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RESEARCH ARTICLE

WiTNNess: An international natural history study of infantile-onset TNNT1 myopathy

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ABSTRACT

Objective: We created WiTNNess as a hybrid prospective/cross-sectional observational study to simulate a clinical trial for infantile-onset TNNT1 myopathy. Our aims were to identify populations for future trial enrollment, rehearse outcome assessments, specify endpoints, and refine trial logistics. **Methods**: Eligible participants had biallelic pathogenic variants of *TNNT1* and infantile-onset proximal weakness without confounding conditions. The primary endpoint was ventilator-free survival. "Thriving" was a secondary endpoint defined as the ability to swallow and grow normally without non-oral feeding support. Endpoints of gross motor function included independent sitting and standing as defined by the Word Health Organization, a novel TNNT1 abbreviated motor score, and video mapping of limb movement. We recorded adverse events, concomitant medications, and indices of organ function to serve as comparators of safety in future trials. **Results**: Sixteen children were enrolled in the aggregate cohort (6 prospective, 10 cross-sectional; median census age 2.3 years, range 0.5–13.8). Median ventilator-free survival was 20.2 months and probability of death or permanent mechanical ventilation was 100% by age 60 months. All six children (100%) in the prospective arm failed to thrive by age 12 months. Only 2 of 16 (13%) children in the aggregate cohort sat independently and none stood alone. Novel exploratory motor assessments also proved informative. Laboratory and imaging data suggest that primary manifestations of TNNT1 deficiency are restricted to skeletal muscle. **Interpretation**: WiTNNess allowed us to streamline and economize the collection of historical control data without compromising scientific rigor, and thereby establish a sound operational framework for future clinical trials.

Introduction

Infantile nemaline rod myopathies are most commonly caused by biallelic mutations of sarcomere genes (MIM# PS161800). One of these, *TNNT1*, encodes the slow-twitch, type 1 isoform of troponin T, a subunit of the complex that mediates actin-myosin binding in response to calcium fluctuations in skeletal muscle.[1] Between 1988

and 2000, Johnston and colleagues studied 71 young children across 33 Old Order Amish families and traced their myopathic phenotype to homozygous truncating mutations in *TNNT1* (c.505G > T; p.Glu180Ter).[2] Online Mendelian Inheritance in Man lists this association as nemaline rod myopathy type 5 (NEM5; OMIM# 605355). In a retrospective study of 106 affected Amish children born between 1923 and 2017,[3] all died before 6 years of age. In other populations, *TNNT1* variants have been linked to lethal infantile muscle disease[4–9] as well as later onset recessive or dominant limb-girdle myopathy,[10–13] and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) currently lists at least 24 "pathogenic" and 12 "likely pathogenic" *TNNT1* variants associated with NEM5.

New treatment platforms hold promise. Adeno-associated virus (AAV)-mediated gene transfer rescues sarcoglycanopathies in mice[14–17] and has entered Phase I trials for limb girdle muscular dystrophy types 2C (*SGCG*; NCT01344798), 2D (*SGCA*; NCT00494195), and 2E (*SGCB*; NCT03652259). This strategy could be applied to the TNNT1 myopathies, which involve a cDNA sized (1.1 kb) for inclusion into AAV particles that need only transfect a single multinucleated cell type (type 1 slow-twitch skeletal myofibers).[18,19] There are both murine and ovine models suitable for testing *TNNT1* replacement and editing therapies,[20,21] but moving from preclinical studies to human trials requires deep understanding of the natural disease course as it evolves over time.[22]

Extensive natural history data about infantile-onset TNNT1 myopathy among Amish and Israeli-Palestinian "founder" populations[2,3,7] are largely retrospective in nature and therefore not suitable for external comparisons. In 2021, investigators published a cross-sectional study of 57 patients with different genetic forms of nemaline rod myopathy and chose candidate measures of motor, pulmonary, and bulbar function.[23] Applying these data to clinical trials is challenging, however, due to the broad spectra of ages (1–57 years), severities, and genotypes (i.e., *ACTA1*, *NEB*, or *TPM2*) represented. Importantly, 17 (30%) participants had no genetic diagnosis, and none had pathogenic variants of *TNNT1*.

We created WiTNNess to generate rigorous prospective natural history data across multiple *TNNT1* genotypes in anticipation of future interventional trials. Our aim was to simulate a clinical trial and thereby identify populations for enrollment, rehearse outcome procedures, specify informative endpoints, and refine operations. Prospective assessments could be performed at relatively low cost in virtually any clinical environment, which allowed us to include children in resource-limited settings.[3,24] We added a cross-sectional arm to reach patients across state and national borders.[5,7,9,25] The hybrid design introduced analytical challenges but optimized the number and diversity of participants.

Finally, the WiTNNess study aligns closely with recent draft guidance by the U.S. Food and Drug Administration (FDA) regarding the design and conduct of externally controlled clinical trials (https://www.fda.gov/media/164960/). This approach recognizes that reducing potential for bias is best addressed in the design phase; in other words, conduct of a historical observational study should closely mimic that of a clinical trial to allow for meaningful statistical comparisons with a future treatment group. WiTNNess satisfies this requirement, and therefore serves as one example of how to accelerate therapeutic development for a rare disorder.

Methods

Study design and oversight

The study was approved by the Penn Medicine-Lancaster General Hospital institutional review board and parents consented to participation on behalf of their children. Children eligible for enrollment had biallelic loss-of-function variants in *TNNT1* and a clinical phenotype consistent with infantile-onset proximal myopathy. Individuals were excluded if they had another known or suspected medical condition (genetic or acquired) that could potentially alter the natural disease course or otherwise interfere with completion of study procedures.

WiTNNess was designed as a hybrid observational natural history study comprised of prospective and cross-sectional arms. Children in the prospective arm were diagnosed during the newborn period and followed longitudinally by a single center (Clinic for Special Children, Strasburg, Pennsylvania). Prospective study visits were conducted almost exclusively in the home setting in the context of strictly palliative care. Additional children who met inclusion criteria were eligible to enroll in the cross-sectional cohort; their study data were captured on a separate case report form (WiTNNess LITE; see Appendix S1) completed by the attending investigator with parental consent. Hereafter, prospective and cross-sectional cohorts are together referred to as the "aggregate cohort."

Procedures

Children in the prospective arm underwent a baseline evaluation and were reassessed at 3-month intervals (Table S1). At each study encounter, we recorded vital signs and anthropomorphic data, conducted a medical history and physical examination, and collected information

about concomitant medications and adverse events (AEs). Blood hemoglobin oxygen saturation (%) and carbon dioxide (mm Hg) were measured using a Masimo Rad-5 Pulse Oximeter (Irvine, California) and Sentec Digital Monitoring System (Lincoln, Rhode Island), respectively. Where possible, AEs were listed according to a unifying diagnosis (e.g., pneumonia) rather than isolated symptoms or signs (e.g., cough, rales). For each AE, the investigator determined its potential relationship to the underlying myopathic disease process, and also recorded its duration, actions taken, outcome, and severity; the latter was based on a 1–5 scale established by the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (https://ctep.cancer.gov/).

Three instruments were used to assess motor function:

1 A motor milestone checklist captured age (in postnatal days/months) at which children achieved each of six major milestones included in the World Health Organization Multicentre Growth Reference Study (WHO-MGRS).[26] These milestones are typically acquired in a sequence: independent sitting, hands-and-knees crawling, standing with assistance, standing independently, walking with assistance, and walking independently. The criteria and procedures used to judge their achievement are described elsewhere[27] and listed in Appendix S1 to this article. The WHO-MGRS milestone checklist was administered at baseline and every 3 months thereafter.
2 The TNNT1 Abbreviated Motor Scale (TAMS) is a 10-item motor battery created specifically for WiTNNess to provide a simple, continuous, quantitative measure of early motor development. It is adapted from the more comprehensive 50-item Neuromuscular Gross Motor Outcome (GRO) developed by Alfano and colleagues.[28] We selected a series of 10 items from the GRO, progressing from easiest to most difficult (Appendix S1). In most healthy infants, these skills unfold serially over the first 9 months of life to culminate in independent sitting; each can be scored rapidly and unambiguously by a study nurse or physician in the home setting. Items are assigned a score of 0, 1, or 2, for a total maximum TAMS score of 20, with higher scores indicating better motor function. We completed the TAMS at baseline and every 3 months thereafter.
3 The Ability Captured Through Interactive Video Evaluation-mini (ACTIVE-mini) system is a non-invasive method of recording movement using a Microsoft KINECT camera (Redmond, Washington).[29] We assigned a uniquely colored tag to each hand and foot and positioned the camera over infants lying supine to measure total spontaneous movement of each limb for 2 min. The ACTIVE-mini was performed at baseline and every 6 months.

For children in the prospective cohort, we tracked anthropomorphic data, vital signs, WHO-MGRS milestones, and TAMS scores longitudinally. For children in the cross-sectional cohort, these same measures were recorded at a single time point (census age). ACTIVE-mini testing was only performed in the prospective study arm.

Serum and whole blood were collected every 6 months for complete blood cell count, total creatinine kinase, aldolase, troponin I, alanine aminotransferase, aspartate aminotransferase, creatinine, and biobanking. We performed a 12-lead electrocardiogram and complete echocardiogram at baseline and every 9 months, and took a chest radiograph in anterior–posterior and lateral projections every 12 months.

For children in the cross-sectional arm, parents and the attending investigator cooperated to complete the WiTNNess LITE case report form (Appendix S1). WiTNNess LITE captured information about each child's region/country of origin, *TNNT1* genotype, growth measurements, vital signs, arterial oxygen saturation, supplemental oxygen requirement, current age or age of death, and cause of death (if applicable). The form also assessed ages (if known) associated with symptom onset, molecular diagnosis, initiation of mechanical feeding and/or respiratory support, and invasive device insertion (e.g., gastrostomy, tracheostomy). Motor milestones and TAMS score were recorded once at census age, which was age of death or, for survivors, age at which the case report form was completed.

Outcomes

The primary outcome was time until death or permanent respiratory support, defined as any invasive (e.g., tracheostomy) or non-invasive (e.g., bi-level positive airway pressure) mechanical ventilatory assistance for ≥16 h daily during ≥14 consecutive days in the absence of a reversible clinical state. The two secondary endpoints were age of independent sitting and the ability to "thrive" at any study visit. We used the WHO-MGRS definition of independent sitting: "child sits up straight with head erect for at least 10 s without using arms or hands to balance the body or support position".[27] Thriving was defined as the ability to swallow normally by bedside assessment and maintain body weight equal to or greater than the WHO 3rd reference percentile for sex and age without requiring non-oral feeding support (i.e., nasogastric or gastrostomy tube).

Exploratory endpoints included other motor milestones as defined by the WHO-MGRS, in particular standing alone (child stands upright on both feet [not on toes] for at least 10 s with back straight, legs supporting 100% of

body weight, in no contact with a person or object), scores on the TAMS administered at baseline and every 3 months, total limb movement volume captured by ACTIVE-mini, and age of gastrostomy or tracheostomy tube insertion. We collated laboratory and cardiac studies, radiographs, AEs, and concomitant medications to establish baseline data for future Phase I clinical trials.

Statistical methods

Statistical tests were performed using Prism 9 (GraphPad Software, San Diego, California). For analyzing event-free survival, each child was assigned a census age defined in one of three ways: (1) age of death or permanent ventilation (aggregate cohort); (2) age of survivors in the prospective cohort as of a December 2022 data cut; or (3) age of each non-ventilated survivor from the cross-sectional cohort when his or her case report form was completed. We compared ventilator-free survival among cohorts using the Mantel–Cox log-rank test. Motor milestones were benchmarked to the WHO-MGRS 1st–99th percentile reference windows for normal development[26] and compared by Mantel–Cox log-rank test to milestones recorded for a group of 56 healthy age-matched Amish and Mennonite children without known neuromuscular disease. Growth measurements were plotted against the WHO 3rd–97th percentile reference standards for age and sex (https://www.who.int/tools/child-growth-standards). Cardiac studies and chest radiographs were interpreted by a board-certified pediatric cardiologist and radiologist, respectively. Descriptive statistics are reported as median and range, except where otherwise noted.

Results

WiTNNess cohorts

Sixteen children were enrolled in WiTNNess between September 2018 and June 2021 (Table 1). Two children from the cross-sectional cohort were the focus of a prior publication[6]; no other WiTNNess participants were represented in previous studies. The prospective arm

Table 1. WiTNNess prospective, cross-sectional, and aggregate cohorts.

	Prospective (n = 6)	Cross-sectional (n = 10)	Aggregate (n = 16)
Age at census, years[a]			
Median (range)	1.4 (0.5–2.4)	5.3 (0.9–13.8)	2.3 (0.5–13.8)
Mean (SD)	1.4 (0.6)	6.4 (4.8)	4.5 (4.5)
Sex, *n* (%)			
Female	2 (33%)	6 (60%)	8 (50%)
Male	4 (67%)	4 (40%)	8 (50%)
Age at diagnosis, months			
Median (range)	0.2 (0–1.9)	12.0 (7.5–145.3)	7.5 (0–145.3)
Mean (SD)	0.5 (0.7)	38.3 (50.7)	20.9 (40.9)
Age of clinical onset, months			
Median (range)	2.6 (0.4–4.5)	6.0 (2.0–8.5)	3.6 (0.4–8.5)
Mean (SD)	2.4 (1.6)	5.5 (2.6)	3.8 (2.6)
Mechanical life support, *n* (%)			
Non-oral gastrostomy tube feeding	2 (33%)	8 (80%)	10 (63%)
Tracheostomy and mechanical ventilation	0 (0%)	6 (60%)	6 (38%)
Deceased, *n* (%)	6 (100%)	0	6 (38%)
Median ventilator-free survival, months	17.1	46.0	20.2
Age of death, range in months	13.5–28.5	*na*	*na*
TNNT1 homozygous genotype (region)[b]			
c.505G > T; p.Glu180Ter (Amish)	6		6
c.574_577delinsTAGTGCTGT; p.Pro192fs (Jerusalem)		6	6
c.661G > T; p.Glu221Ter (Italy)		2	2
c.272_281del; p.Lys91ArgfsTer6 (India)		1	1
c.129-5C > G (Honduras)		1	1

SD, one standard deviation.
[a]Census age (in years) is defined as time of death for prospective cohort or age at time of WiTNNess assessment for cross-sectional cohort.
[b]The relevant founder population or geographical region is listed in parentheses.

comprised six children of Amish ancestry diagnosed with homozygous *TNNT1* c.505G > T alleles at a median age of 6.5 days (range 1–59 days). The cross-sectional arm included 10 children originating from communities in Palestine, Israel, India, Italy, and Honduras, all diagnosed with homozygous truncating or null *TNNT1* alleles at median age 9.5 months (range 4.0–145.3) (Table 1). The prospective as compared to cross-sectional cohort was characterized by earlier age at molecular diagnosis, younger census age, a higher proportion of males, fewer surviving members, and lower utilization of mechanical feeding and respiratory support (Fig. 1).

Primary endpoint: Ventilator-free survival

All six children (100%) in the prospective cohort died of respiratory failure at median age 17.1 months (range 13.5–28.5) (Fig. 2). All children in the cross-sectional arm were alive at census but six (60%) were permanently dependent on mechanical ventilation (median age 21 months), comprising an overall median ventilator-free survival of 20.2 months for the aggregate cohort (n = 16). Ventilator-free survival was shorter for children in the prospective as compared to the cross-sectional cohort (Mantel–Cox log-rank: chi-square = 5.06, p = 0.0245) but similar to median survival of 18.3 months for 88 *TNNT1* c.505G > T homozygotes ascertained retrospectively by Fox and colleagues (chi-square <0.014, p = 0.907).[3] Mechanical breathing support was only used for children in the cross-sectional arm, who had a 100% probability of tracheostomy insertion by age 5 years (Fig. 3). Gastrostomy tube placement was more common in this group (n = 8, 80%), but did not appear to prolong ventilator-free survival (chi-square = 1.27, p = 0.260).

Secondary endpoint: Growth

Median birth weight for the prospective cohort was 3.15 kg (range, 2.11–3.80). Growth began to decelerate between 5 and 10 months of age (Fig. 4). Two children in the prospective arm had gastrostomy tubes inserted at 6.1 and 9.7 months of age. By the age of 12 months, all 6 (100%) failed to thrive, defined here as the inability to maintain weight ≥ 3rd WHO reference percentile without mechanical and/or non-oral feeding support. In the aggregate cohort (n = 16), the probability of gastrostomy was 100% by 45 months of age (Fig. 3). Despite gastrostomy insertion at an early age, all surviving children in the cross-sectional cohort continued to exhibit profound growth failure at census age.

Disease course and motor outcomes

All six newborns in the prospective cohort presented with tremors of the limbs and mandible which diminished

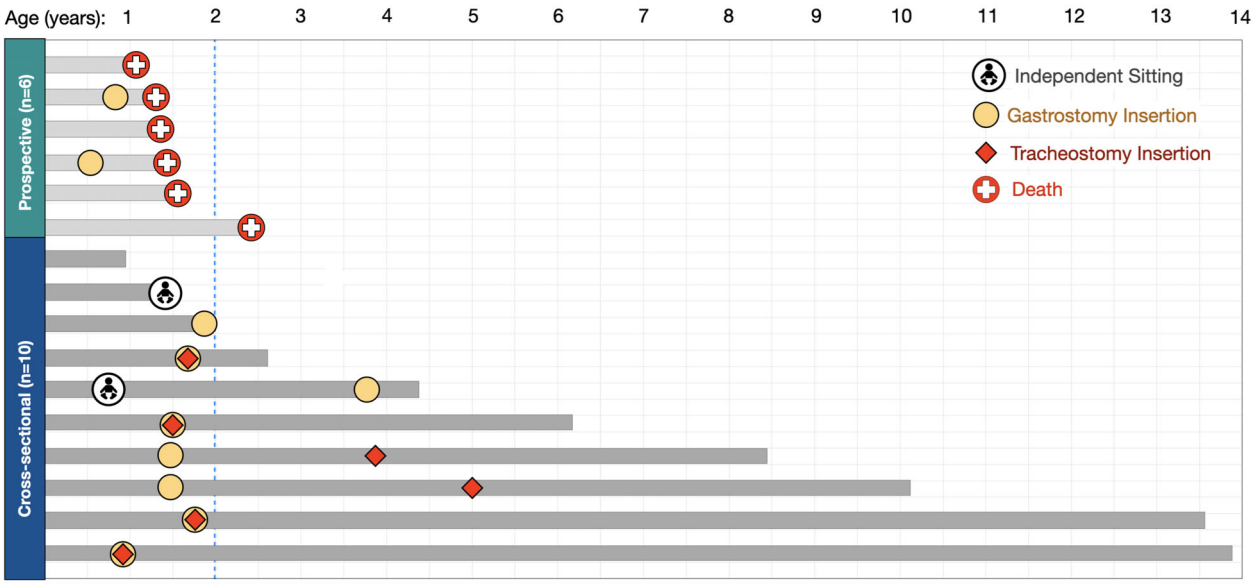

Figure 1. Overview of WiTNNess outcomes. Each bar represents a child in the prospective (light gray, n = 6) or cross-sectional (dark gray n = 10) arm. Bar length indicates census age (in years; upper border) and symbols show patient age at time of gastrostomy insertion (orange circles), tracheostomy insertion (red diamonds), independent sitting (white/black icons), or death (white/red crosses). The dashed blue vertical line (birth to age 2 years) marks the proposed time window for future clinical trials.

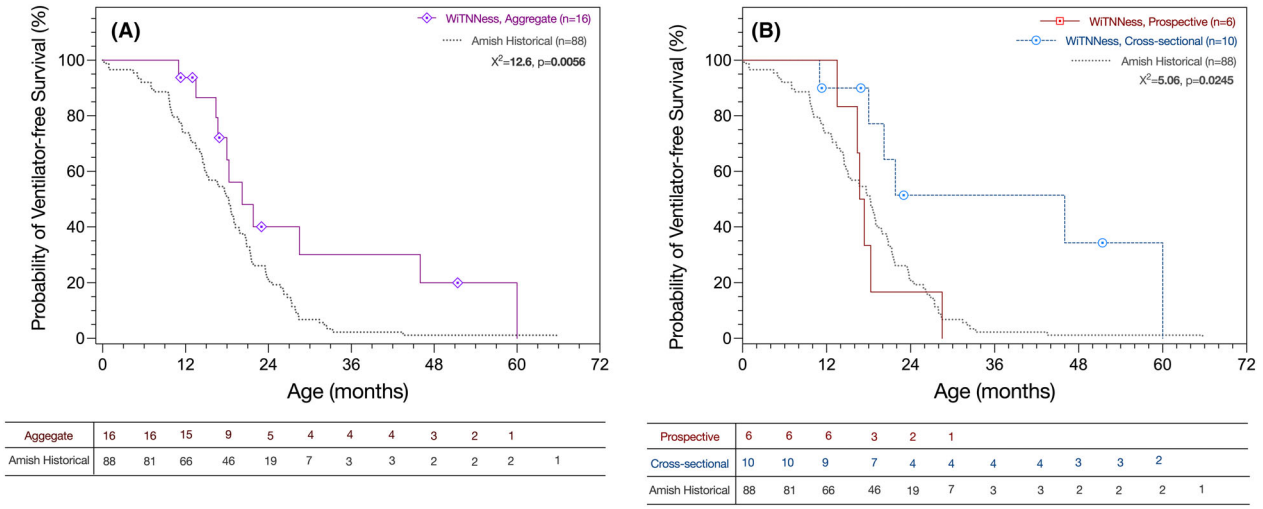

Figure 2. Primary endpoint: Ventilator-free survival. (A) Median ventilator-free survival was 20.2 months for children in the aggregate WiTNNess cohort (purple diamonds, $n = 16$) and longer than median ventilator-free survival of 88 Amish children with TNNT1 myopathy ascertained retrospectively (18.3 months; gray dotted line).[3] (B) This difference was explained by longer survival of children in the cross-sectional (46.0 months; blue circles, $n = 10$) as compared to prospective (17.1 months; red line, $n = 6$) arm.

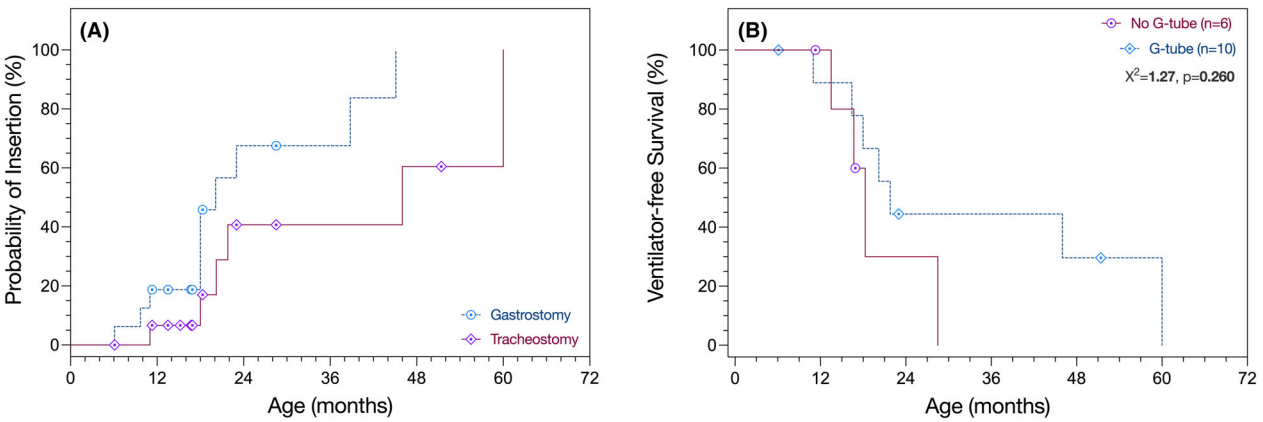

Figure 3. Mechanical life support. (A) For surviving children in the aggregate cohort, the probability of gastrostomy tube or tracheostomy tube insertion reached 100% by ages 45 and 60 months, respectively. (B) Gastrostomy feeding did not significantly prolong ventilator-free survival.

over the first few weeks of life. Hypotonia and proximal weakness were evident by median age 2.6 months (range 0.4–4.5). These were followed by muscle wasting and the emergence of secondary musculoskeletal changes. Progressive contractures came to limit movement of the neck, shoulders, hips, and knees. Evolving deformity of the thoracic cage was accompanied by shallow, restrictive breathing. Movements of the face, forearms, and hands were generally well preserved until the terminal phase of disease.

Only 2 (13%) of 16 participants sat independently at ages 9 and 17 months (Fig. 5A); both children were from the cross-sectional group but statistically this outcome did not differ between cohorts (Mantel–Cox log-rank: chi-square = 0.97, $p = 0.3248$). No child achieved hands-and-knees crawling or independent standing (Fig. 5B). For children in the prospective cohort, TAMS (highest possible score of 20) reached a maximum score of 9–12 between ages 7 and 12 months and decreased progressively thereafter (Fig. 5C). The TAMS scale was completed for nine children in the cross-sectional cohort and showed a similar age-related pattern. No child scored higher than 12 at any age, and none achieved the ability to lift their head (item #8) or prop on their forearms (item #9) from a prone position. The ACTIVE-mini system recorded a consistent pattern of greater arm as

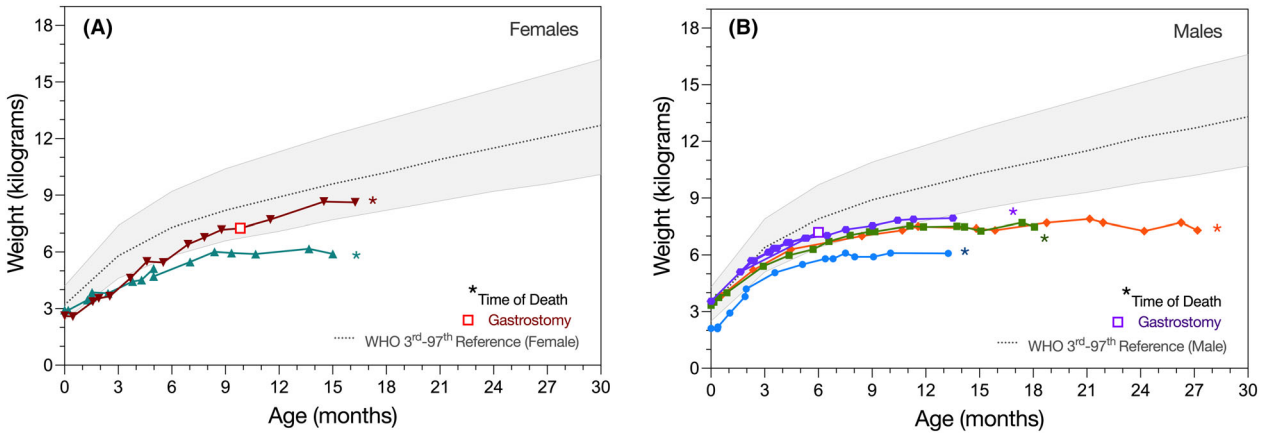

Figure 4. Secondary endpoint: Thriving. "Thriving" was a secondary endpoint defined as the ability to maintain weight at ≥3rd WHO reference percentile for sex and age (gray shading) without non-oral nutritional support (i.e., nasogastric or gastrostomy feeding). According to this definition, all six (100%) children in the prospective cohort failed to thrive by age 12 months. Each colored line represents a female (A) or male (B) child; asterisks and squares indicate the timing of death or gastrostomy insertion, respectively.

compared to leg movement, progressing to near absence of all limb movement by age 2 years (Fig. 5D).

Children with TNNT1 myopathy exhibited no apparent cognitive impairments. Indeed, those surviving in the cross-sectional cohort, although profoundly debilitated, were able to receive age-appropriate schooling and maintain strong social attachments. Using advanced assistive technologies responsive to small movements of the eyes or hands, they learned to navigate power chairs, communicate effectively with friends, and engage in social media. At the time of this writing, the eldest is 17 years of age.

Biomarkers

During the 5–9 months preceding death, children in the prospective cohort developed a distinctive thoracic deformity characterized by pectus carinatum, intercostal narrowing, flattening of the thoracic contour ("triangulation" of the chest wall), and scoliosis (Fig. 6A). Cephalocaudal shortening of the thoracic cavity (Fig. 6B) was associated with worsening hypoxemia and hypercarbia (Fig. 6C), although baseline respiratory rate remained within normal limits (Fig. S1), indicating chronic central adaptation to impaired gas exchange.

Five of six children in the prospective cohort had blood biomarkers drawn every 6 months comprising a total of 10 samples (Table S2); one family declined venipuncture. Aldolase was mildly elevated in all sera (median 20.4 U/L, range 14.5–24.2; normal reference range: 3.4–11.3 U/L) but creatine kinase was consistently normal (median 82.5 U/L, range 59–201; normal reference range 45–240 U/L). Blood hemoglobin concentrations and platelet counts were normal excepting one (10%) value each below the lower reference limit. The lowest observed platelet count was 130,000 cells/μL. Alanine aminotransferase (ALT) was normal in all 10 (100%) serum samples, but aspartate aminotransferase (AST) was elevated in eight (80%) of them, on average 2.3-fold higher than ALT.

Four children in the prospective cohort had electrocardiograms and echocardiograms to investigate any potential effects of TNNT1 deficiency on cardiac structure or function. These studies generally revealed tachycardia but otherwise normal cardiac anatomy and performance (Table S3). At the terminal stage of disease, advanced restrictive lung disease was associated with signs of pulmonary hypertension and right heart failure (e.g., shortened pulmonary artery acceleration time). Troponin I, a subunit of the troponin complex and circulating marker of myocardial injury, was normal in all samples (median 0.01 ng/mL, range < 0.01–0.06).

Adverse events and concomitant treatments

Between September 2019 and December 2022, we recorded 50 adverse events (AEs) among six children in the prospective cohort (Table 2). The median number of AEs was 8 per child (range 4–14). Each child had at least one AE and one serious AE. Respiratory (n = 30, 60%) and gastrointestinal (n = 11, 22%) complications were most common. Sixteen AEs (32%) were serious; 15 of these (94%) were attributed to myopathic disease and 13 (81%) were respiratory in nature. Respiratory failure (n = 10, 20%) was the most common serious AE and proved fatal in six cases.

There were 62 palliative interventions for children in the prospective cohort. The main indications for medical

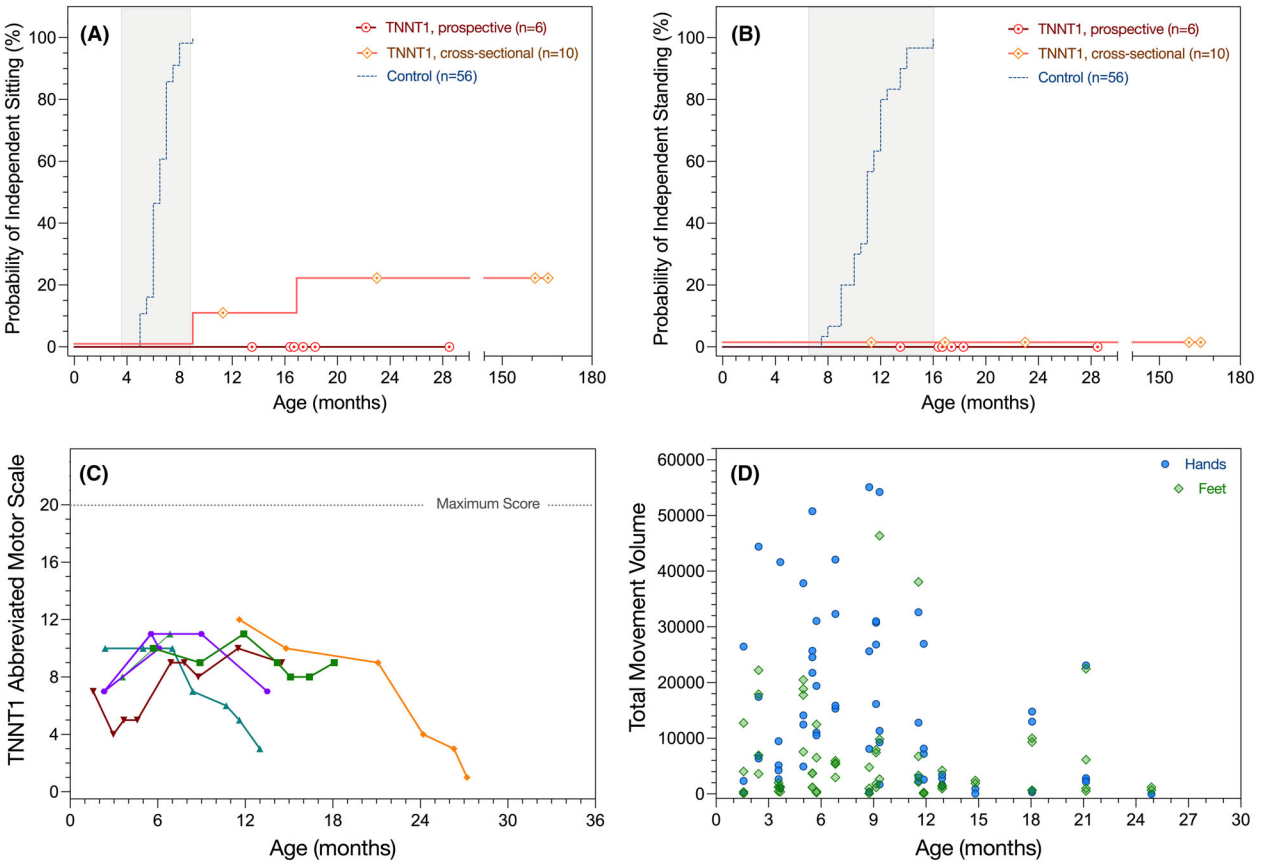

Figure 5. Secondary and exploratory motor outcomes. (A) Independent sitting for ≥10 s was achieved by 2 (13%) of 16 WiTNNess participants, shown as red circles (prospective cohort, $n = 6$) or diamonds (cross-sectional cohort, $n = 10$). Both children who sat independently were from the cross-sectional group, but statistically this outcome did not differ between cohorts (Mantel–Cox log-rank: chi-square = 0.97, $p = 0.3248$). For comparison, gray shading indicates the 1st–99th percentile reference window for this milestone (WHO-MGRS), and the blue dashed line shows time to independent sitting for 56 normally developing Amish and Mennonite children. (B) No child in WiTNNess achieved independent standing. (C) The TNNT1 abbreviated motor scale (TAMS) characterized early motor development using 10 maneuvers adapted from the Neuromuscular Gross Motor Outcome Scale[28] (maximum score of 20, gray dashed line). No child in WiTNNess achieved a TAMS score exceeding 12 at any time point (each line represents one child from the prospective cohort). (D) The Ability Captured Through Interactive Video Evaluation-mini (ACTIVE-mini) system recorded volume and speed of movement in all four limbs from supine position. Spontaneous limb movement was greater in arms (blue circles) as compared to legs (green diamonds) and became negligible after 24 months of age.

intervention were sinopulmonary tract infection ($n = 24$, 39%) and dyspnea ($n = 16$, 26%). Sinopulmonary infections were the most common indication for enteral antibiotic therapy ($n = 20$, 32%) and sometimes accompanied by reactive airways, which were treated with nebulized bronchodilators ($n = 7$), oral prednisolone ($n = 5$), or inhaled corticosteroids ($n = 3$). We treated children empirically for dyspnea if they exhibited signs of restless agitation, emotional distress, or insomnia in the setting of respiratory failure. Specific interventions for dyspnea, alone or in combination, included enteral morphine ($n = 5$), benzodiazepines ($n = 5$), supplemental oxygen by nasal cannula ($n = 4$), and furosemide ($n = 1$).

Discussion

WiTNNess was designed to accelerate therapeutic development for TNNT1 myopathy and set a foundation for future clinical trials.[30] We curated prospective data in the home setting without costly equipment or personnel,[31] and used a streamlined case report form to ease cross-sectional data sharing across state and national borders. With proper foresight, we believe a similar hybrid strategy could be applied to other rare, infantile-onset nemaline myopathies, many of which are suitable targets for gene therapy (e.g., *ACTA1* [1.5 kb], *TPM2* [1.2 kb], *ADSSL1* [2.0 kb], *TNNT3* [1.1 kb], etc.).[23,32–34]

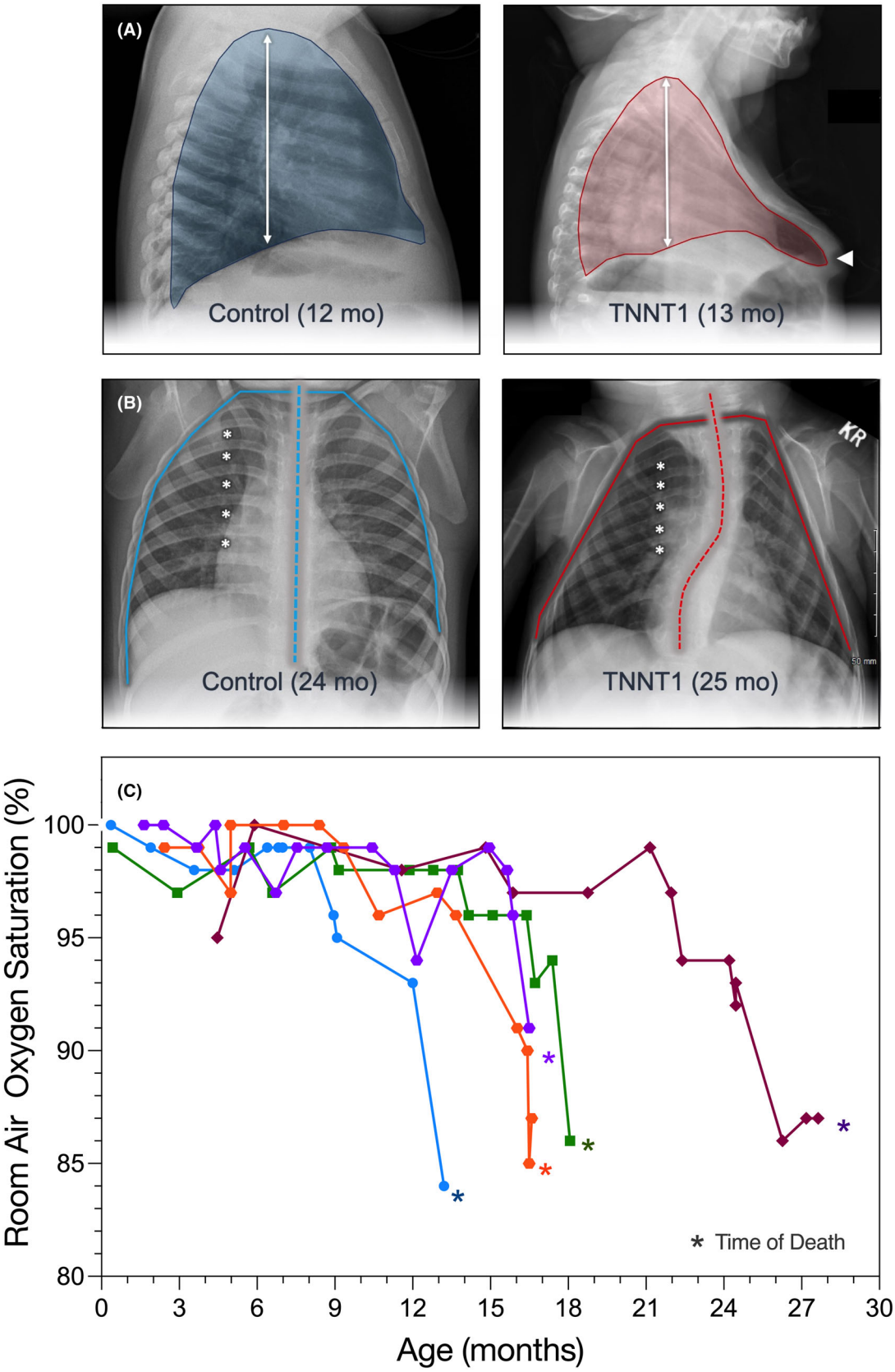

Figure 6. Biomarkers: Chest deformity and blood gases. (A) Lateral chest radiograph of a child with TNNT1 myopathy (*right panel*, age 12 months) compared to a healthy child (*left panel*, age 13 months) shows pectus carinatum (white arrowhead) and cephalocaudal shortening of the thoracic cavity (white arrows). (B) Posterior–anterior chest radiograph at an older age (*right panel*, 25 months) shows contracture of the intercostal spaces (asterisks), flattening of the thoracic contour (solid lines), and scoliosis (dashed line). (C) These anatomical changes were accompanied by progressive hypoxemia and hypercarbia in the months preceding death (asterisks).

Table 2. Adverse events by system category in prospective cohort.[a]

Adverse event by category	Patients (n = 6) N (%)	Events (n = 50) n (%)
Respiratory		
Respiratory failure	6 (100)	10 (20)
Upper respiratory tract infection	4 (67)	7 (14)
Pneumonia	3 (50)	5 (10)
Bronchiolitis	2 (33)	3 (6)
Reactive airway disease	1 (17)	2 (4)
Cough	2 (33)	2 (4)
Croup	1 (17)	1 (2)
Gastrointestinal		
Dysphagia	2 (33)	4 (8)
Gastrostomy insertion	2 (33)	2 (4)
Gastroesophageal reflux	1 (17)	1 (2)
Vomiting	1 (17)	1 (2)
Inguinal hernia	1 (17)	1 (2)
Constipation	1 (17)	1 (2)
Gastroenteritis	1 (17)	1 (2)
Infectious NOS		
Acute otitis media	4 (67)	5 (10)
Fever/pyrexia	2 (33)	2 (4)
Hematological		
Microcytic anemia	1 (17)	1 (2)
Thrombocytopenia	1 (17)	1 (2)
Central nervous system		
Hypersomnolence	2 (33)	2 (4)

NOS, not otherwise specified.
[a]Adverse events are reported from 12 September 2019 to 12 December 2022.

Combining prospective and cross-sectional cohorts introduced methodological and analytical challenges. We accepted these limitations to make enrollment accessible to the greatest number and diversity of affected children, including those from communities historically underrepresented in clinical studies.[24,35] One strength of this approach was the ability to observe a relatively concordant phenotype among 16 children comprising five different pathogenic *TNNT1* genotypes encountered in diverse clinical environments. We nevertheless took care to represent prospective and cross-sectional groups separately in Table 1 as well as Figs. 1, 2, and 5, so as not to obscure differences between them. Based on these differences, we cannot exclude the possibility that the "Amish" *TNNT1* c.505G > T allele,[2,3] exclusively represented in the prospective cohort, is associated with an unusually severe clinical phenotype.

TNNT1 myopathy exemplifies a challenge faced by the broader rare disease community.[24,35,36] Most gene-based therapies target a small clinical population.[37] This is juxtaposed to substantial development and manufacturing costs,[38] as well as an implicit expectation that such agents will be administered in highly resourced urban settings (https://patienteducation.asgct.org/gene-therapy-101/gene-therapy-centers). Consequently, people who are poor, uninsured, or otherwise disadvantaged often have difficulty securing access to life-saving therapies. Not infrequently, these same people are underrepresented in patient registries and natural history studies.[35,39] Through WiTNNess, we addressed these disparities from an early stage, hoping to promote more equitable enrollment in future clinical trials.

Ventilator-free survival and the composite endpoint of thriving emerged as robust clinical trial endpoints. Children in the prospective arm were treated with a strictly palliative focus and therefore followed a relatively natural disease course. All six died of respiratory failure before 29 months of age. Children in the cross-sectional arm survived longer with more intensive medical support, but our data indicate that fewer than 30% of newborns with biallelic loss-of-function *TNNT1* variants survive beyond age 2 years without mechanical ventilation. Thus, while the composite endpoint demonstrates an important difference in *actual* survival between cohorts, the proxy of mechanical ventilatory dependence minimizes that difference, revealing a more consistent biological phenotype among all participants.

A similar consideration applies to the composite endpoint of thriving. In a previous natural history study of TNNT1 myopathy, we ascertained growth retrospectively without specifically considering the role of non-oral and/or mechanically assisted feeding.[3] Here, we tracked growth prospectively using the composite endpoint of thriving employed in gene therapy trials for SMA,[40,41] which requires normal swallowing, age-appropriate weight, and independence from feeding devices. According to this definition, all six children in the prospective arm failed to thrive within the first year of life.[40,41] The same applies to nearly all children in the cross-sectional arm if gastrostomy serves as a proxy for growth failure.

The motor milestone checklist consisted of six benchmarks selected by the WHO as "universal, fundamental to the acquisition of self-sufficient erect locomotion, and simple to test and evaluate".[26] These endpoints are supported by open access methods[27] and universal reference intervals,[26,42] and have been successfully adapted to a number of multinational neuromuscular trials.[41,43,44] In contrast, the TAMS was designed specifically for WiTNNess as a "fit-for-purpose" tool (Appendix S1). This scale is similar in principle to the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders used in therapeutic trials for SMA,[41,45,46] and proved easy for a physician or physical therapist to administer in about 5 min. Scores followed a consistent pattern across *TNNT1* genotypes and, importantly, reached a "ceiling" value of 12 (maximum possible score 20) for all participants. The ACTIVE-mini system vividly tracked cessation of limb movements over the first 2 years of life but required special equipment and software that prolonged visits and introduced technical challenges. Simple wearable devices might serve the same purpose in future versions of the protocol.[47]

The design of WiTNNess aligned with recent FDA draft guidance about externally controlled clinical trials for new biological products (https://www.fda.gov/media/164960/). The FDA states that for rare, intractable disorders with a grave prognosis, it is reasonable to use data from an outside comparator group if (1) a blinded trial design is unreasonable or impractical; (2) baseline demographics resemble those of a treatment group; (3) historical control and treatment groups are observed at similar ages over a comparable time window; and (4) external control data include information about concomitant therapies and relevant AEs. Data collected through WiTNNess satisfy these requirements and should therefore enable meaningful statistical comparisons with future treatment cohorts.

In conclusion, the ongoing WiTNNess study establishes a sound operational framework for therapeutic trials focused on children with infantile-onset TNNT1 myopathy. The protocol allowed us to identify specific underserved populations for future trial enrollment, rehearse a full battery of assessments, specify outcome measures of both safety and efficacy, and refine trial logistics within a relevant timeframe. Beyond its immediate application to nemaline rod myopathies, we hope WiTNNess can serve as a model for other investigators who strive to both democratize and economize the development of precision therapies without compromising scientific rigor.

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Author Contributions

Author contributions include: (1) conception and design: KAS, VJC, SE, KWB; (2) data curation and analysis: KAS, VJC, EB, ME, AB, LEB, DC, KWB; (3) drafting a significant portion of the manuscript or figures: KAS, EB, KWB; and (4) data acquisition: KAS, VJC, EB, ME, AB, LEB, KB, MY, SE, NF, AD, EB, LL, DC, LPL, MI, LNA, KWB.

Conflict of Interest Statement

LPL is co-inventor of the ACTIVE-mini system. No other authors have relevant conflicts of interest to disclose.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Table 1.
Appendix S1.

