Supplementary Appendix

Supplement to: Cohen JL, Chakraborty P, Fung-Kee-Fung K, et al. In utero enzyme-replacement therapy for infantile-onset Pompe's disease. N Engl J Med 2022;387:2150-8. DOI: 10.1056/NEJMoa2200587

This appendix has been provided by the authors to give readers additional information about the work.

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Supplemental Methods

Tippi MacKenzie and her team at the UCSF Center for Maternal-Fetal Precision Medicine designed the study. Jennifer Cohen, Pranesh Chakraborty, Priya Kishnani and Tippi MacKenzie vouch for the data and analysis.

Sample collection and clinical monitoring: Research samples were collected from mother, affected fetus, postnatal infant, and historical positive and negative controls, using appropriate IRB-approved research protocols: Duke Pro00010830 and Pro00007612.

Postnatal enzyme replacement therapy (ERT): Following delivery, the patient received standard of care treatment for cross reactive immunologic material (CRIM) negative infantile-onset Pompe disease (IOPD) which included immune tolerance induction (ITI)[1] (4 doses of rituximab, 9 doses of methotrexate, and monthly IVIG) initiated concurrently with the first postnatal dose of alglucosidase alfa. Alglucosidase alfa was continued at 20mg/kg/dose, administered every other week until 9.6 months of age when the dose was increased to 40mg/kg/dose every other week; it was then increased to 40mg/kg/dose weekly at 11.3 months of age. The patient received a second course of rituximab (administered monthly) beginning at 11 weeks of age, which was subsequently spaced to every two months at 27 weeks of age, and every three months at 43 weeks of age.

IgG anti-ERT antibody (ADA) titer measurements: Maternal blood samples were collected before each umbilical vein ERT administration, after delivery, and 7 months after delivery, and tested for IgG ADA. Umbilical vein blood was collected before each in utero ERT (IUERT) infusion and tested for IgG ADA. We analyzed ADA levels at each in utero infusion, with the plan to halt IUERT if levels were high (levels of ≥1:12,800). Infant blood was collected at delivery and monthly thereafter for analysis of IgG ADA titers. All IgG ADA titers were analyzed at LabCorp.

Acid alpha-glucosidase (GAA) enzyme activity levels: Umbilical vein blood (fetal plasma) samples were collected before each IUERT administration and tested for GAA enzyme activity. Fetal plasma samples were analyzed by two independent experienced biochemical genetics laboratories— Duke University (**Figure 3B**) and University of Washington (**Supplemental Figure S3A**)—to measure trough GAA enzyme activity levels before each in utero infusion. The difference in scale for these two graphs (both in nmol/h/mL) is due to the different substrates

and methods used by the two laboratories.

Glucose tetrasaccharide (Glc_4) levels: Amniotic fluid was collected before each IUERT infusion to measure Glc_4 levels. Gestational age-matched unaffected control amniotic fluid samples were collected for comparison of Glc_4 levels. Values were plotted as µmol/L (**Figure 3C**) and mmol/molCr (**Supplemental Figure S3B**). We confirmed that the current patient had normal amniotic fluid volumes throughout the pregnancy, with a maximal vertical fluid pocket (MVP) ranging from 4.0cm-6.9cm from 24+3/7 to 32+6/7 weeks gestation, with a median MVP of 6.4cm. Following delivery, postnatal infant urine was routinely collected for Glc_4. These levels were compared to those of previously published cohorts of CRIM-negative and CRIM-positive IOPD patients treated with ERT at ≤4 weeks of age (following newborn screening (NBS)).[2,3]

Creatine kinase levels: Following delivery, infant blood was collected periodically to monitor creatine kinase levels in a clinical laboratory setting.

Motor assessments: Motor skills were assessed using Alberta Infant Motor Scale (AIMS) and Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND), a standardized measure of infant strength; these were compared to those in a published cohort of four CRIM-negative IOPD individuals diagnosed at ≤4 weeks of age by NBS.[3]

Cardiac assessments: Fetal echocardiograms were completed every 2-5 weeks to monitor the cardiac status. Postnatal echocardiograms were analyzed at birth and every 1-2 months to monitor Left Ventricular Mass Index and other cardiac parameters.

Placental histology: After delivery, the current patient's placenta was collected for histopathologic analysis and all cut slides underwent light microscopy (Periodic Acid-Schiff (PAS) staining) or underwent electron microscopy (EM) analysis. The light microscopy slides were compared to those of another patient with IOPD and a healthy control, which were cut and analyzed by a perinatal pathologist. The EM slides were compared to previously published

IOPD patient placentas[4,5] and reviewed by board-certified anatomic pathologists with experience in placental pathology.

Supplemental Table S1. Motor skills assessment for Sibling 3 in comparison to previously published CRIM-negative newborn screening (NBS) infantile-onset Pompe disease (IOPD) cohort[3]

Patient	Motor assessments	Age	CGA
CRIM neg IOPD NBS 1	Central hypotonia	1m 14d	1m
CRIM neg IOPD NBS 2	Age-appropriate gross motor skill development	25d	4d
CRIM neg IOPD NBS 3	AIMS <5th percentile and delayed milestones	1m	21d
CRIM neg IOPD NBS 4	AIMS 10-25th percentile	1m 6d	29d
Current IUERT patient (Sibling 3)	AIMS 25-50th percentile; CHOP-INTEND 53/64	2m 4d	1m 17d

CGA: corrected gestational age; **m**: months; **d**: days; **AIMS**: Alberta Infant Motor Scale; **CHOP-INTEND**: Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders

Supplemental Table S2. Motor skills trajectory of Sibling 3 (IUERT)

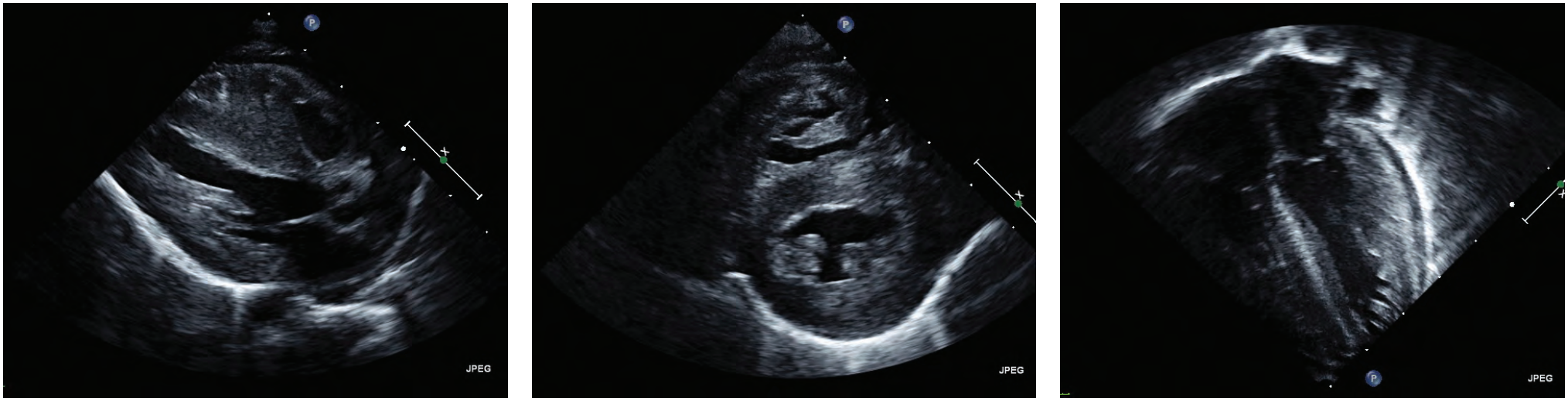
Age, (CGA)	**AIMS Score**	**AIMS Percentile Rank for CGA**	**CHOP-INTEND**
2m 4d, (1m 17d)	7	25-50th percentile	53/64
3m 29d, (3m 12d)	15	50th percentile	58/64
5m 24d, (5m 7d)	17	10-25th percentile	64/64
7m 5d, (6m 18d)	29	50th percentile	64/64
10m 13d*	50	50-75th percentile	64/64

CGA: corrected gestational age; **m**: months; **d**: days; **AIMS**: Alberta Infant Motor Scale; **CHOP-INTEND**: Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders

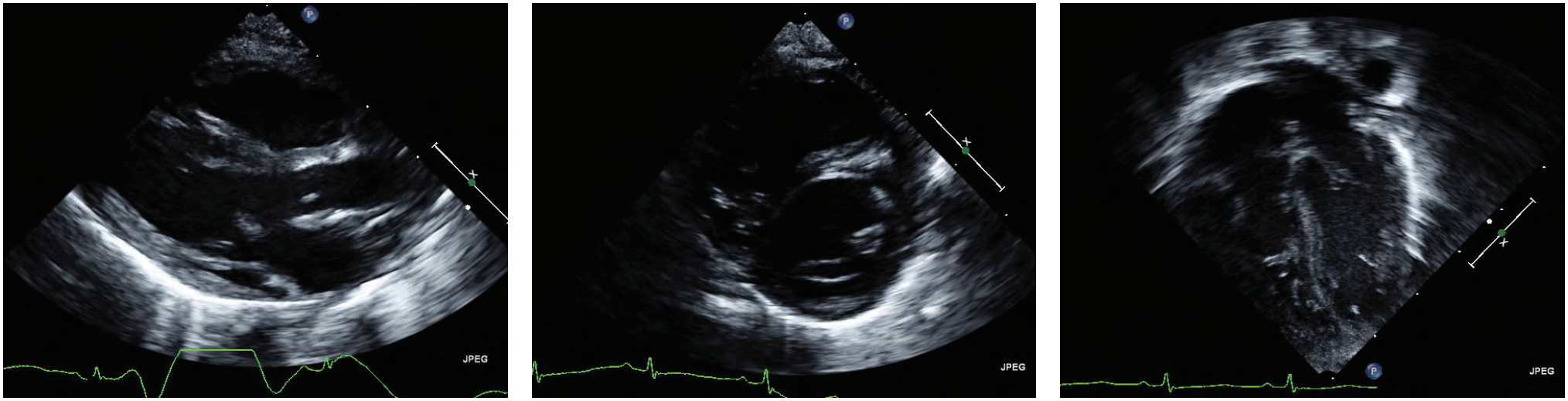
*CGA not applicable at this chronologic age; higher dose of 40mg/kg/dose every other week began at 9.6 months of age

Supplemental Figure S1A. Postnatal echocardiograms for Sibling 2 (untreated) and Sibling 3 (IUERT)

Sibling 2 (untreated), 17 days of age



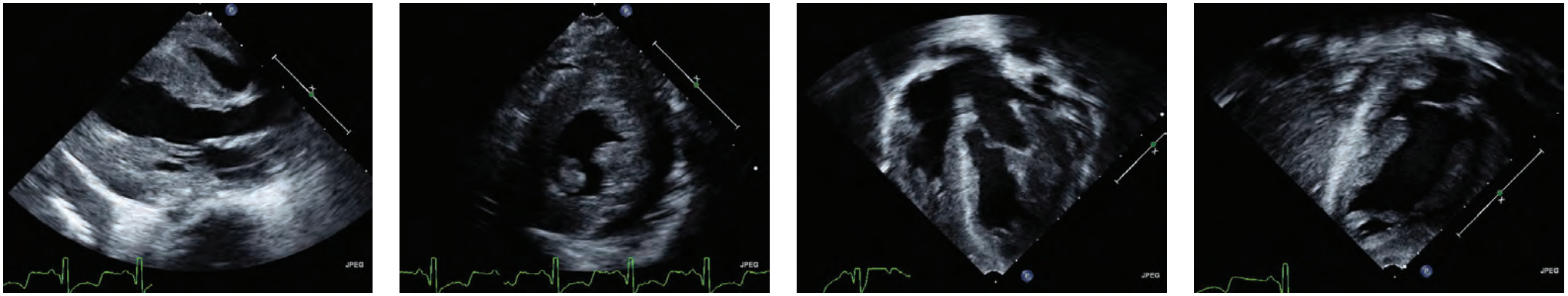
Sibling 3 (IUERT), 1 month of age



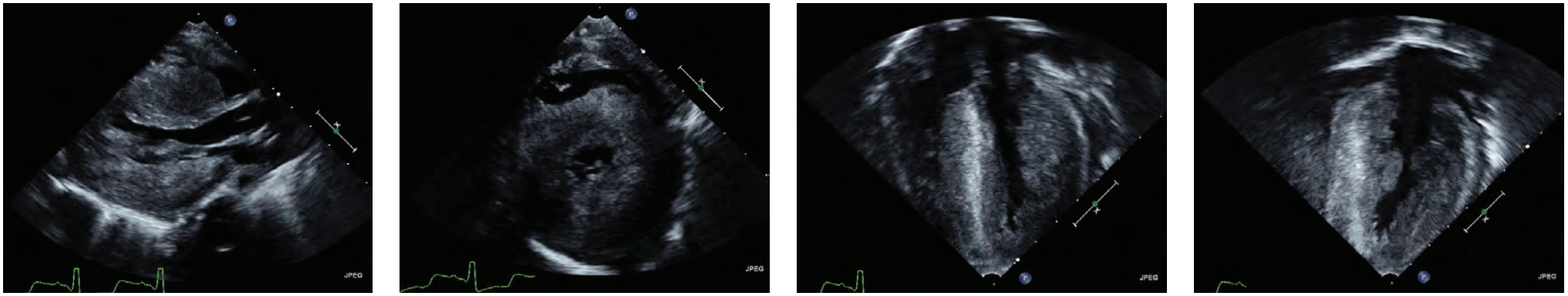
Supplemental Figure S1A. Echocardiogram images of Sibling 2 (untreated) and Sibling 3 (IUERT) from within the first month of life; multiple views shown. Note the moderate-severe hypertrophy in Sibling 2 compared to normal echocardiogram images in Sibling 3. (See primary **Figure 1D** for Left Ventricular Mass Index graph)

Supplemental Figure S1B. Echocardiograms at 6 months of age for all three siblings

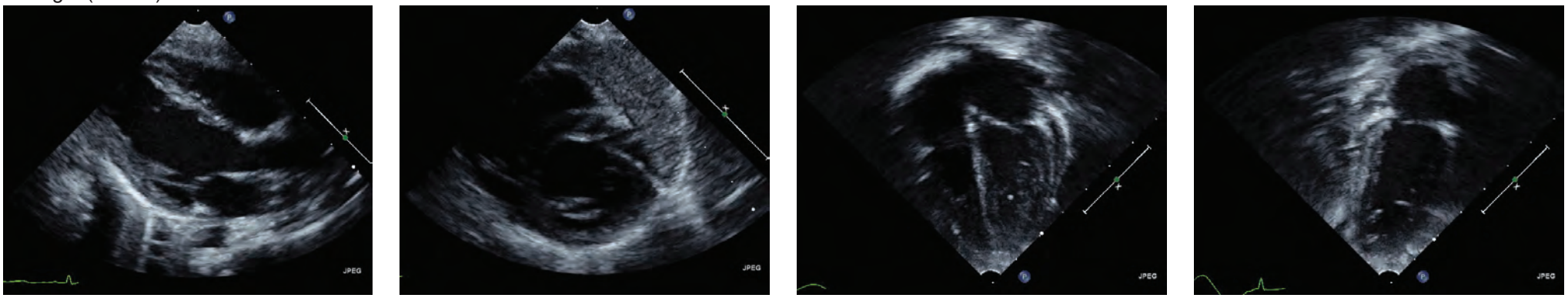
Sibling 1 (proband)



Sibling 2 (untreated)



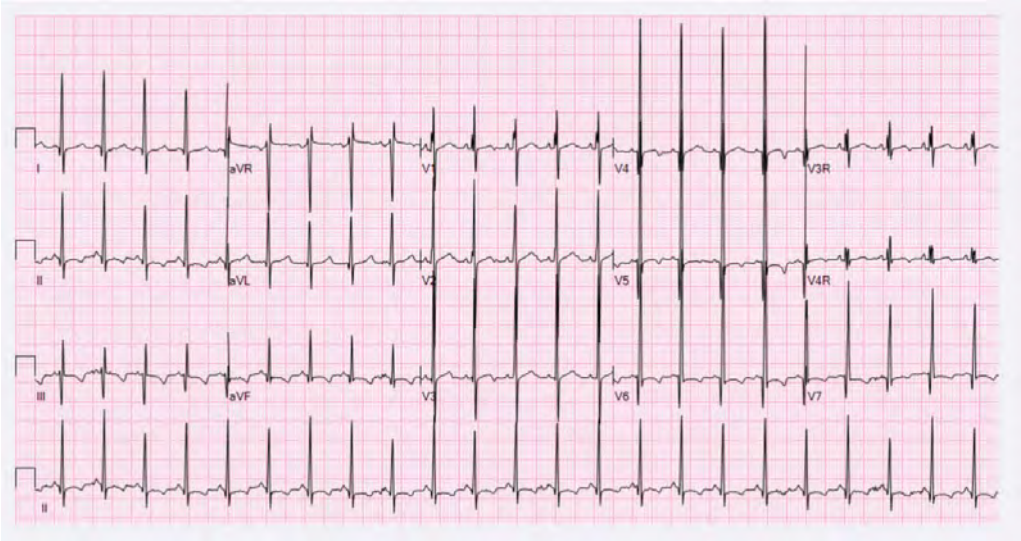
Sibling 3 (IUERT)



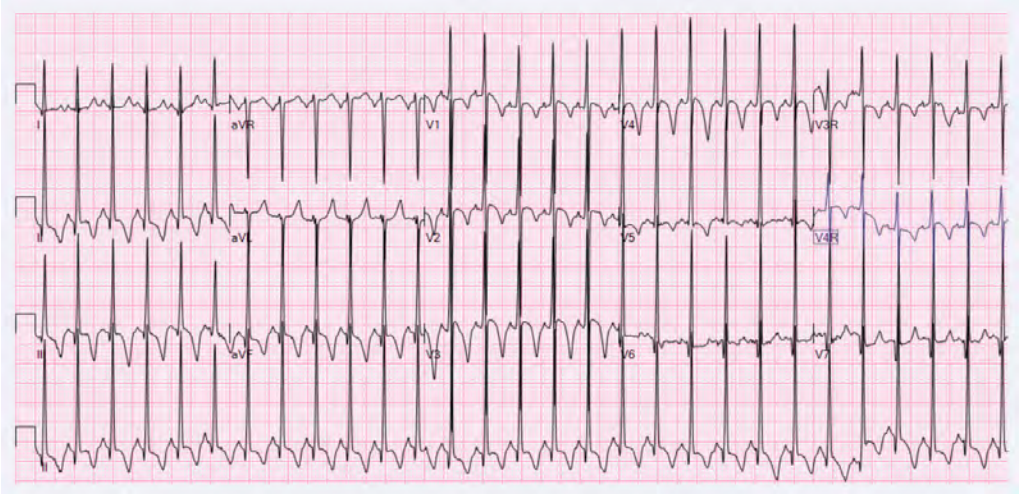
Supplemental Figure S1B. Sibling 1 (proband), Sibling 2 (untreated), and Sibling 3 (IUERT) echocardiogram images at approximately 6 months of age; multiple views shown. Note the hypertrophy of Sibling 1 at time of diagnosis (prior to receiving treatment), and the progressed hypertrophy in Sibling 2, compared to the maintenance of normal echocardiogram images in Sibling 3.

Supplemental Figure S2. Electrocardiograms (ECG) for all three siblings

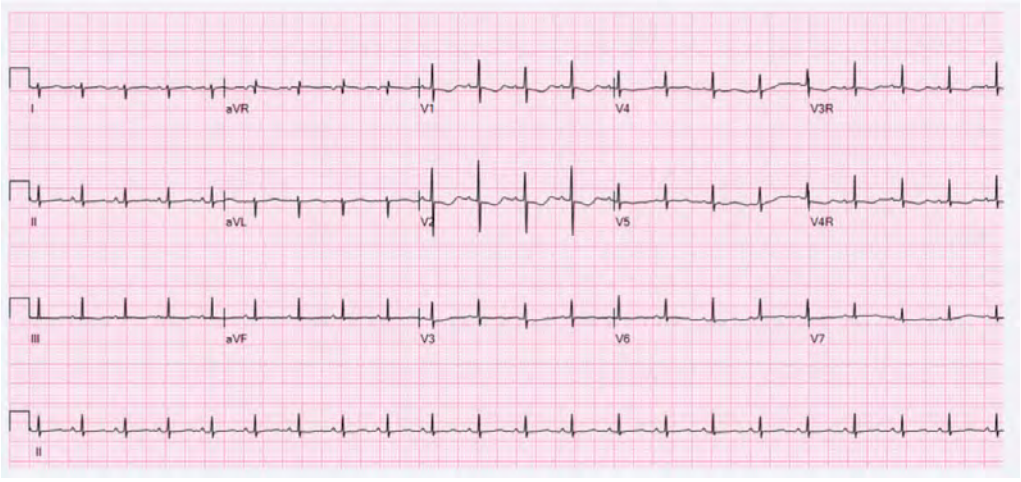
a) Sibling 1 (Proband), 6 months, ECG half-standard



b) Sibling 2 (untreated), 4 months, ECG half-standard

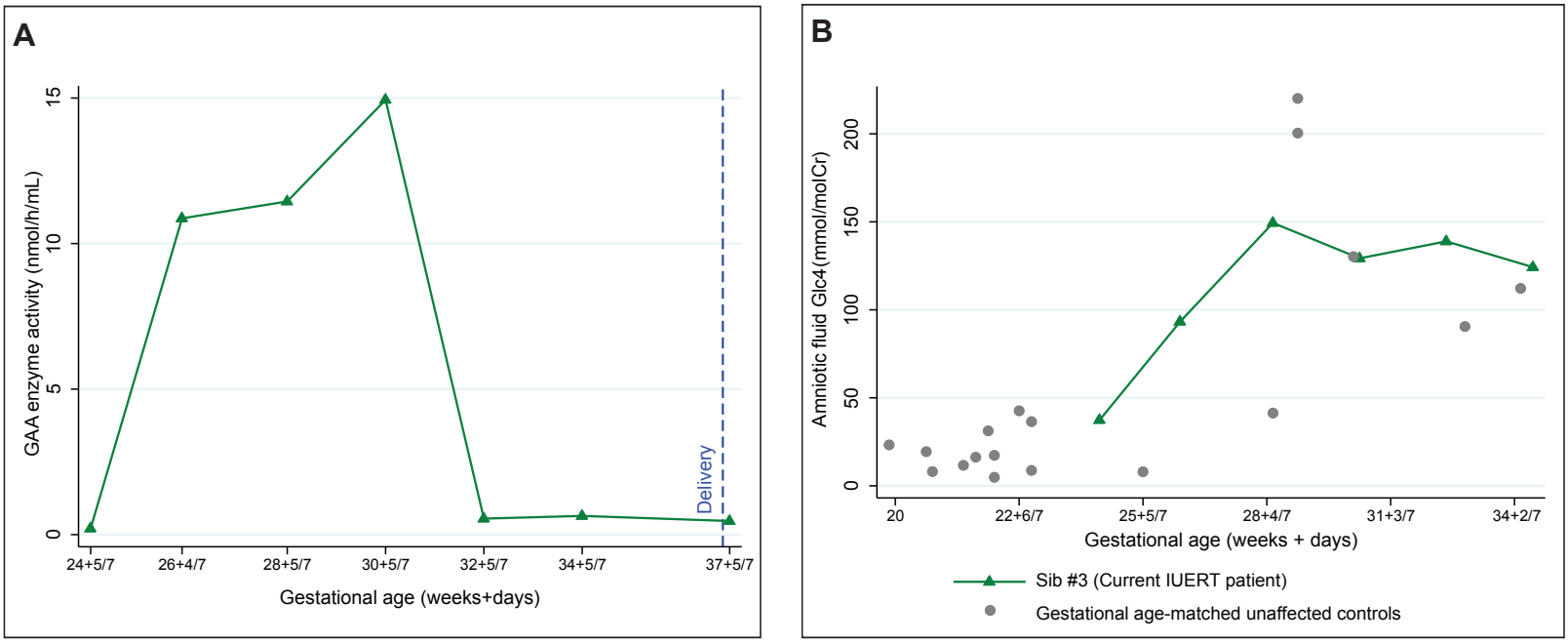


c) Sibling 3 (IUERT), 6 months, ECG half-standard



Supplemental Figure S2. Half standard electrocardiograms (ECGs) of a) Sibling 1 demonstrating severe biventricular hypertrophy with T wave inversion in the inferolateral leads and b) Sibling 2 (untreated) also demonstrating severe biventricular hypertrophy with T wave inversion in the inferolateral leads and c) Sibling 3 (IUERT) demonstrating a normal pediatric ECG with no hypertrophy or T wave inversion. Half standardization was chosen due to the fact that the voltages on the ECG for the affected siblings were too large to make the ECG interpretable or comparable.

Supplemental Figure S3. Additional prenatal laboratory data for Sibling 3 (IUERT)



Supplemental Figure S3. Additional prenatal laboratory data for Sibling 3 (IUERT). **A.** Plasma acid alpha-glucosidase (GAA) enzyme activity trough levels (obtained before each enzyme replacement therapy (ERT) infusion) in current patient's fetal plasma over the course of treatment, performed by a research lab at University of Washington.[6] These results correlate closely to the results in primary **Figure 3B**, performed by a clinical lab at Duke University; Pearson correlation = 0.951925; RSQ = 0.906162. **B.** The current patient's Glc_4 levels in amniotic fluid (corrected for creatinine concentration) over the course of in utero ERT (IUERT) infusions compared to unaffected controls (gray) that are gestational age-matched to our patient. This figure indicates concordance with unaffected controls and the predicted gestational age rise in this biomarker over time.

References

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