney is a member of the North American Fabry Registry Board, consults and/or has been an investigator and coordinator in clinical trials sponsored by 4DMT, Amicus Therapeutics, Chiesi Farmaceutici, Protalix BioTherapeutics, Sangamo, Sanofi-Genzyme, Spark Therapeutics, and Takeda-Shire. These activities have been monitored and found to be in compliance with the conflict of interest policies at the Emory University School of Medicine.

R.J. Hopkin is a member of the Fabry Registry Advisory Board and the FollowMe registry, consults with Sanofi and Amicus Therapeutics, Chiesi Farmaceutici and Freeline, and has been an investigator in clinical trials sponsored by Amicus Therapeutics, Idorsia, Protalix BioTherapeutics, Sangamo Therapeutics, Sanofi, and Takeda. These activities have been monitored and found to be in compliance with the conflict of interest policies at Cincinnati Children's Hospital Medical Center.

C. Auray-Blais has received research grants from Shire/Takeda, Sanofi-Genzyme, BioMarin Pharmaceuticals Inc. and Amicus Therapeutics. She has been a consultant for Amicus Therapeutics Inc. and Biomarin Pharmaceuticals Inc. She has received financial support for service contracts from Protalix Biotherapeutics, Avrobio, Moderna Therapeutics, Sigilon Therapeutics, 4D- Molecular Therapeutics, and Chiesi Farmaceutici. She has received honoraria and traveling funds for lectures from Amicus Therapeutics, Shire/Takeda, Sanofi-Genzyme, BioMarin Pharmaceuticals Inc. and traveling funds from Waters Corp for lectures given. These activities have been disclosed at the Faculty of Medicine and Health Sciences at the Université de Sherbrooke.

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A.M. Atherton is an employee at Amgen, Inc and has stock in the company.

M.F. Houde is an employee at Takeda.

All other authors declare no conflicts of interest.

Additional Information

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