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A Prospective Study of Milestones in Juvenile PLA2G6-Associated Neurodegeneration (PLAN)

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A Prospective Study of Milestones in Juvenile *PLA2G6*-Associated Neurodegeneration (PLAN)


THESIS


submitted in partial satisfaction of the requirements
for the degree of


MASTER OF SCIENCE

in Genetic Counseling


by


Leila Karlene Rahim Schwanemann

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2023

# TABLE OF CONTENTS

# LIST OF ABBREVIATIONS

INAD   Infantile Neuroaxonal Dystrophy

NBIA   Neurodegeneration with Brain Iron Accumulation

PLAN   *PLA2G6*-Associated Neurodegeneration

# LIST OF FIGURES

# LIST OF TABLES

# ACKNOWLEDGEMENTS

To the NBIA community, my thesis committee, and family and friends—thank you.

# ABSTRACT OF THE THESIS

A Prospective Study of Milestones in Juvenile *PLA2G6*-Associated Neurodegeneration (PLAN)

by

Leila Karlene Rahim Schwanemann

Master of Science in Genetic Counseling

University of California, Irvine, 2023

Professor John Jay Gargus, Chair

Juvenile *PLA2G6*-Associated Neurodegeneration (PLAN), an ultra-rare disorder characterized by variable features, is often discussed alongside its well-characterized counterpart, infantile PLAN. Despite detailed accounts of single cases of juvenile PLAN (n=1) and recent small cohorts (n=3 or 4), a comprehensive summary of its natural history remains elusive, presenting challenges in diagnosis.

This study, utilizing data from Oregon Health & Science University, involves a semiannual questionnaire with 16 participants attending visits, ranging from one to 11. The questionnaire covered milestones, encompassing the regression of early developmental milestones (e.g., sitting, walking, etc.) and the progression of other disease-related milestones (e.g., feeding tube, seizures, etc.). The primary objective was to provide a quantitative review of the presence or absence of features relative to their age at onset, providing a detailed description of juvenile PLAN and its progression.

The primary findings, represented by medians, were that individuals experienced frequent falls around 7.2 years, swallowing difficulties at 11.4 years, and abnormal autonomic function at 12.7 years. A cluster of gross motor skill loss spanned from 12.7 to 16.9 years, with wheelchair

use beginning at 12.8 years. Fine motor skill loss appeared between 15.1 to 16.7 years, while seizures typically manifested at 15.6 years. Gastrostomy/feeding tube intervention was introduced at 15.9 years, and notably, none of the participants required a tracheostomy.

During follow-up study visits, participants were questioned about regression relative to their skillset six months earlier. An analysis over five-year intervals showed a rising trend in regression reporting: 13% between ages 5.0 and 9.9 years, 44% between ages 10.0 and 14.9 years, and a substantial 95% between ages 15.0 and 19.9 years. Participants provided detailed descriptions of their regression symptoms extending beyond the initial questionnaire, encompassing changes in behavior, cognition, and dystonia, among other aspects. A retrospective analysis of medical records for a subset of participants (n=8) encompassed these features and more.

This analysis improves the understanding of juvenile PLAN, thereby shortening the diagnostic odyssey, providing timely family support, informing best practices in PLAN, and laying the groundwork for future clinical trials to assess the statistical significance of interventions, such as gene therapy or devices.

# 1.  INTRODUCTION

## 1.1. Overview of Juvenile *PLA2G6*-Associated Neurodegeneration (PLAN)

Neurodegeneration with Brain Iron Accumulation (NBIA) encompasses a group of disorders characterized by abnormal iron deposition in specific regions of the brain. One of these disorders is an ultra-rare autosomal recessive condition called *PLA2G6*-associated neurodegeneration (PLAN) caused by mutations in the *PLA2G6* gene (Morgan et al., 2006). PLAN leads to the progressive loss of essential motor skills, including walking, talking, and eating. Individuals affected may eventually require the use of a wheelchair, feeding tube, or tracheostomy tube. PLAN is debilitating, often results in premature death, and currently, there is no known cure. To date, there are three PLAN subtypes (Figure 1), however, there is a spectrum of overlapping phenotypes within these subtypes (Gregory et al., 2008b; Gregory et al., 2009; Hayflick et al., 2018; Kurian & Hayflick, 2013).

**Figure 1**

*Flowchart of Medical Eponyms and Disease Nomenclature*
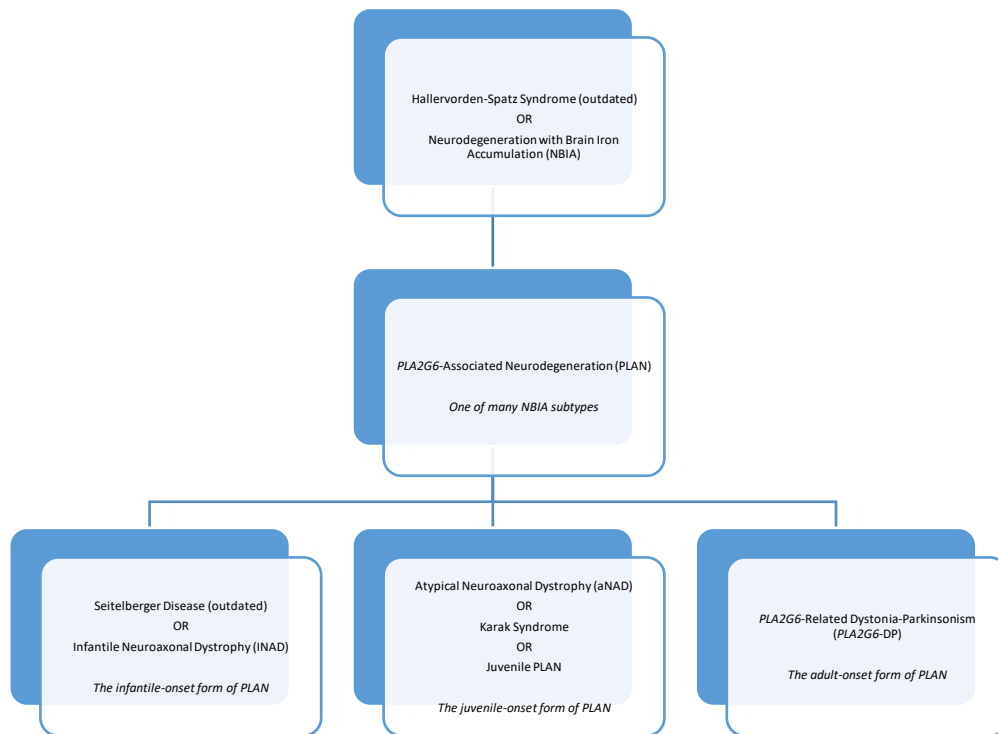


Due to the estimated prevalence of 1:1,000,000 (Gregory et al., 2009), the natural

history of juvenile PLAN is not well-characterized in the literature, leading to diagnostic

challenges. Some studies have described large cohorts of individuals with homozygous or

compound heterozygous *PLA2G6* mutations, but most of these cases have a clinical diagnosis of

infantile neuroaxonal dystrophy (INAD), or rarely *PLA2G6*-DP, rather than juvenile PLAN, as

seen in Morgan et al., 2006 (6 of 38) and Gregory et al., 2008b (6 of 79). Juvenile PLAN cases are

frequently compared to INAD for reference. INAD has a relatively well-established and uniform

clinical presentation, whereas juvenile PLAN has been described as having a wide range of

phenotypes, later onset of symptoms, milder presentation, and slower progression:

- "As more people are discovered to have mutations in *PLA2G6*, we anticipate that the phenotypic spectrum of [juvenile PLAN] will probably expand…" (Gregory et al., 2008b, p.1407).

- "One other patient was diagnosed with the onset of PLAN in childhood, and our report highlights the diagnostic challenges associated with this [juvenile] PLAN subtype" (p.183). "[Juvenile PLAN] is less common than INAD, and the phenotype is more heterogeneous rendering the diagnosis challenging…" (Illingworth et al., 2014, p.187).

- "However, further studies are needed to better explain the clinical variability associated with the *PLA2G6* variants, especially regarding the 'intermediate severity' phenotype of childhood-onset PLAN" (Romani et al., 2015, p.184)

Many publications have reported single cases of juvenile PLAN among cohorts of individuals with biallelic *PLA2G6* mutations such as Cheng et al., 2022 (1 case out of 6), Cif et al., 2014 (1 case out of 1), Erro et al., 2016 (1 case out of 3), Illingworth et al., 2014 (1 case out of 5), Jain et al., 2019 (1 case out of 1), Kim et al., 2015 (1 case out of 2), and Romani et al., 2015 (1 case out of 17). Other studies have described a few cases of juvenile PLAN with a focus on the clinical phenotype as seen in Salih et al., 2013 (5 cases out of 11), Toth-Bencsik et al., 2021 (3 cases out of 5), and Wan et al., 2022 (4 cases out of 5).

## 1.2. Presenting Signs and Symptoms

Typically, pregnancy, birth, and early developmental milestones are reported as normal. The age at onset of symptoms can vary widely, with reports ranging from as early as 1.5 years (Gregory et al., 2008b; Romani et al., 2015) and as late as the end of the second decade (Kurian

& Hayflick, 2013). Initial signs and symptoms often include cerebellar ataxia or gait instability. However, others may exhibit autistic features such as speech delay or diminished social interaction. For instance, one patient presented at 3-years-old with toe-walking and lower extremity spasticity, whereas another individual maintained a diagnosis of autism until his parents noticed toe-walking and gait instability at 8-years-old (Gregory et al., 2008b).

Other early signs and symptoms may encompass frequent falls, cognitive decline affecting school performance, dysarthria, or neurobehavioral disturbances. (Gregory et al., 2008b; Mubaidin et al., 2003; Salih et al., 2013). One child's presentation, among other features, included their first epileptic seizure (Toth-Bencsik et al., 2021).

### 1.3. Clinical Features

Described as a slowly progressive psychomotor disorder, juvenile PLAN includes a constellation of symptoms such as ataxia, spasticity, dystonia, dysarthria, contractures that may require surgical release, and spastic tetraparesis in the later stages of the disease. Other manifestations may include cognitive decline, neuropsychiatric features, and seizures. In the early stages, visual symptoms may present as strabismus and nystagmus. With disease progression, patients may also develop signs such as optic nerve pallor, eventually leading to optic atrophy (Gregory et al., 2008b; Gregory et al., 2009; Hayflick et al., 2018; Illingworth et al., 2014; Kurian & Hayflick, 2013; Mubaidin et al., 2003; Nardocci et al., 1999; Salih et al., 2013). There have been mentions of hyperthermia (Cif et al., 2014) and abnormal autonomic function (Ma et al., 2019; Toth-Bencsik et al., 2021), although additional details regarding these phenotypes remain unclear. The lifespan with this condition is unknown; however, Gregory et

al. (2008b) reported an individual who died at 23-years-old, while Salih et al. (2013) reported

another individual who developed multiple bed sores and osteomyelitis at 27-years-old,

ultimately succumbing to septicemia from the bed sores at 28-years-old.

### 1.4. Neuropathologic Features

As part of a comprehensive neurological evaluation, a brain MRI (magnetic resonance

imaging) may be recommended to better understand the underlying pathology contributing to

a patient's neurological symptoms. In juvenile PLAN, two prominent features may become

apparent: (1) An early finding is degeneration of the cerebellum, called cerebellar atrophy,

which plays a crucial role in motor control and coordination. (2) As the disorder progresses, iron

accumulates in a portion of the brain that controls movement called the basal ganglia,

particularly the globus pallidus and substantia nigra. The detection of high brain iron, regardless

of the presence or absence of cerebellar atrophy, indicates the need for *PLA2G6* sequencing

(and other NBIA disorders).

Another neuropathologic feature is the presence of widespread axonal swellings known as

spheroids in both the central and peripheral nervous systems (CNS and PNS). Previously, the

presence of spheroids in the PNS was considered a hallmark feature of INAD (Seitelberger,

1952). Historically, to confirm the clinical diagnosis, biopsies, such as muscle, skin, conjunctiva,

etc., were performed. However, with the discovery of *PLA2G6* mutations, it became evident

that there was not always a direct correlation between mutations in *PLA2G6* mutations and the

presence of axonal spheroids in the PNS, therefore, Morgan et al. (2006) recommended that

molecular diagnoses be the preferred approach for subsequent diagnostic testing in cases suspected to have PLAN.

## 1.5. Diagnostic Challenges

Due to its initial presentation and slow progression, juvenile PLAN can pose diagnostic challenges, leading to various initial misdiagnoses. In some cases, individuals have received an initial diagnosis of static encephalopathy or hereditary spastic paraplegia.

Prior to gene discovery, Nardocci et al. (1999) examined a group of individuals who had initially been clinically diagnosed with INAD. However, four of them were described as having an atypical clinical picture that was somewhat milder, characterized by slower progression of both motor disability and mental impairment. The primary clinical features observed in these cases were hypotonic-areflexic tetraparesis associated with cerebellar signs, without evidence of pyramidal dysfunction. Their fairly stable condition was described as resembling static encephalopathy followed by neurologic deterioration.

Upon discovery of the causative gene (Morgan et al., 2006), it was notable that the initial clinical diagnoses of the six cases ultimately diagnosed with juvenile PLAN were more general, using terms like NBIA or the closely related Karak syndrome (Mubaidin et al., 2003). In a subsequent publication that identified an additional six juvenile PLAN cases, these individuals had previously been clinically diagnosed with idiopathic NBIA (Gregory et al., 2008b). Most of these individuals presented with high brain iron and nonspecific clinical features.

In the most recent publications, at least five patients were initially diagnosed with hereditary spastic paraplegia. This diagnosis was primarily based on their initial symptoms,

which included spasticity and hyperreflexia, or spastic rigidity, in their lower limbs, as reported

by Cheng et al. (2022) and Wan et al. (2022).

These diagnostic challenges highlight the complexity of identifying juvenile PLAN,

particularly in its early stages, and the importance of genetic testing for an accurate diagnosis.

In recent years, many are now diagnosed through whole exome sequencing (WES) (Hayflick et

al., 2018). Although the genotype-phenotype correlation has been described as loose,

individuals with juvenile PLAN tend to have missense mutations (Gregory et al.,2008b; Gregory

et al., 2009; Hayflick et al., 2018; Kurian & Hayflick, 2013).

### 1.6. Other *PLA2G6* Disorders

#### 1.6.1. Infantile Neuroaxonal Dystrophy (INAD)

INAD is often referred to as the infantile-onset form of PLAN due to its early presentation of

symptoms occurring between six months to three years of age. Many children with INAD

experience psychomotor delay or regression, leading to a failure to acquire or a loss of early

developmental milestones such as walking. A characteristic feature of INAD, not reported in

genetically and clinically confirmed cases of juvenile PLAN post gene discovery in 2006, is the

early presentation of truncal hypotonia. Common progressive features shared between the two

disorders include cognitive decline and spastic tetraparesis, initially presenting as hyperreflexia

and progressing to areflexia. Overlapping visual features include nystagmus, strabismus, and

optic atrophy. The progression of INAD is rapid, causing a significantly shortened a lifespan,

often within the first decade of life (Gregory et al., 2008a).

7

**1.6.2. *PLA2G6*-Related Dystonia-Parkinsonism (*PLA2G6*-DP)**

*PLA2G6*-DP is often referred to as the adult-onset form of PLAN, usually starting in adolescence or early adulthood. Mild intellectual impairment is common. Initial symptoms may include gait disturbance or neuropsychiatric changes, which is consistently followed by dystonia and Parkinsonism, and may be accompanied by rapid cognitive decline. Other symptoms can include dysarthria and abnormal autonomic function. Overlapping features between *PLA2G6*-DP and juvenile PLAN include neurobehavioral/neuropsychiatric disturbances and abnormal autonomic function. Although Parkinsonism appears to be a distinct feature of *PLA2G6*-DP, juvenile PLAN also exhibits bradykinesia, tremors, etc. (Gregory et al., 2008a; Hayflick et al., 2018).

**1.7. Outdated Medical Eponyms**

The first opportunity to describe NBIA occurred in 1922 when five affected sisters from a sibship of twelve were identified. Their symptoms included progressive dysarthria and dementia, and death before age 25. An autopsy was performed on two of the sisters, which revealed a brown discoloration (i.e., iron) in specific portions of their brains, particularly the globus pallidus and substantia nigra (Hallervorden & Spatz, 1922; as cited in Pearce, 2006).

In the late 19th century to early 20th century, it was common practice to honor the physicians credited with playing a major role in identifying a disorder (Ferguson & Thomas, 2014), therefore, the eponym Hallervorden-Spatz syndrome (i.e., NBIA), attributed to Julius Hallervorden and Hugo Spatz was coined. However, the use of this eponym has been in decline since the neurologic community became aware of Hallervorden and Spatz's subsequent roles

within the Nazi Party that was controlled by Adolf Hitler in Germany during the Third Reich (Shevell, 1992; Shevell, 2012).

During World War II (WWII; 1939 to 1945) the Nazi euthanasia program set the stage for the Holocaust, the genocide of six million European Jews. Run by members of the Schutzstaffel (or SS, a Nazi paramilitary organization), vulnerable German citizens (children and adults, hospitalized or institutionalized, with mental and physical disabilities) were targeted and murdered. In a postwar report, Hallervorden shared his perspective on this process, "I heard that they were going to do that, and so I went up to them and told them, 'Look here now, boys. If you are going to kill all those people, at least take the brains out so that the material can be utilized'". Hallervorden actively participated in the program by removing brain specimens from euthanized victims. He is not the only physician to benefit from this material with multiple scientific publications, but Hallervorden is noted to be the most active participant (Hughes, 2007). Julius Hallervorden was a senior physician and pathologist at the Brandenburg State Hospitals, Brandenburg-Goerden was one of six elimination centers. One year prior to the program, four postmortem brain examinations were performed at Brandenburg-Goerden. Over the next six years, 1260 postmortem brain examinations were performed at Brandenburg-Goerden. Hallervorden also acted as the chair of neuropathology at the Kaiser Wilhelm Institute for Brain Research (KWIBR) during WWII (Shevell & Peiffer, 2001). The director of KWIBR was Hallervorden's lifetime mentor and friend, Hugo Spatz (Shevell, 1992; Voges & Kupsch, 2021).

In the postwar report, Hallervorden confirmed that he had examined 698 brain specimens from the euthanasia program at KWIBR (Hughes, 2007), whereas Spatz, his superior, denied any association between KWIBR and the euthanasia program (Hughes, 2007; Voges & Kupsch,

2021). Spatz's role is less clear, but since his death, there is growing evidence to suggest that he was involved in the euthanasia program. One piece of evidence includes the presence of brain specimens from euthanasia victims in his autopsy collection, however, how those specimens were added to his collection has not been delineated (Voges & Kupsch, 2021).

Another NBIA eponym associated with the Nazi regime is Seitelberger disease (i.e., INAD). Franz Seitelberger, a former member of the SS (Neuberger & Stacher, 1999), examined the brains of euthanasia victims in the post-WWII era, ultimately earning his PhD under the supervision of Hallervorden (Dahlkamp, 2003; as cited in Kondziella, 2009). Although Seitelberger was not directly involved in the euthanasia program, he continued to advance his scientific career for decades by utilizing brain specimens acquired through similar means from other collections (Kondziella, 2009). Due to the deeply troubling history associated with these eponyms and individuals, terms like Hallervorden and Spatz syndrome, as well as Seitelberger disease, are now considered outdated.

## 1.8. Purpose of Research

This research exclusively focuses on juvenile PLAN cases, with no inclusion of INAD or *PLA2G6*-DP cases. The primary aim was to provide clinical insights utilizing both prospective (n=16) and preliminary retrospective (n=8) data from individuals with *PLA2G6* mutations whose clinical presentations most closely align with juvenile PLAN. This approach contributes to a more comprehensive understanding of the natural history of juvenile PLAN.

## 2. METHODS

This human-subjects research project, UCI IRB (University of California, Irvine Institutional Review Board) #1097, was reviewed, confirmed to fall under the category of 'minimal risk (expedited)', and approved by the UCI IRB (Appendix A; Appendix B).

### 2.1. Clinical Data and Eligibility Factors

This research study utilized two sets of data: prospective and retrospective. The prospective data was obtained from the IRB-approved natural history study called NBIAready (Neurodegeneration with Brain Iron Accumulation): Online Collection of Natural History Patient-reported Outcome Measures (protocol e10832) at Oregon Health & Science University (OHSU). Individuals or their proxies participating in NBIAready complete a survey (Appendix C) every six months for a period of five to 10 years, where they report on specific milestones such as regression of early developmental milestones and progression of disease-related milestones. UCI IRB #1097 focused on the analysis of data for individuals (n=16) with a confirmed genetic diagnosis of *PLA2G6*-associated neurodegeneration (PLAN) whose age of onset and clinical features aligned most closely with juvenile PLAN, as opposed to infantile neuroaxonal dystrophy (INAD) or *PLA2G6*-related dystonia parkinsonism, as determined by OHSU.

In addition, as part of UCI IRB #1097, a retrospective detailed medical chart review was conducted for eight of the 16 participants who provided consent. The relevant medical records such as genetics, neurology, and pediatrician notes were transferred from OHSU to UCI, and additional medical records were obtained (if necessary). The purpose of this review was to

focus on the same milestones as NBIAready while also expanding the scope to investigate other phenotypic abnormalities that might be present in multiple individuals.

## 2.2. Recruitment Methods

The NBIA Center of Excellence at OHSU shared an IRB-approved UCI recruitment flyer (Appendix D) to the 16 individuals enrolled in NBIAready who had juvenile PLAN. This flyer informed them about an opportunity to participate in UCI IRB #1097 by signing an updated consent form for OHSU (protocol e10832) and a new consent form for UCI IRB #1097. By signing these consent forms, the participants agreed to have their identifiable research data and medical records transferred from OHSU to UCI. Out of the 16 families, eight families provided their consent to participate. Other participants were lost to follow-up and did not provide their consent. At UCI, a Data Recipient Usage Agreement, overseen by Sponsored Projects, was coordinated to facilitate the transfer of de-identified research data for eight participants, and identifiable research data and medical records for the eight participants that consented.

To recruit additional subjects for the retrospective portion of the UCI study, the NBIA Center of Excellence at OHSU distributed the flyer to eligible participants registered in their NBIA Repository (protocol e7232). The flyer was also shared through NBIA advocacy and support groups, such as the INADcure Foundation (https://www.inadcure.org/). Furthermore, it was shared among colleagues, such as clinical genetics experts, who have affiliations with electronic distribution lists restricted to medical professionals and scientists. Additionally, the flyer was shared with members of the National Society of Genetic Counselors (NSGC) by

purchasing a Research Survey E-Blast & Reminder service. However, no additional subjects were recruited, likely due to the ultra-rare nature of juvenile PLAN.

## 2.3. Informed Consent Process

Signed informed consent and child assent (if applicable) were obtained from all research participants that had the capacity to provide consent/assent. Signed parental consent for research participants aged 0-17 was obtained regardless of the research participant's capacity to provide assent. Surrogate consent from a legally authorized representative was obtained from research participants aged 18 years or older who were cognitively impaired or medically incapacitated. For deceased research participants of all ages, surrogate consent was obtained.

If the lead researcher had reason to believe that the potential subject's decision-making capacity may be impaired, the lead researcher utilized a Decision-Making Capacity Assessment Tool (Appendix E) provided by the UCI Office of Research HRP (Human Research Protections). If the research participant lacked the capacity to consent, the lead researcher made a reasonable effort to describe the research to the participant in a manner consistent with the standard consent process and indicate the intent to obtain surrogate consent. The lead researcher used the Investigator Certification of Surrogate Decision Makers for Potential Subject's Participation in University of California Research form (Appendix F) to document surrogate consent.

## 2.4. Research Procedures

A research participant's involvement for the retrospective portion of UCI IRB #1097 included one study visit via Zoom. Participants or their proxies provided information about their doctors (e.g., pediatrician, neurologist, genetics, etc.) and the UCI study team prepared a

medical records release form(s) to collect additional medical records (if necessary) for the retrospective portion of the research study.

### 2.5. Data Collection and Management

The lead researcher was given access to a folder on the OHSU secure server and subsequently downloaded and stored the files on the UCI secure server, which adheres to the security requirements outlined in the HIPAA (Health Insurance Portability and Accountability Act of 1996) regulations.

Prospective data was provided in the form of an Excel spreadsheet, while retrospective data was supplied as PDF files. If the subject provided written authorization to release their protected health information from an institution other than OHSU, additional digital records were obtained directly from the healthcare providers' institution. These records were securely downloaded and stored on the UCI secure server.

The prospective data was analyzed and visualized using Excel and GraphPad Prism 9 software, whereas the retrospective data was analyzed using Excel.

While performing a detailed review of medical records for the retrospective portion, phenotypic abnormalities were extracted and recorded in an Excel spreadsheet. To ensure standardization, The Jackson Laboratory HPO (Human Phenotype Ontology) terms (https://hpo.jax.org/app/) were used as a guideline to standardize the clinical features identified in the medical records.

Other relevant information was abstracted from the medical records such as services received. These services, which do not necessarily correspond to HPO terms, were categorized accordingly. Examples may include early intervention, physical/speech/occupational therapy, an IEP (Individualized Education Program) which allows students with disabilities to receive specialized instruction and related services, among others.

## 2.6. Statistical Analysis

Prospective data analysis involved utilizing information such as the age at which participants lost the ability to perform specific motor skills. This data underwent analysis and was visualized through Kaplan-Meier curves and scatter plots using GraphPad Prism 9, or longitudinal plots and grouped columns via Excel. Descriptive statistics, including measures like the minimum, median, maximum, etc., were applied to summarize the data. The retrospective data was collated and analyzed using descriptive statistics.

## 2.7. Confidentiality of Research Data

The identifiable research data and medical records were securely stored and accessed electronically through the UCI secure server, which meets HIPAA security requirements. An NIH (National Institutes of Health) Certificate of Confidentiality (CoC) (Appendix G) was obtained to further protect the privacy of research participants by prohibiting disclosure of identifiably, sensitive research information to anyone not connected to research except when the participant consents or in a few other specific situations. The identifiable information for UCI IRB #1097 will be destroyed after publication, presentation, or end of protocol for all participants.

# 3. RESULTS

## 3.1. Prospective Data

### 3.1.1. Demographics

In the prospective data, a total of 16 participants were included. The participants' ages at enrollment ranged from 3.5 to 21.7 years. Each participant underwent between one and 11 PLANready study visits. Of the 16 participants, 13 were female, while the remaining three were male. Table 1 provides a summary of the number of study visits for each subject, along with their ages at their first and most recent visit. In Figure 2 each subject was represented by a unique color, and each dot corresponds to a study visit, indicating the subject's age at that point in time.

**Table 1**

PLANready Study Participants

| Subject | Visit (N) | Age (Years) | |
| --- | --- | --- | --- |
| | | First Visit | Last Visit |
| 24 | 1 | 21.7 | - |
| 44 | 11 | 5.9 | 10.6 |
| 47 | 6 | 9.8 | 12.3 |
| 49 | 10 | 16.9 | 21.4 |
| 56 | 5 | 15.6 | 17.6 |
| 63 | 8 | 12.8 | 16.3 |
| 66 | 8 | 14.2 | 17.7 |
| 67 | 6 | 12.7 | 16.3 |
| 88 | 6 | 5.5 | 7.2 |
| 95 | 3 | 7.4 | 8.9 |
| 96 | 6 | 3.5 | 5.0 |
| 110 | 4 | 11.4 | 12.9 |
| 137 | 2 | 20.2 | 20.7 |

**Table 1 Continued**

PLANready Study Participants

| Subject | Visit (N) | Age (Years) | |
|---------|-----------|-------------|------------|
| | | First Visit | Last Visit |
| 147 | 2 | 10.6 | 11.1 |
| 156 | 1 | 10.6 | - |
| 166 | 1 | 7.2 | - |

**Figure 2**

*PLANready Study Visits*
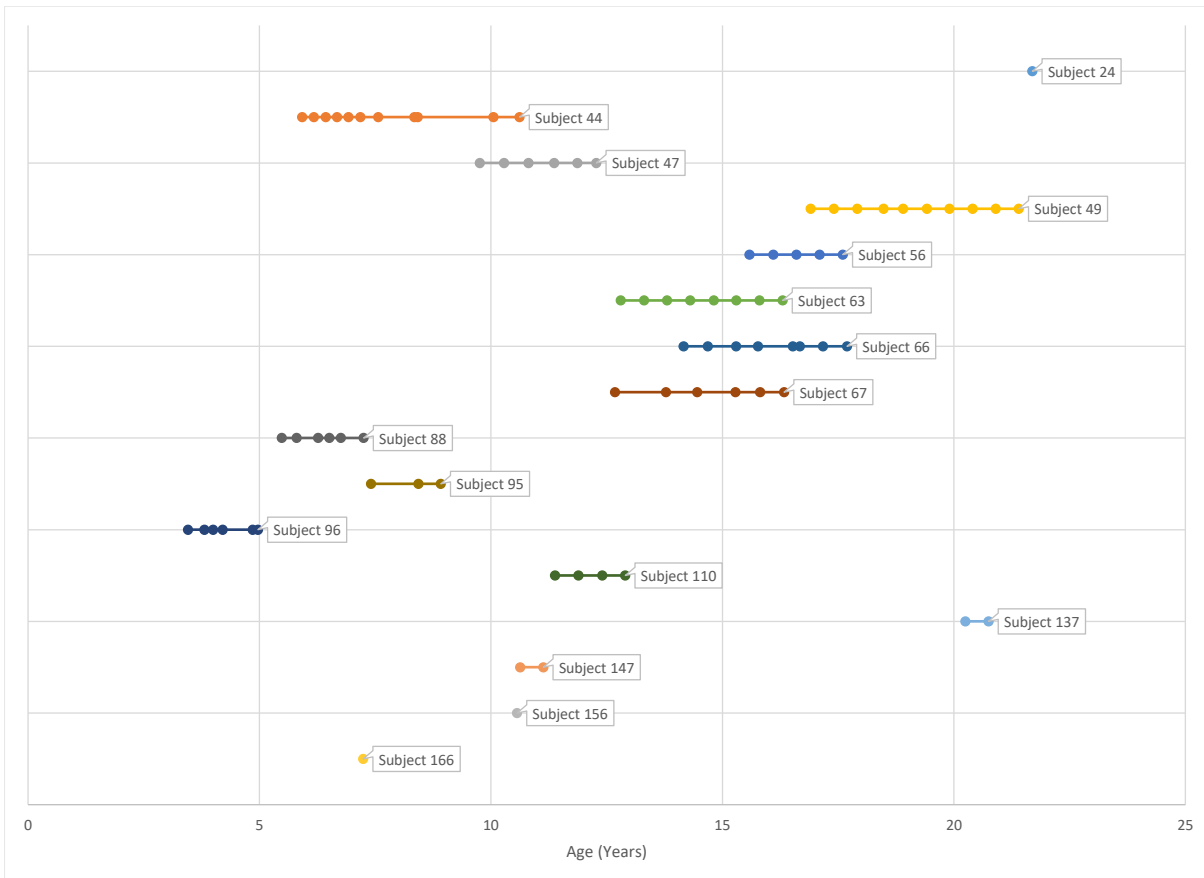


*Note.* Each subject was represented by a unique color, and each dot corresponds to a study visit, indicating the subject's age at that point in time.

### 3.1.2. Overview of Graphs and Statistics

The objective of PLANready was to capture regression of motor skills and progression of disease-related features in real-time. The PLANready survey can be divided into two categories. The first category includes 20 questions related to regression of early developmental milestones such as sitting, standing, and walking. The second category contains 11 questions related to progression of disease-related milestones such as wheelchair use, gastrostomy tube, tracheostomy, etc. The data was analyzed and visualized in four ways: scatter plots, longitudinal plots, Kaplan-Meier curves, and grouped columns.

### 3.1.2.1. Scatter Plots

In the scatter plots (e.g., Figure 3), each study participant was represented once with a gray dot and categorized into one of three groups. The category 'has the skill' represents the age of an individual who still has the skill at their most recent PLANready study visit. 'First lost the skill' represents the age at loss of skill for an individual who reported loss of the skill after visit 1 (i.e., visits 2, 3, 4, etc.), allowing for real-time data capture of regression. 'Lost the skill' represents the age at enrollment for an individual who reported that loss of the skill occurred before visit 1, resulting in data captured at enrollment, at an older age than when it first occurred. A blue dot represents a participant that experienced fluctuations in this motor ability. Descriptive statistics such as the minimum, median, maximum, etc. can be found in Tables 2-4.

**Figure 3**

*Scatter Plot: Regression of Milestone 1*
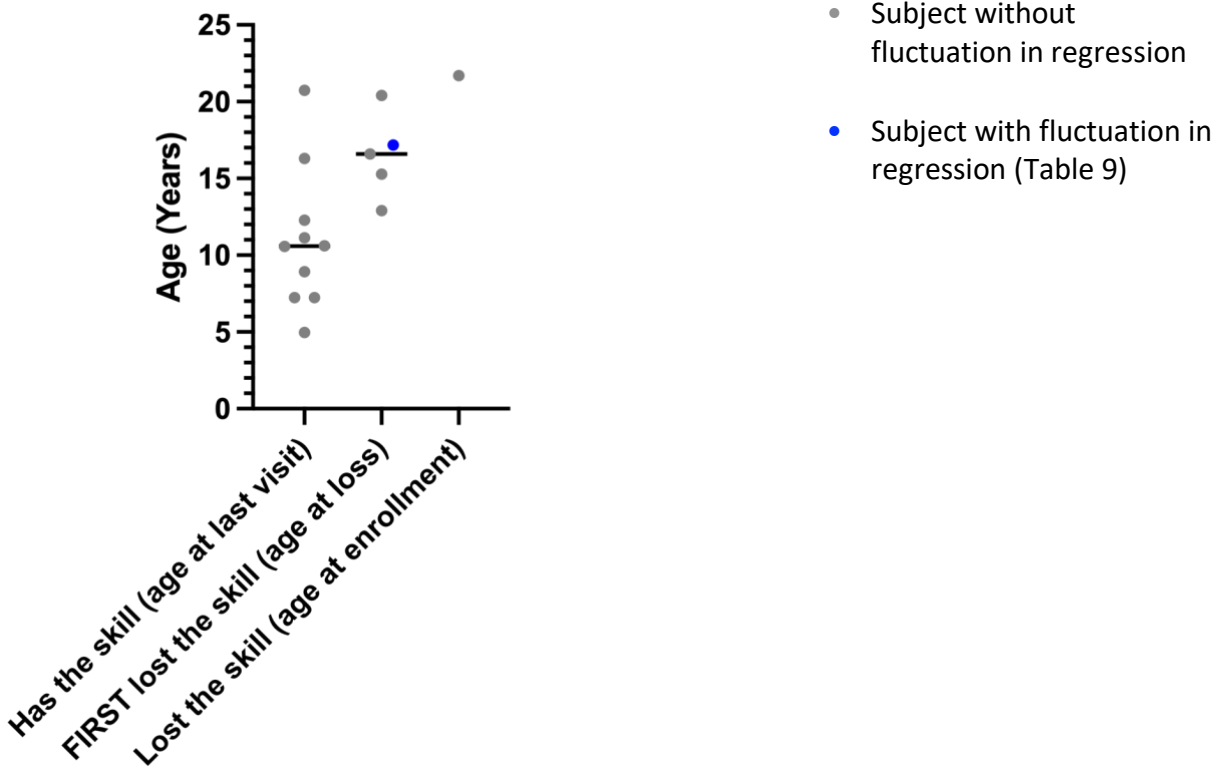


*Note.* Milestone 1—'Grasps a toy or other object when it is touched to the backs or tips of the fingers'. Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Table 2**

*Scatter Plot Statistics for Regression of Early Developmental Milestones (1-20)*

| PLANready Milestone | Category | Sub (N) | Min (Years) | Max (Years) | Med (Years) |
|---|---|---|---|---|---|
| 1. Grasps a toy or other object when it is touched to the backs or tips of the fingers | Has the skill[a] | 10 | 5.0 | 20.8 | 10.6 |
| | FIRST lost the skill[b] | 5 | 12.9 | 20.4 | 16.6 |
| | Lost the skill[c] | 1 | 21.7 | - | - |

**Table 2 Continued**

*Scatter Plot Statistics for Regression of Early Developmental Milestones (1-20)*

| PLANready Milestone | Category | Sub (N) | Min (Years) | Max (Years) | Med (Years) |
|---|---|---|---|---|---|
| 2. Reaches toward objects placed in front of him/her and tries to grab them | Has the skill[a] | 13 | 5.0 | 20.8 | 11.1 |
| | FIRST lost the skill[b] | 2 | 16.6 | 20.4 | - |
| | Lost the skill[c] | 1 | 21.7 | - | - |
| 3. Picks up small objects, like a Cheerio or raisin | Has the skill[a] | 9 | 5.0 | 16.3 | 10.6 |
| | FIRST lost the skill[b] | 5 | 12.4 | 19.9 | 15.8 |
| | Lost the skill[c] | 2 | 20.3 | 21.7 | - |
| 4. Holds head upright & steady for several seconds when held in sitting position, no bobbing action | Has the skill[a] | 14 | 5.0 | 21.7 | 11.7 |
| | FIRST lost the skill[b] | 2 | 16.6 | 20.4 | - |
| | Lost the skill[c] | 0 | - | - | - |
| 5. Rolls over independently | Has the skill[a] | 10 | 5.0 | 16.3 | 10.6 |
| | FIRST lost the skill[b] | 0 | - | - | - |
| | Lost the skill[c] | 6 | 12.7 | 21.7 | 16.3 |
| 6. Sits up alone for at least 5 seconds without help from another person | Has the skill[a] | 11 | 5.0 | 20.8 | 10.6 |
| | FIRST lost the skill[b] | 2 | 16.6 | 16.7 | - |
| | Lost the skill[c] | 3 | 12.7 | 21.7 | 16.9 |
| 7. Pulls self to stand without assistance | Has the skill[a] | 8 | 5.0 | 12.3 | 9.7 |
| | FIRST lost the skill[b] | 2 | 11.9 | 13.3 | - |
| | Lost the skill[c] | 6 | 12.7 | 21.7 | 16.3 |
| 8. Get into a sitting position by him/herself (from lying down or standing with support) | Has the skill[a] | 9 | 5.0 | 16.3 | 10.6 |
| | FIRST lost the skill[b] | 0 | - | - | - |
| | Lost the skill[c] | 7 | 11.4 | 21.7 | 15.6 |
| 9. Walks 5 steps independently (without support) | Has the skill[a] | 5 | 5.0 | 12.3 | 8.9 |
| | FIRST lost the skill[b] | 1 | 11.1 | - | - |
| | Lost the skill[c] | 10 | 5.5 | 21.7 | 13.5 |

**Table 2 Continued**

*Scatter Plot Statistics for Regression of Early Developmental Milestones (1-20)*

| PLANready Milestone | Category | Sub (N) | Min (Years) | Max (Years) | Med (Years) |
|---|---|---|---|---|---|
| 10. Stands balanced on one foot for at least one second | Has the skill[a] | 4 | 5.0 | 12.3 | 8.9 |
| | FIRST lost the skill[b] | 3 | 8.9 | 20.8 | 13.3 |
| | Lost the skill[c] | 9 | 5.5 | 21.7 | 12.7 |
| 11. Smiles back in response to being smiled at or spoken to (but not touched) | Has the skill[a] | 15 | 5.0 | 21.7 | 12.3 |
| | FIRST lost the skill[b] | 1 | 20.4 | - | - |
| | Lost the skill[c] | 0 | - | - | - |
| 12. Can child indicate a want to parent without crying? Can include pointing, reaching, making sounds, moving arms, pulling, saying a word, others | Has the skill[a] | 14 | 5.0 | 21.7 | 11.7 |
| | FIRST lost the skill[b] | 2 | 17.6 | 20.4 | - |
| | Lost the skill[c] | 0 | - | - | - |
| 13. Waves 'bye-bye' when someone waves to him/her | Has the skill[a] | 10 | 5.0 | 16.3 | 10.6 |
| | FIRST lost the skill[b] | 3 | 16.6 | 20.4 | 16.7 |
| | Lost the skill[c] | 3 | 12.7 | 21.7 | 20.3 |
| 14. Feeds him/herself finger foods WITHOUT help | Has the skill[a] | 9 | 5.0 | 16.3 | 10.6 |
| | FIRST lost the skill[b] | 4 | 14.7 | 19.4 | 16.0 |
| | Lost the skill[c] | 3 | 11.4 | 21.7 | 20.3 |
| 15. Feeds him/herself with fork or spoon without help and gets most of the food into his/her mouth | Has the skill[a] | 9 | 5.0 | 16.3 | 10.6 |
| | FIRST lost the skill[b] | 2 | 13.8 | 20.8 | - |
| | Lost the skill[c] | 5 | 11.4 | 21.7 | 15.6 |
| 16. Vocalizes - small throaty sounds or short vowel sounds (crying does not count) | Has the skill[a] | 14 | 5.0 | 21.7 | 11.7 |
| | FIRST lost the skill[b] | 2 | 13.8 | 16.7 | - |
| | Lost the skill[c] | 0 | - | - | - |

**Table 2 Continued**

*Scatter Plot Statistics for Regression of Early Developmental Milestones (1-20)*

| PLANready Milestone | Category | Sub (N) | Min (Years) | Max (Years) | Med (Years) |
|---|---|---|---|---|---|
| 17. Laughs out loud | Has the skill[a] | 14 | 5.0 | 21.7 | 11.7 |
| | FIRST lost the skill[b] | 2 | 14.5 | 17.6 | - |
| | Lost the skill[c] | 0 | - | - | - |
| 18. Makes single syllable sounds with consonant and vowel like 'ga', 'ma', or 'ba' | Has the skill[a] | 11 | 5.0 | 21.7 | 10.6 |
| | FIRST lost the skill[b] | 4 | 13.8 | 20.4 | 17.1 |
| | Lost the skill[c] | 1 | 20.3 | - | - |
| 19. Uses 2 words other than mama, dada, or the names of other family or pets | Has the skill[a] | 12 | 5.0 | 21.7 | 10.9 |
| | FIRST lost the skill[b] | 2 | 17.6 | 20.4 | - |
| | Lost the skill[c] | 2 | 12.7 | 20.3 | - |
| 20. Combines at least 2 words to make a meaningful phrase that indicates an action, like 'play ball' or 'want drink' or 'go bye-bye' | Has the skill[a] | 12 | 5.0 | 21.7 | 10.9 |
| | FIRST lost the skill[b] | 2 | 17.6 | 20.4 | - |
| | Lost the skill[c] | 2 | 12.7 | 20.3 | - |

Sub, subjects; Min, minimum; Max, maximum; Med, median
[a] Age at last visit
[b] Age at loss
[c] Age at enrollment

**Table 3**

*Scatter Plot Statistics for Regression of Early Developmental Sub-Milestone 3*

| PLANready Sub-Milestone | Category | Sub (N) | Min (Years) | Max (Years) | Med (Years) |
|---|---|---|---|---|---|
| How does your child pick up small objects? | Pincer[a] | 5 | 5.0 | 12.3 | 10.6 |
| | FIRST rake[b] | 4 | 6.3 | 18.9 | 15.1 |
| | Rake[c] | 3 | 7.2 | 11.4 | 10.6 |

Sub, subject; Min, minimum; Max, maximum; Med, median
[a] Age at last visit
[b] Age at transition
[c] Age at enrollment

**Table 4**

*Scatter Plot Statistics for Progression of Disease-Related Milestones (21-31)*

| PLANready Milestone | Category | Sub (N) | Min (Years) | Max (Years) | Med (Years) |
|---|---|---|---|---|---|
| 21. Uses wheelchair/stroller | No[a] | 4 | 5.0 | 12.3 | 8.9 |
| | Yes[b] | 1 | 8.4 | - | - |
| | Yes[c] | 11 | 5.5 | 21.7 | 12.8 |
| 22. Falls frequently while walking (at least once/day) | No[a] | 2 | 10.6 | 12.3 | - |
| | Yes[b] | 0 | - | - | - |
| | Yes[c] | 3 | 3.5 | 7.4 | 7.2 |
| 23. Has seizures (or treated for them) | No[a] | 9 | 5.0 | 21.7 | 11.1 |
| | Yes[b] | 2 | 15.8 | 15.8 | - |
| | Yes[c] | 5 | 5.9 | 20.3 | 15.6 |
| 24. Requires diet modifications (soft diet, blending, dicing, thickening liquids, etc.) | No[a] | 8 | 5.0 | 17.7 | 10.9 |
| | Yes[b] | 3 | 8.4 | 17.4 | 15.3 |
| | Yes[c] | 5 | 5.5 | 21.7 | 15.6 |
| 25. Has swallowing difficulty / coughing with foods or liquids | No[a] | 7 | 5.0 | 16.3 | 10.6 |
| | Yes[b] | 4 | 6.4 | 15.8 | 11.4 |
| | Yes[c] | 5 | 11.4 | 21.7 | 16.9 |

**Table 4 Continued**

*Scatter Plot Statistics for Progression of Disease-Related Milestones (21-31)*

| PLANready Milestone | Category | Sub (N) | Min (Years) | Max (Years) | Med (Years) |
|---|---|---|---|---|---|
| 26. Has a gastrostomy tube (feeding tube) | No[a] | 11 | 5.0 | 20.8 | 11.1 |
| | Yes[b] | 4 | 8.4 | 20.9 | 15.9 |
| | Yes[c] | 1 | 21.7 | - | - |
| 27. Has optic nerve atrophy or 'pale' optic nerve | No[a] | 13 | 5.0 | 21.7 | 11.1 |
| | Yes[b] | 2 | 10.3 | 13.3 | - |
| | Yes[c] | 1 | 15.6 | - | - |
| 28. Has constipation (or uses treatments for it) | No[a] | 3 | 7.3 | 16.3 | 12.3 |
| | Yes[b] | 0 | - | - | - |
| | Yes[c] | 13 | 3.5 | 21.7 | 11.4 |
| 29. Has gastroesophageal reflux (or uses treatments for it) | No[a] | 10 | 5.0 | 20.8 | 11.7 |
| | Yes[b] | 4 | 5.8 | 19.4 | 11.9 |
| | Yes[c] | 2 | 15.6 | 21.7 | - |
| 30. Has a tracheostomy | No[a] | 16 | 5.0 | 21.7 | 12.6 |
| | Yes[b] | 0 | - | - | - |
| | Yes[c] | 0 | - | - | - |
| 31. Has abnormal autonomic function (such as cold hands/feet or sudden changes in core body temperature) | No[a] | 3 | 5.0 | 12.3 | 10.6 |
| | Yes[b] | 0 | - | - | - |
| | Yes[c] | 13 | 5.5 | 21.7 | 12.7 |

Sub, subject; Min, minimum; Max, maximum; Med, median
[a] Age at last visit
[b] Age at transition
[c] Age at enrollment

### 3.1.2.2. Longitudinal Plots

With longitudinal plots (e.g., Figure 4), each subject was represented by a unique color, and each dot corresponds to a study visit, indicating the subject's age at that point in time. For the early developmental milestones (1-20), the presence of a skill was denoted by a dot at the top of the plot, while the absence of a skill was represented by a dot at the bottom. If there was a real-time observation of regression in a motor skill, the transition was indicated with a negative slope. However, during subsequent visits, if the subject reported fluctuations in the regression of that motor skill, it was represented with a positive slope. An example was subject 66 whom initially reported regression of milestone 1 at 17.2 years, but at their next visit at 17.7 years, they reported that they had this skill. Detailed fluctuations for all PLANready milestones are available in Appendix H.

**Figure 4**

*Longitudinal Plot—Regression of Milestone 1*
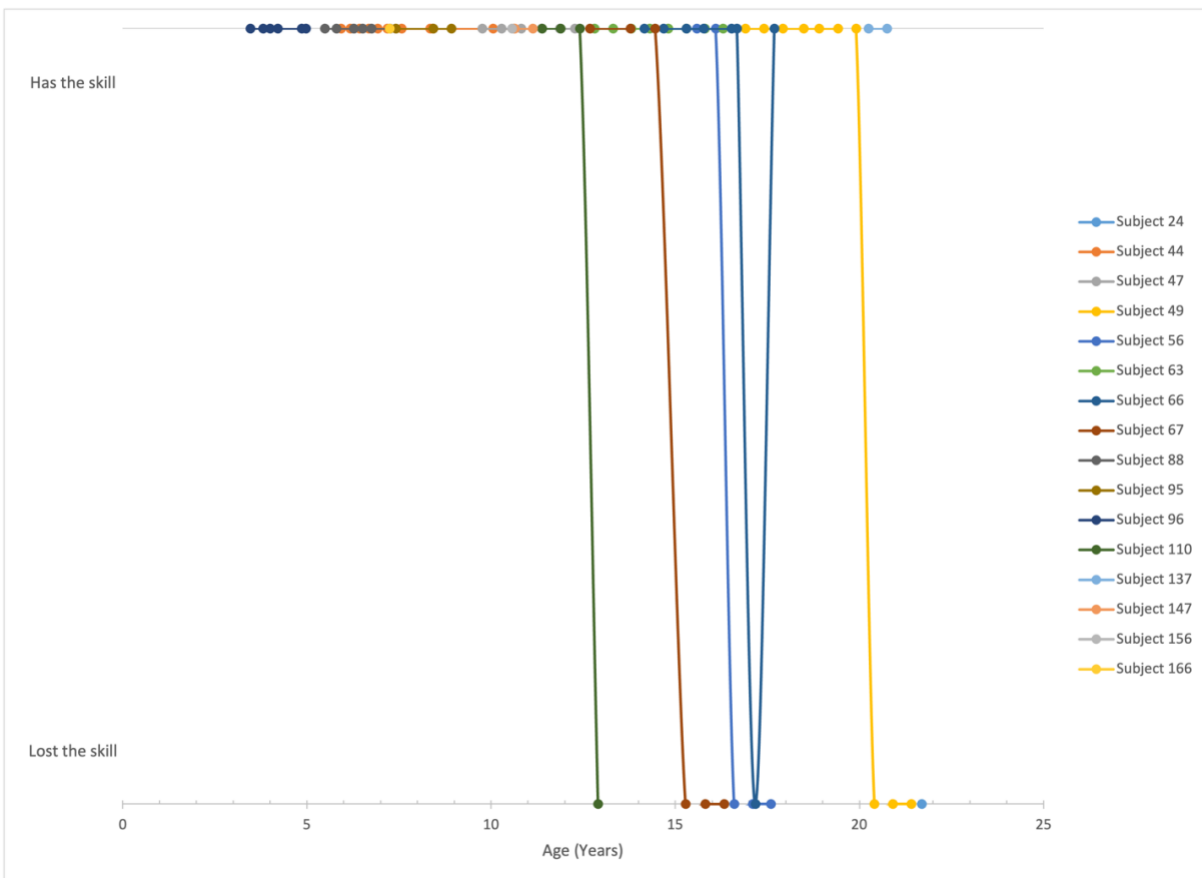


*Note.* Milestone 1—'Grasps a toy or other object when it is touched to the backs or tips of the fingers'. Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 1, and a positive slope indicates fluctuations in regression (Table 9).

For the disease-related milestones (21-31), such as reaching the point of first wheelchair use, if captured in real-time, the progression of the disorder was shown with a negative slope and fluctuations were represented with a positive slope.

### 3.1.2.3. Kaplan-Meier Curves

Kaplan-Meier curves include vertical drops that represent regression of an early developmental milestone (1-20), or progression of a disease-related milestones (21-31) captured in real-time relative to the subject's age. A detailed review of Figure 5 reveals that all individuals appear to retain this motor skill until the age of 12.9 years. The retention rate declines to 86% between 12.9 to 15.3 years, and further declines to 71% from 15.3 to 16.6 years, and so forth (Table 5). A detailed analysis for each milestone's Kaplan-Meier curve can be found in Appendix H. Enrollment data has been omitted from the dataset to avoid left-censored data. Red rectangles demonstrate the age of an individual at their most recent study visit who have never reported loss of a particular motor skill. A blue 'x' on the x-axis correlates with a vertical drop in the curve, indicating the subject's loss of a motor skill in real-time, however, in subsequent visits, that subject reported fluctuations in the loss of that motor skill (Appendix H). Tables 6 and 7 present Kaplan-Meier curve descriptive statistics for regression of early developmental milestones, and progression of disease-related milestones, respectively.

**Table 5**

*Kaplan-Meier Curve Analysis—Regression of Milestone 1*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 12.9 | 100 |
| 12.9 | to | 15.3 | 86 |
| 15.3 | to | 16.6 | 71 |
| 16.6 | to | 17.2 | 54 |
| 17.2 | to | 20.4 | 36 |
| 20.4 | to | 20.7 | 18 |

**Figure 5**

*Kaplan-Meier Curve—Regression of Milestone 1*
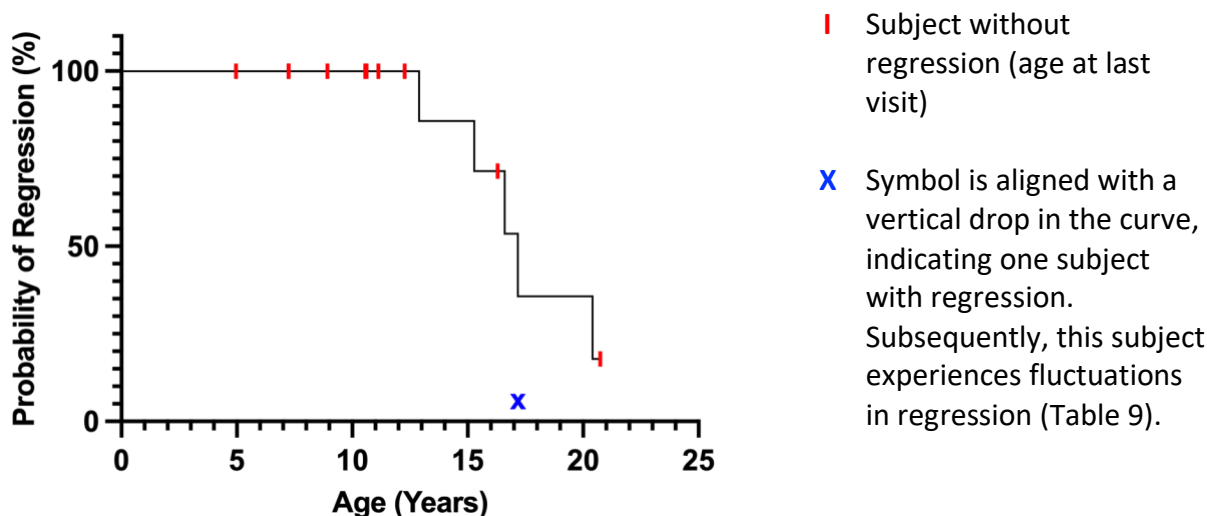


| | Subject without regression (age at last visit) |
|---|---|
| **X** | Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Table 9). |

*Note.* Milestone 1—'Grasps a toy or other object when it is touched to the backs or tips of the fingers'. If present, a vertical drop in the solid black line represents real-time regression of milestone 1 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Tables 5 and 6.

**Table 6**

*Kaplan-Meier Curve Statistics for Regression of Early Developmental Milestones (1-20)*

| PLANready Milestone | Sub (N) | Reg (N) | Range (Years) | | Med (Years) | Fluc (N) |
|---|---|---|---|---|---|---|
| 1. Grasps a toy or other object when it is touched to the backs or tips of the fingers | 15 | 5 | 12.9 | 20.4 | 16.6 | 1 |
| 2. Reaches toward objects placed in front of him/her and tries to grab them | 15 | 2 | 16.6 | 20.4 | - | 0 |
| 3. Picks up small objects, like a Cheerio or raisin | 14 | 5 | 12.4 | 19.9 | 15.8 | 1 |

28

**Table 6 Continued**

*Kaplan-Meier Curve Statistics for Regression of Early Developmental Milestones (1-20)*

| PLANready Milestone | Sub (N) | Reg (N) | Range (Years) | | Med (Years) | Fluc (N) |
|---|---|---|---|---|---|---|
| 4. Holds head upright & steady for several seconds when held in sitting position, no bobbing action | 16 | 2 | 16.6 | 20.4 | - | 0 |
| 5. Rolls over independently | 10 | 0 | - | - | - | 0 |
| 6. Sits up alone for at least 5 seconds without help from another person | 13 | 2 | 16.6 | 16.7 | - | 1 |
| 7. Pulls self to stand without assistance | 10 | 2 | 11.9 | 13.3 | - | 0 |
| 8. Get into a sitting position by him/herself (from lying down or standing with support) | 9 | 0 | - | - | - | 0 |
| 9. Walks 5 steps independently (without support) | 6 | 1 | 11.1 | - | - | 0 |
| 10. Stands balanced on one foot for at least one second | 7 | 3 | 8.9 | 20.7 | 13.3 | 0 |
| 11. Smiles back in response to being smiled at or spoken to (but not touched) | 16 | 1 | 20.4 | - | - | 1 |
| 12. Can child indicate a want to parent without crying? Can include pointing, reaching, making sounds, moving arms, pulling, saying a word, others | 16 | 2 | 17.6 | 20.4 | - | 0 |
| 13. Waves 'bye-bye' when someone waves to him/her | 13 | 3 | 16.6 | 20.4 | 16.7 | 1 |

**Table 6 Continued**

*Kaplan-Meier Curve Statistics for Regression of Early Developmental Milestones (1-20)*

| PLANready Milestone | Sub (N) | Reg (N) | Range (Years) | | Med (Years) | Fluc (N) |
|---|---|---|---|---|---|---|
| 14. Feeds him/herself finger foods WITHOUT help | 13 | 4 | 14.7 | 19.4 | 16.0 | 1 |
| 15. Feeds him/herself with fork or spoon without help and gets most of the food into his/her mouth | 11 | 2 | 13.8 | 20.7 | - | 0 |
| 16. Vocalizes - small throaty sounds or short vowel sounds (crying does not count) | 16 | 2 | 13.8 | 16.7 | - | 2 |
| 17. Laughs out loud | 16 | 2 | 14.5 | 17.6 | - | 1 |
| 18. Makes single syllable sounds with consonant and vowel like 'ga', 'ma', or 'ba' | 15 | 4 | 13.8 | 20.4 | 17.1 | 3 |
| 19. Uses 2 words other than mama, dada, or the names of other family or pets | 14 | 2 | 17.6 | 20.4 | - | 0 |
| 20. Combines at least 2 words to make a meaningful phrase that indicates an action, like 'play ball' or 'want drink' or 'go bye-bye' | 14 | 2 | 17.6 | 20.4 | - | 0 |

Sub, subject; Reg, regression; Range, age range of regression; Med, median; Fluc, fluctuate

**Table 7**

*Kaplan-Meier Curve Statistics for Progression of Disease-Related Milestones (21-31)*

| PLANready Milestone | Sub (N) | Prog (N) | Range (Years) | | Med (Years) | Fluc (N) |
|---|---|---|---|---|---|---|
| 21. Uses wheelchair/stroller | 5 | 1 | 8.4 | - | - | 0 |

**Table 7 Continued**

*Kaplan-Meier Curve Statistics for Progression of Disease-Related Milestones (21-31)*

| PLANready Milestone | Sub (N) | Prog (N) | Range (Years) | | Med (Years) | Fluc (N) |
|---|---|---|---|---|---|---|
| 22. Falls frequently while walking (at least once/day) | 2 | 0 | - | - | - | 0 |
| 23. Has seizures (or treated for them) | 11 | 2 | 15.8 | 15.8 | - | 1 |
| 24. Requires diet modifications (soft diet, blending, dicing, thickening liquids, etc.) | 11 | 3 | 8.4 | 17.4 | 15.3 | 1 |
| 25. Has swallowing difficulty / coughing with foods or liquids | 11 | 4 | 6.4 | 15.8 | 11.3 | 2 |
| 26. Has a gastrostomy tube (feeding tube) | 15 | 4 | 8.4 | 20.9 | 15.9 | 0 |
| 27. Has optic nerve atrophy or 'pale' optic nerve | 15 | 2 | 10.3 | 13.3 | - | 2 |
| 28. Has constipation (or uses treatments for it) | 3 | 0 | - | - | - | 0 |
| 29. Has gastroesophageal reflux (or uses treatments for it) | 14 | 4 | 5.8 | 19.4 | 11.9 | 2 |
| 30. Has a tracheostomy | 16 | 0 | 5.0 | 21.7 | - | 0 |
| 31. Has abnormal autonomic function (such as cold hands/feet or sudden changes in core body temperature) | 3 | 0 | 0.0 | 0.0 | - | 0 |

Sub, subject; Prog, progression; Range, age range of regression; Med, median; Fluc, fluctuate

### 3.1.2.4. Grouped Columns

In grouped columns (e.g., Figure 6), for any particular milestone, if a subject reported no regression or progression, it was assumed they had not experienced regression or progression prior to visit 1 and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 were marked as 'no regression/progression'). If a subject reported regression or progression, their previous status was marked as 'unknown' due to the uncertainty about when it had initially occurred (e.g., visit 1 occurred at age 10, ages 1-9 were marked as 'unknown').

The number of participants at each time point shown on these bar graphs was not always equal to 16, as indicated in Table 8, because not all individuals in the study have reached that particular age. The number of participants varies based on the age group being considered.

**Figure 6**

*Grouped Columns—Regression of Milestone 1*
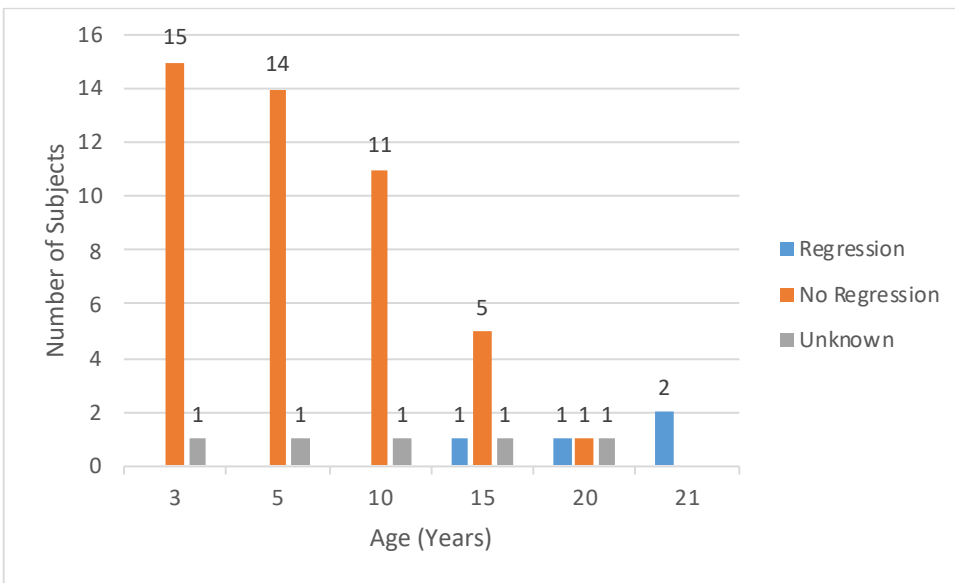


*Note.* At visit 1, if a subject reported no regression to milestone 1, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Table 8**

*PLANready Grouped Columns*

| Age Group (Years) | Subjects[a] (N) |
|:---:|:---:|
| 3 | 16 |
| 5 | 15 |
| 10 | 12 |
| 15 | 7 |
| 20 | 3 |
| 21 | 2 |

[a] Not all individuals in the study have reached certain ages, leading to unequal participant counts.

Grouped columns enable us to examine specific time points and assess whether a particular skill was present or absent at a specific age. The focus was on determining the presence or absence of the skill at each age, rather than identifying the exact age at which a subject first lost the skill.

### 3.1.2.5. Summary of Prospective Data

Two graphs provide a high-level overview for regression of early developmental milestones or progression of disease-related milestones, emphasizing medians when available. In Figure 7, milestones 1-20, the medians for individuals with the skill (green circles) were organized in ascending order. To the right of each milestone, a red circle indicates real-time skill loss (i.e., first lost the skill), and a yellow circle represents loss captured at enrollment (i.e., lost the skill), with a preference for real-time loss. Absence of circles indicates a sample size for skill

loss (real-time or enrollment data) less than three, and these instances have been excluded

from the graph.

**Figure 7**

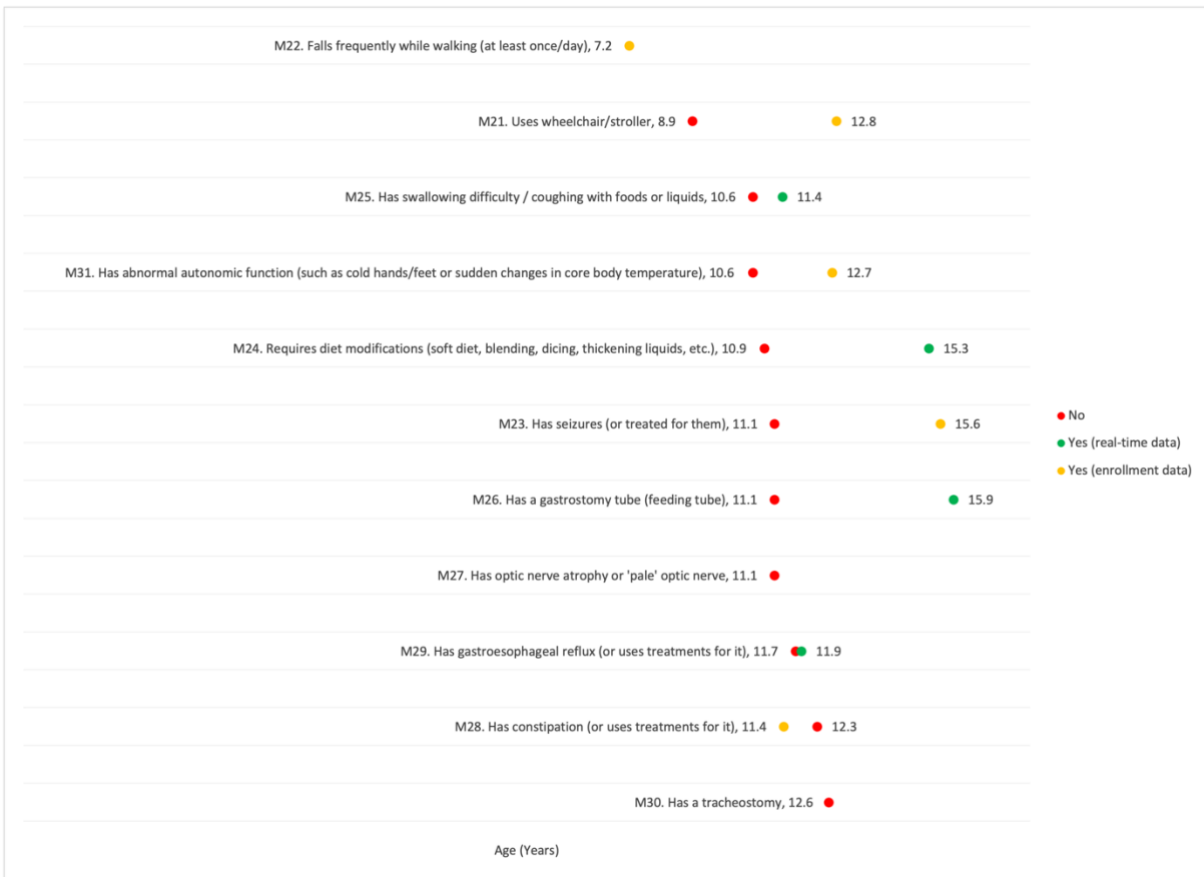*A Summary of PLANready Milestones with Medians 1-20*



*Note.* The foundation of the graph is built upon the organization of medians for 'has the skill', arranged in ascending order (green circles). To the right, the median for 'first lost the skill' is represented by red circles, or 'lost the skill' by yellow circles, with a preference for 'first lost the skill' as it signifies real-time data, in contrast to enrollment data.

In Figure 8, milestones 21-31, medians for individuals not experiencing these disease-

related milestones (red circles) were organized in ascending order. The red circles are

accompanied by a green circle or yellow circle, representing the median age for onset of a

disease-related feature, where green circles represent real-time data, and yellow circles

represent enrollment data, with a preference for real-time data. If a circle is absent, it indicates

that the sample size for the onset (real-time or enrollment data) was less than three.

**Figure 8**

*A Summary of PLANready Milestones with Medians 21-31*



*Note.* The foundation of the graph is built upon the organization of medians for 'no',
arranged in ascending order (red circles), except for milestone 22, where there is no median
in the 'no' category. To the right or left, the median for 'Yes (real-time data)' is represented
by green circles, or 'Yes (enrollment data)' by yellow circles, with a preference for 'Yes (real-
time data)'.

### 3.1.3. Data Fluctuations

Fluctuations in regression were observed in 10 out of 20 early developmental milestones, and were reported by three participants: subjects 49, 66, and 67. A similar pattern was noticed in seven out of 11 disease-related milestones, and was reported by seven participants: subjects 44, 47, 63, 66, 67, 88, and 96. For example (Table 9), subject 66 first reported loss of the ability to grasp objects (milestone 1, or M01) at 17.2 years, but reported having the ability at 17.7 years. A comprehensive review of fluctuations for each milestone is provided in Appendix H.

**Table 9**

*Fluctuations—Regression of Milestone 1*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 66      | 1     | 14.2        | No         |
|         | 2     | 14.7        | No         |
|         | 3     | 15.3        | No         |
|         | 4     | 15.8        | No         |
|         | 5     | 16.5        | No         |
|         | 6     | 16.7        | No         |
|         | 7     | 17.2        | Yes        |
|         | 8     | 17.7        | No         |

### 3.1.4. Regression

Out of the 16 participants in PLANready, 13 participated in follow-up visits (visits 2, 3, 4, etc.). During these follow-up visits, it was asked whether the individual with PLAN had experienced regression since the last online study visit. Among the participants, 12 reported

experiencing regression at one or more follow-up visits, indicating a recurrent pattern of regression. Only one subject consistently responded 'no' to regression during all their follow-up visits.

For participants who reported experiencing regression ('yes' responses), they were given an open text field to describe their symptoms. These responses were carefully edited for length and clarity. Each symptom was then categorized into one of 10 domains: motor skills (Table 10), speech (Table 11), appetite / swallowing / feeding tube (Table 12), genitourinary (Table 13), tremors (Table 14), behavior (Table 15), cognition (Table 16), eyes (Table 17), seizures (Table 18), and other (Table 19). Each corresponding table provides a detailed account of the symptoms the subjects were encountering.

In Figure 9, the first row, represented by blue dots, accounts for 64 return visits involving 13 subjects. Among these 64 visits, the second row of orange dots represents a subject answering 'yes' to experiencing regression since the last study visit. The third row of gray dots represents a subject responding 'no' to experiencing regression since the last study visit. Furthermore, the ten domains were organized in ascending order based on the earliest reported instance of regression within each respective domain. For instance, in the case of motor skills, the first dot appears at 4.0 years. In contrast, for seizures, the first dot emerges at 16.3 years. This organization allows for a clear visualization of regression or progression within each domain over time.

**Figure 9**

*Free-Text Responses on Regression Organized into Data Points by Domain*
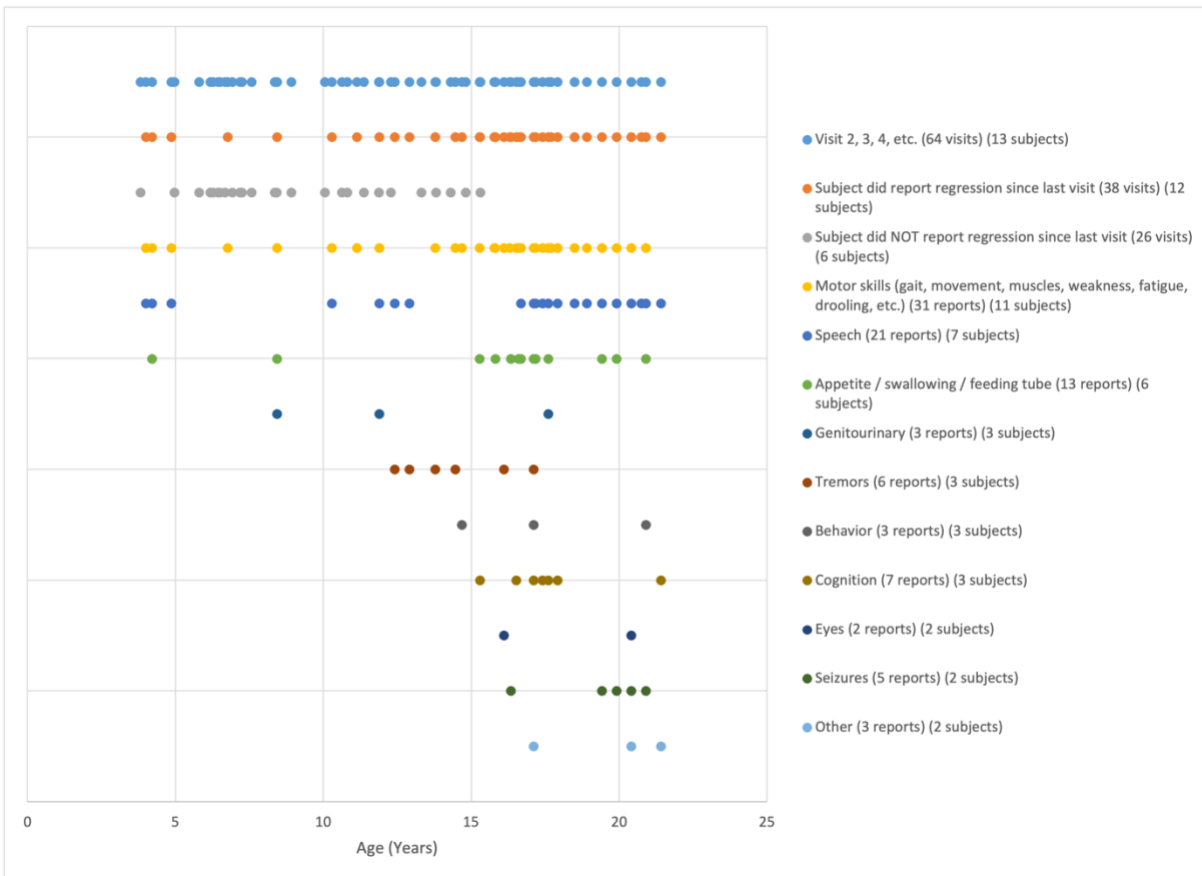


*Note.* During visits 2, 3, 4, etc., participants were asked about experiencing regression since their last visit, with response options of yes or no, and were provided with an open text field to elaborate on their regression if applicable. The first row, blue dots, corresponds to visits 2, 3, 4, etc. (64 visits across 13 subjects). The second row, orange dots, corresponds to visits where participants reported regression (38 visits across 12 subjects), whereas the third row, gray dots, corresponds to visits where participants reported no regression (26 visits across 6 subjects). The subsequent rows (rows 4 to 13) organize these regression dots by domain; row 4 represents visits where a participant reported regression in motor skills, row 5 encompasses visits with reported regression in speech, and so forth.

**Table 10**

*Free-Text Responses Regarding Regression—Motor Skills*

| Age (Years) | Motor Skills[a] (31 Reports) (11 Subjects) | Related Milestone(s) |
|---|---|---|
| 4.0 | Starting to trip and bump into things a little more. | |
| 4.2 | Tripping and **falling** more often. | M22 |
| 4.9 | A little in gross motor and fatigues quicker. | |
| 6.8 | Now uses hands to move around legs. | |
| 8.4 | Mobility, **walking**. | M09 |
| 10.3 | Muscles in feet are tighter. | |
| 11.1 | More difficulty with **walking**. Increased weakness in legs. | M09 |
| 11.9 | Use of **walking frame** has become too difficult. | M09 |
| 13.8 | Slight regression in motor skills. | |
| 14.5 | Much more spastic in upper extremities. | |
| 14.7 | Increased fatigue, movements have increased. | |
| 15.3 | Motor skills have declined. | |
| 15.8 | Slightly weaker, needs an afternoon nap. | |
| 15.8 | Decreased ability to navigate stairs. Stair lift installed. More difficulty transitioning from the floor to couch, needs assistance from a parent. | |
| 16.1 | Stopped using hands freely, they are now in a claw-like state, keeps them tucked up against body. Overall body stiffness. | |
| 16.3 | More trouble turning around while walking, even with the assistance of a walker. Has become more reliant on **wheelchair** for transitions such as switching seats or changing travel direction. | M21 |
| 16.5 | Muscle strength. | |
| 16.6 | Arms now non-functioning. Mouth action is non-stop (i.e., tardive dyskinesia). | |
| 16.7 | Much weaker, drooling a bit more. | |
| 17.1 | Loss of arm control, increased arm spasms. | |
| 17.2 | Tired. | |
| 17.4 | Increased right side weakness. | |
| 17.6 | Dystonia has moved to include neck which twists head painfully back. Arms and hands now completely useless. | |
| 17.7 | Getting a bit weaker and tires easily, drooling when tired. | |
| 17.9 | Gradual decline in gross and fine motor skills, **core strength**. | M06 |
| 18.5 | Slower motor skills. **Loss of core strength when seated.** | M06 |
| 18.9 | Reduced energy. | |

**Table 10 Continued**

*Free-Text Responses Regarding Regression—Motor Skills*

| Age (Years) | Motor Skills[a] (31 Reports) (11 Subjects) | Related Milestone(s) |
|---|---|---|
| 19.4 | General weakness. | |
| 19.9 | Increased dystonia. | |
| 20.4 | **Loss of head control.** Increased dystonia. | M04 |
| 20.9 | A little independent movement of head and limbs. | |

*Note.* Participants' responses were carefully edited for length and clarity.

[a] Includes gait, movement, muscles, weakness, fatigue, drooling, etc.

**Table 11**

*Free-Text Responses Regarding Regression—Speech*

| Age (Years) | Speech (21 Reports) (7 Subjects) | Related Milestone(s) |
|---|---|---|
| 4.0 | Struggling with speech a little more, getting 'stuck' with words, especially when excited. | |
| 4.2 | Speech stuttering. | |
| 4.9 | A little in speech. | |
| 10.3 | Increased slurring. | |
| 11.9 | Speech has deteriorated. | |
| 12.4 | Speech, in volume and clarity has deteriorated. | |
| 12.9 | Speech has deteriorated further. | |
| 16.7 | Speech harder to understand. | |
| 17.1 | Ability to speak has decreased markedly. | |
| 17.2 | Slurred speech. | |
| 17.4 | Speech delays. | |
| 17.6 | Ability to verbalize has decreased and can now only make **throaty sounds**. | M16 |
| 17.9 | Gradual decline in speech. | |
| 18.5 | Slower speech. | |
| 18.9 | Reduced speech. | |
| 19.4 | Problems with speech. | |
| 19.9 | Speech loss. | |
| 20.4 | Loss of speech. | |
| 20.7 | Further loss of speech, **can only make sounds**. | M16, M18 |
| 20.9 | No longer verbal. | |

**Table 11 Continued**

*Free-Text Responses Regarding Regression—Speech*

| Age (Years) | Speech (21 Reports) (7 Subjects) | Related Milestone(s) |
|---|---|---|
| 21.4 | Loss of speech. | |

*Note.* Participants' responses were carefully edited for length and clarity.

**Table 12**

*Free-Text Responses Regarding Regression—Appetite / Swallowing / Feeding Tube*

| Age (Years) | Appetite / Swallowing / Feeding tube (13 Reports) (6 Subjects) | Related Milestone(s) |
|---|---|---|
| 4.2 | Less appetite. | |
| 8.4 | Eating. | |
| 15.3 | **Swallowing difficulties.** | M25 |
| 15.8 | Now has **trouble swallowing, button placed for nutrition.** Still eats by mouth but it must be **soft foods**. | M24, M25, M26 |
| 16.3 | Slight **regression with swallowing**. Has **gastrostomy tube** to supplement nutrition, cannot take calories by mouth. | M25, M26 |
| 16.6 | Not sure if **PEG was inserted** since last assessment but there is one now. | M26 |
| 16.7 | Not eating as much. | |
| 17.1 | Almost totally reliant on nutritional supplements via **PEG tube**. | M26 |
| 17.2 | A little trouble eating. | |
| 17.6 | **Unable to manage anything by mouth, coughs, and chokes on oral secretions.** | M25 |
| 19.4 | **Problems with swallow.** | M25 |
| 19.9 | **Compromised swallow.** | M25 |
| 20.9 | **Compromised swallowing.** | M25 |

*Note.* Participants' responses were carefully edited for length and clarity.

**Table 13**

*Free-Text Responses Regarding Regression—Genitourinary*

| Age (Years) | Gastrointestinal (3 Reports) (3 Subjects) | Related Milestone(s) |
|---|---|---|
| 8.4 | Bathroom accidents. | |
| 11.9 | **Bowel movement much less regular.** | <u>M28</u> |
| 17.6 | Unable to urinate by self and is catheterized. | |

*Note.* Participants' responses were carefully edited for length and clarity.

**Table 14**

*Free-Text Responses Regarding Regression—Tremors*

| Age (Years) | Tremors (6 Reports) (3 Subjects) | Related Milestone(s) |
|---|---|---|
| 12.4 | Tremor in both arms has increased. | |
| 12.9 | Tremor in left hand has increased, cannot use left hand to help with standing transfers. | |
| 13.8 | Periodic shaking in the hands. | |
| 14.5 | Started having Parkinson-like tremors. | |
| 16.1 | Head tremors have increased. | |
| 17.1 | Tremors. | |

*Note.* Participants' responses were carefully edited for length and clarity.

**Table 15**

*Free-Text Responses Regarding Regression—Behavior*

| Age (Years) | Behavior (3 Reports) (3 Subjects) | Related Milestone(s) |
|---|---|---|
| 14.7 | A bit more irritable and depressed. | |
| 17.1 | Agitation. | |
| 20.9 | Regressed rapidly over the last six months. | |

*Note.* Participants' responses were carefully edited for length and clarity.

**Table 16**

*Free-Text Responses Regarding Regression—Cognition*

| Age (Years) | Cognition (7 Reports) (3 Subjects) | Related Milestone(s) |
|---|---|---|
| 15.3 | Loss of memory with schoolwork (math, spelling). | |
| 16.5 | Cognition. | |
| 17.1 | Increase with dementia-like symptoms. | |
| 17.4 | Memory problems. | |
| 17.6 | State of alertness has decreased and is often in their own world. | |
| 17.9 | Gradual decline in working memory. | |
| 21.4 | Loss of awareness. | |

*Note.* Participants' responses were carefully edited for length and clarity.

**Table 17**

*Free-Text Responses Regarding Regression—Eyes*

| Age (Years) | Eyes (2 Reports) (2 Subjects) | Related Milestone(s) |
|---|---|---|
| 16.1 | Now legally blind after a **rapid degeneration of optic nerve**. | M27 |
| 20.4 | Light sensitivity. | |

*Note.* Participants' responses were carefully edited for length and clarity.

**Table 18**

*Free-Text Responses Regarding Regression—Seizures*

| Age (Years) | Seizures (5 Reports) (2 Subjects) | Related Milestone(s) |
|---|---|---|
| 16.3 | A couple of **seizures** six months ago. | M23 |
| 19.4 | Increased **seizure** activity. | M23 |
| 19.9 | Increased **seizures**. | M23 |
| 20.4 | Increased **seizures**. | M23 |

**Table 18 Continued**

*Free-Text Responses Regarding Regression—Seizures*

| Age (Years) | Seizures (5 Reports) (2 Subjects) | Related Milestone(s) |
|---|---|---|
| 20.9 | Increased **seizure** activity which became difficult to control with medication. | M23 |

*Note.* Participants' responses were carefully edited for length and clarity.

**Table 19**

*Free-Text Responses Regarding Regression—Other*

| Age (Years) | Other (3 Reports) (2 Subjects) | Related Milestone(s) |
|---|---|---|
| 17.1 | Increasing and distressing symptoms. | |
| 20.4 | Rapid progression this year. Nonspecific pain. | |
| 21.4 | Skin and gum deterioration. | |

*Note.* Participants' responses were carefully edited for length and clarity.

## 3.2. Preliminary Retrospective Data

A review of the medical records for eight participants highlighted the diagnostic challenges of juvenile PLAN, particularly, the variability in the initial presentation of features, and the diagnostic odyssey. Below is a preliminary report summarizing findings on two participants.

For subject 147, his parents initially became concerned at 3.5 years due a noticeable change in behavior. His atypical behavior included panic attacks characterized by yelling, screaming, and crying, along with insomnia, episodic hyperventilation featuring stereotyped patterns, and socially inappropriate behavior, such as blurting out irrelevant remarks. By 4.0

years, he received diagnoses of anxiety, autism, and attention deficit hyperactivity disorder, leading to interventions involving medication and therapy, although the latter proved ineffective. In kindergarten, he was diagnosed with learning disabilities (NOS).

While subject 147's phenotype expands to encompass motor symptoms, as outlined below, it aligns with previous cases documented in the literature (Cif et al., 2014; Erro et al., 2016). These reports highlight a recurring pattern where individuals initially manifest a neuropsychiatric phenotype.

His parents observed the onset of balance issues, dragging both feet, and falls, prompting a brain MRI at 6.5 years, initially reported as normal. However, a second opinion at 6.9 years on the brain MRI revealed cerebellar atrophy. The accompanying physical examination noted gait disturbance, presenting as both tip-toe and broad-based gaits, lower limb spasticity and hyperreflexia, fine motor deterioration, intention tremor, and abnormal saccadic eye movements. Trio whole exome sequencing (WES) identified compound heterozygous variants of uncertain significance (VUS) (c.1634A>G, p.Lys545Arg; c.1942G>A, p.Gly648Arg) in *PLA2G6*, but due to the absence of progressive visual loss and iron accumulation in the brain, a diagnosis of juvenile PLAN was not confirmed.

At 8.5 years, the classification of p.Lys545Arg was upgraded to likely pathogenic, yet the diagnosis of juvenile PLAN remained uncertain. The condition progressed, with the emergence of dysarthria and lower limb muscle weakness at 8.6 years, prompting the use of a walker at 8.9 years. At 10.5 years, trio WES was pursued at a second laboratory for a second opinion on the remaining *PLA2G6* variant classified as a VUS and to identify other potential contributing

variants. A repeat brain MRI was ordered, revealing iron accumulation, and subsequently confirming the diagnosis of juvenile PLAN.

Subject 63 followed a different trajectory with the onset of symptoms, which were limited to motor impairments. Her initial referral to a muscular dystrophy clinic occurred at 5.3 years, prompted by mildly elevated creatine kinase levels. Documentation at 5.5 years included a waddling and unsteady gait, falls, hyporeflexia in the upper extremities, areflexia in the lower extremities, and weakness in the neck flexors, elbow flexors, and quadriceps. Over the next four months, there was a progression involving limited ankle dorsiflexion and ankle contracture(s), ultimately leading to a working diagnosis of myopathy.

At 6.2 years, generalized low muscle tone was noted, and discussions regarding a wheelchair evaluation were underway. Elevated aspartate aminotransferase (consistent with previous report in Romani et al., 2015), alanine aminotransferase, and alkaline phosphatase levels were documented at 6.9 years. A tip-toe gait manifested at 7.0 years, and a subsequent brain MRI at 7.1 years showed normal results.

There was a gap in the medical record spanning from 7.1 to 13.0 years. However, at 13.0 years, subject 63 was documented as using walker. By 13.2 years, she was reluctant to use a wheelchair at school. To manage the risk of falls, she opted for hand-in-hand ambulation with her peers. Remarkably, aside from anxiety, her symptoms seem to be confined to the loss of gross motor skills.

# 4. DISCUSSION

The objective of this study was to utilize two distinct datasets—prospective (n=16) and retrospective (n=8)—comprising a total of 16 participants with *PLA2G6* mutations, whose clinical presentations most closely aligned with juvenile PLAN, contributing to a better understanding of its natural history.

## 4.1. Prospective Data

In the context of juvenile PLAN, it was expected that individuals would initially achieve early developmental milestones, followed by regression of these early developmental milestones and progression of disease-related milestones. The primary objective of PLANready was to capture these transitions in real-time to better describe the natural history of juvenile PLAN. This analysis focuses on the scatter plot data presented in Tables 2-4.

The descriptive statistics for regression or progression of milestones were referred to as enrollment data or real-time data. Enrollment data reflects regression or progression that occurred before visit 1, but the timing was unknown, resulting in these transitions being documented at an older age, whereas real-time data reflects regression or progression that occurred after visit 1, resulting in these transitions being documented at the age when they occurred.

The study generally observed a pattern where the medians for milestones followed an order from youngest to oldest:

- No regression or progression.

- Transition occurred after visit 1 (real-time data).

- Transition occurred before visit 1 (enrollment data).

However, this pattern was not observed when comparing real-time data and enrollment data for several milestones. For example, individuals that reported loss of the ability to stand balanced on one foot (milestone 10, or M10). The real-time median for regression of this milestone captured during the study (13.3 years) was greater than the median age at enrollment (12.7 years) for individuals who had already lost this milestone before enrolling. However, the real-time data only included three participants, whereas the enrollment data included nine participants. It is possible that comparable sample sizes would yield different results.

Another example pertains to the presence or absence of constipation (M28). The median age for individuals without constipation was 12.3 years (n=3), while the median age for those with constipation was 11.4 years (n=13). It is also important to consider the known variability in the presentation of juvenile PLAN with this milestone. Given that not everyone develops the same features of the condition, some individuals may never experience constipation. As they age, the median age for those without constipation would naturally increase as well.

The remainder of the discussion focuses on milestones categorized within their respective domains such as gross motor skills, fine motor skills, speech/social/communication skills, and other disease-related milestones. These milestones are organized in ascending order concerning their medians to observe clustering of milestones around similar ages.

### 4.1.1. Gross Motor Skills

This section focuses on enrollment data due to the larger sample sizes in comparison to real-time data. The pattern observed reveals a sequence of events, starting with frequent falls in childhood, using a wheelchair in adolescence, and, ultimately, the loss of stationary gross motor skills. The milestones under consideration are 4, 5, 6, 7, 8, 9, 10, 21, and 22.

Individuals started experiencing frequent falls at 7.2 years (M22, range 3.5-7.4 years, n=3). After a 5.5 year-gap occurred, the loss of gross motor skills persisted from 12.7 to 16.9 years:

- Loss of the ability to stand balanced on one foot at 12.7 years (M10, range 5.5-21.7 years, n=9).

- Wheelchair use at 12.8 years (M21, range 5.5-21.7 years, n=11).

- Loss of the ability to walk five steps at 13.5 years (M09, range 5.5-21.7 years, n=10).

- Loss of the ability to transition from lying down or standing to sitting at 15.6 years (M08, range 11.4-12.7 years, n=7).

- Loss of the ability to roll over at 16.3 years (M05, range 12.7-21.7 years, n=6).

- Loss of the ability to pull self to a standing position at 16.3 years (M07, range 12.7-21.7 years, n=6).

- Loss of the ability to sit at 16.9 (M06, range 12.7-21.7 years, n=3).

Despite the smaller sample sizes, comparisons were made between real-time medians and enrollment medians, revealing similar conclusions for both sets. For example, the loss of the ability to stand balanced on one foot, reported above at 12.7 years, showed loss of the

ability in real-time occurring at a relatively similar age of 13.3 years. Likewise, for loss of the

ability to sit, reported above at 16.9 years, two individuals reported loss of the ability in real-

time at 16.6 and 16.7 years. These cases highlight the similarities between enrollment data and

real-time data for certain milestones. In addition, Cif et al. (2014) described one individual who

experienced the loss of independent sitting at 15-years-old, further emphasizing the

consistency of these milestones.

There were a few milestones where the sample sizes were too small to calculate a real-

time median, meaning that there were very few participants that reached these milestones

during their participation in the study, such as loss of the ability to walk five steps, first use of a

wheelchair, or loss of the ability to pull self to stand. However, it was observed that the real-

time data points for these milestones were lower than the corresponding enrollment median

and the real-time data points fell within the enrollment range. The observation provides some

reassurance regarding the validity of utilizing the descriptive statistics available for enrollment

data when real-time data is limited.

For a few milestones, there were no instances of regression or progression captured in

real-time, such as the beginning of experiencing frequent falls, loss of the ability to transition

from lying down or standing to sitting, or loss of the ability to roll over. Therefore, the

enrollment data can serve as a foundational point that can be further refined over time as

individuals continue their participation in PLANready for the foreseeable future.

The final milestone involves the loss of the ability to hold the head upright (M04). This

milestone was the least impacted, with only two participants reporting regression (at 16.6 and

20.4 years); this was the latest age of onset of regression of the milestones evaluated in this study. In contrast, 14 participants, spanning ages 5.0-21.7 years, did not report regression for this milestone. However, 10 individuals were not within the age range of the two individuals who reported loss (10 individuals were younger than 16.6 years), and the remaining individuals were relatively within the same age range (17.2, 17.7, 20.7, and 21.7 years) as the individuals who lost the skill at 16.6 and 20.4 years.

### 4.1.2. Fine Motor Skills

In the analysis of fine motor skill regression, the focus was directed towards real-time median losses due to the larger sample sizes relative to enrollment median losses, except for milestone 15. The observed pattern suggests a natural sequence of regression, starting with tasks involving the fingers, then progressing to the hands, and finally extending to the upper extremities. The milestones under consideration are 1, 2, 3, sub-milestone 3, 13, 14, and 15, with medians clustering around 15.1-16.7 years:

- Loss of pincer grasp, transitioned to rake grasp at 15.1 years (sub-milestone 3, range 6.3-18.9 years, n=4).

- Loss of the ability to feed him/herself with a fork or spoon at 15.6 years (M15, range 11.4-21.7 years, n=5).

- Loss of the ability to pick up small objects at 15.8 years (M03, range 12.4-19.9 years, n=5).

- Loss of the ability to feed him/herself finger foods at 16.0 years (M14, range 14.7-19.4 years, n=14).

- Loss of the ability to grasp an object at 16.6 years (M01, range 12.9-20.4 years, n=5).

- Loss of the ability to wave 'bye-bye' at 16.7 years (M13, range 16.6-20.4 years, n=3).

- Loss of the ability to reach toward objects (M02) with two individuals in real-time at 16.6 and 20.4 years, and one individual at enrollment at 21.7 years.

Regarding loss of the ability to feed him/herself with a fork or spoon, the real-time data points were compared to the range of ages for those who had already lost this skill at the time of enrollment (i.e., enrollment range). Two individuals reported real-time loss of the ability at 13.8 and 20.8 years, and these ages fall within enrollment range of 11.4-21.7 years. This alignment provided confidence in the reliability of the enrollment data when real-time data was limited.

### 4.1.3. Speech / Social / Communication

The data focuses on individuals whose speech, social, and communication skills remained unaffected. Minimal participants experienced regression during the study (real-time data), and a few had already lost these skills at enrollment (enrollment data). Brief mentions will be made of participants reporting a loss of these abilities in real-time. Enrollment data will be referenced only when explicitly noted. Drawing conclusions from the limited cases of regression in speech, social, or communications skills was not feasible at present, and the focus remains on reviewing individual case studies.

The milestones considered include speech milestones 16, 18, 19, and 20, social milestones 11 and 17, and communication milestone 12. These data points will be presented in

ascending order of the median age within their respective domains at which these skills were last observed.

For the speech milestones, individuals had the ability to make single syllable sounds at 10.6 years (M18, range 5.0-21.7 years, n=11) with loss of the ability at 17.1 years (range 13.8-20.4 years, n=4). Individuals had the ability to use two words other than family or pet names (M19, range 5.0-21.7 years, n=12) and combine at least two words to make a meaningful phrase at 10.9 years (M20, range 5.0-21.7 years, n=12). Two individuals reported loss of these abilities at 17.6 and 20.4 years. Furthermore, two individuals reported loss of these abilities at enrollment at 12.7 years, signifying that loss of these abilities can occur even earlier than the data captured in real-time. Individuals had the ability to make vocalizations at 11.7 years (M16, range 5.0-21.7 years, n=14). Two individuals reported loss of the ability at 13.8 and 16.7 years.

One individual reported having the ability to perform all speech-related milestones one month before their passing at 21.8 years. In contrast, another participant reported loss of the ability to make single syllable sounds, to use two words other than family or pet names, and to combine at least two words to make a meaningful phrase, three months before their passing at 17.8 years.

For the social milestones, individuals had the ability to laugh out loud at 11.7 years (M17, range 5.0-21.7 years, n=14). Two individuals reported loss of the ability at 14.5 and 17.6 years. Individuals had the ability to smile back in response at 12.3 years (M11, range 5.0-21.7 years, n=15). One individual reported loss of the ability at 20.4 years.

For the communication milestone, individuals had the ability to indicate a want at 11.7 years (M12, range 5.0-21.7 years, n=14). Two individuals reported loss of the ability at 17.6 and 20.4 years.

### 4.1.4. Disease Milestones

When considering progression of disease-related milestones (23-31), three milestones (26, 27, and 29) saw a larger sample size of progression captured in real-time compared to the sample size of progression captured at enrollment. On the other hand, five milestones (23, 24, 25, 28, and 31) exhibited a larger sample size of progression at enrollment compared to what was captured in real-time. The subsequent discussion provides insights into each of these larger sample sizes (except for milestone 25 which will focus on the smaller sample size of data captured in real-time (n=4) since it was nearly identical to the sample size of data captured at enrollment (n=5)), arranged in ascending order based on median age.

Individuals reported swallowing difficulty and constipation at 11.4 years (M25, range 6.4-15.8 years, n=4; M28, range 3.5-21.7 years, n=13), gastroesophageal reflux at 11.9 years (M29, range 5.8-19.4 years, n=4), abnormal autonomic function at 12.7 years (M31, range 5.5-21.7 years, n=13), seizures and diet modifications at 15.6 years (M23, range 5.9-20.3 years, n=5; M24, range 5.5-21.7 years, n=5), and a gastrostomy/feeding tube at 15.9 years (M26, range 8.9-20.9 years, n=4).

For milestones related to seizures, diet modifications, constipation, and abnormal autonomic function where a larger sample size was seen in the data captured at enrollment, a comparison was conducted with the smaller real-time data to gauge their correlation. Among

individuals with seizures at enrollment, the median age was 15.6 years, similar to the age of onset for the two individuals who developed seizures in real-time during the study (15.8 years for both). Diet modifications had a median age at enrollment of 15.6 years, slightly higher than the median age in real-time at 15.3 years (range 8.4-17.4 years, n=3). While the availability of real-time data was limited for certain milestones, the minimal difference between capturing the age at enrollment, or the age in real-time provides reassurance within the enrollment data available to date. For individuals that either did or did not have constipation or abnormal autonomic function at enrollment, and their status remained the same for subsequent visits.

It was not possible to draw significant conclusions about optic nerve atrophy (M27) as two individuals reported onset in real-time at 10.3 and 13.3 years, and one individual reported the presence of this feature at enrollment (age at onset unknown) at 15.6 years. However, the majority of individuals, n=13, have not reported optic nerve atrophy.

Lastly, regarding a tracheostomy (M30), it is worth noting that a tracheostomy for palliative treatment is recommended as needed for cases of atypical neuroaxonal dystrophy (aNAD) / juvenile *PLA2G6*-associated neurodegeneration (PLAN) to reduce the risk of aspiration pneumonia (Gregory et al., 2008a). Interestingly, in PLANready, none of the participants or their proxies reported a tracheostomy intervention. This observation applies to both living (n=14) and deceased (n=2) participants.

If participants answered in the affirmative to having abnormal autonomic function, an open text field was provided to describe their symptoms. Of the 13 participants who reported autonomic symptoms, five exclusively reported cold symptoms, eight reported cold/hot

symptoms, which could occur either separately or simultaneously. However, none of the participants exclusively reported hot symptoms.

Cold symptoms included cold feet (n=12), cold legs (n=6), blue feet (n=3), blue legs (n=2), cold hands (n=5), blue hands (n=1), and swollen feet (n=1). Parents provided a variety of potential explanations, including poor circulation, heightened sensitivity to cold weather (or air conditioning), and prolonged periods of sitting. Notably, one individual experienced a drop in their core body temperature to 93-95 degrees F, which had necessitated a couple of hospitalizations. To relieve cold symptoms there were reports of vigorous rubbing, using an electric blanket, wearing compression socks / ankle foot orthotics, rest with feet up, etc. One subject was seemingly unaware of their cold extremities and complains of pain when their feet were splashed with warm water.

In relation to hot symptoms, there were various reports of hot/warm torso/extremities (n=8), sweating (n=2), flushing (n=2), and heat rashes (n=2). One subject's hot flushes appear to correlate with dystonia, particularly when their dystonia was severe. Another subject experiences hot feet that appear to be associated with eventful nights, characterized by factors like pain and sleep disruption. To relieve hot symptoms there were reports of sponge baths, wearing less clothes, using less blankets, short hair, etc.

Three subjects noted their intolerance to cold/hot weather. In contrast, two subjects reported having cold/hot symptoms regardless of the weather (e.g., having hot feet as a baby and not using socks even in winter).

Other features included a resting heart rate of 110 bpm (n=1), poor skin quality on toes and bruising under nails (n=1), and flares where face/scalp turn red, subject complains head hurts and scalp itches (n=1).

The autonomic features outlined above could potentially be shared characteristics among other individuals, however, the initial prompt was intentionally broad to gain a preliminary understanding of the symptoms that participants are encountering, with the intent of utilizing this information to guide future research studies.

### 4.1.5. Data Fluctuations

For some of these milestones, such as constipation (M28), it seems reasonable to expect that an individual may experience a period of constipation that resolves over time but then appears again at a later date. However, for other milestones, the observation of a fluctuation in the data for a participant raises the question about potential contributing factors such as a prior illness or hospitalization that might have influenced their symptoms, a new treatment, or a change in treatment such as a change in the dosage of a medication. Moreover, considering that parents might share the responsibility of completing the questionnaires from home, discrepancies in reported symptoms may arise. A comprehensive review of fluctuations for each milestone is provided in Appendix H.

### 4.1.6. Regression

At follow-up study visits, participants or their proxies were asked whether they had experienced regression since their last study visit, and if so, to provide a detailed description of their symptoms (Tables 10-19). For instance, prospective speech milestones primarily focused

on significant areas of speech loss, such as the loss of the ability to vocalize (M16), make single syllable sounds (M18), use two words other than family names or pet names (M19), or combine at least two words to make a meaningful phrase (M20). However, the information provided by participants or their proxies in Table 11 indicates that the onset of speech regression can occur earlier and may encompass features such as getting stuck, stuttering, slurring, reduced volume, slower speech, etc.

It is necessary to emphasize that the features represented here may have manifested at even earlier ages than indicated. However, the purpose of the regression question was not to determine when symptoms originally appeared but rather to highlight instances where a subject experienced an increase in the frequency or severity of their symptoms as shown in Figure 9. Additionally, it captures features in other domains that were not documented in PLANready, such as the genitourinary system (Table 13), tremors (Table 14), cognition (Table 16), etc.

Examining Figure 9, certain patterns emerge. There is a noticeable increase in the frequency of overlapping gray dots, indicating no regression, occurring between 6.2-7.2 years, which corresponds to three study participants. There is an overlap of orange dots, indicating regression, between 15.8-17.9. years, involving five participants. The last recorded instance of no regression was at 15.3 years. Participants responding 'yes' to regression from 15.8-21.4 years encompassed data from six participants.

Additionally, the responses from participants or their proxies included other features such as tremors (Table 14). It is possible that these symptoms may be shared across a broader group of participants, a point that could be explored in future research studies.

Some of the areas of regression were related to PLANready milestones (as noted in Tables 10-13, 17, and 18), but it is important to mention other changes as well. These details provide insight into the day-to-day experience of the disorder and how individuals and their families adapted. At 6.8 years, one individual began using their hands to move their legs. At 15.8 years, one individual was losing their ability to navigate stairs, necessitating the installation of a stair lift in their home. At 16.1 years, another individual stopped using their hands freely and maintained a claw-like posture, keeping their hands tucked against their body, and at 16.6 years, reported non-stop mouth actions due to tardive dyskinesia, etc.

Upon reviewing regression data points in five-year increments, notable patterns emerge. Between ages 5.0 and 9.9 years, out of 15 follow-up visits, two subjects reported regression (13%), while 13 subjects reported no regression (87%). Moving to the 10.0 to 14.9 years range with 18 follow-up visits, eight subjects reported regression (44%), and 10 reported no regression (56%). In the 15.0 to 19.9 years range, encompassing 22 follow-up visits, 21 subjects reported regression (95%), with only one reporting no regression (5%). Notably, from age 15.8 onwards, all 23 follow-up visits reported subjects experiencing regression.

**4.2. Preliminary Retrospective Data**

The descriptions of both subjects 63 and 147 offer valuable insights, underscoring the variability in juvenile PLAN and the challenges associated with this diagnosis, especially when case studies predominantly involve descriptions of a single participant. Understanding the

complexities of this disorder is challenging. To address this, a subsequent publication involving this larger cohort of eight individuals, aims to provide a comprehensive and succinct list of juvenile PLAN features, including information on frequency and the age at onset when available. The intention is to streamline this information to facilitate the diagnosis of juvenile PLAN and ultimately reduce the diagnostic odyssey faced by individuals with this rare disorder.

### 4.3. Summary of Data

The age range of individuals that participated in PLANready was 3.5 to 21.7 years. Two out of eight individuals passed at 17.8 and 21.8 years. When real-time data was limited for regression of a motor skill or progression of a disease-related milestone, enrollment data became a reliable source and is marked with an asterisk (*). The medians are organized in ascending order:

Frequent falls (M22*) occurred at least once per day at 7.2 years, the onset of swallowing difficulties (M25) or constipation (M28*) at 11.4 years, gastroesophageal reflux (M29) at 11.9 years, the presence of abnormal autonomic function (M31*) at 12.7 years, loss of gross motor skills (described below) from 12.7 to 16.9 years, wheelchair use at 12.8 years (M21*), loss of fine motor skills (described below) from 15.1 to 16.7 years. The onset of seizures (M23*) or diet modifications (M24*) occurred at 15.6 years, and a gastrostomy/feeding tube (M26) at 15.9 years. No one required a tracheostomy (M30).

The loss of gross motor skills clustering from 12.7 to 16.9 years include loss of the ability to stand balanced on one foot at 12.7 years (M10*), walk five steps at 13.5 years (M09*), transition from lying down to standing or sitting at 15.6 years (M08*), roll over at 16.3 years

(M05*), pull self to standing at 16.3 years (M07*), or sit at 16.9 years (M06*). Then, the loss of fine motor skills cluster from 15.1 to 16.7 years, and include the transition from pincer to rake grasp at 15.1 years (sub-milestone 3), loss of the ability to feed him/herself with a fork/spoon at 15.6 years (M15*), pick up small objects at 15.8 years (M03), feed him/herself finger foods at 16.0 years (M14), grasp an object at 16.6 years (M01), wave 'bye-bye' at 16.7 years (M13), or make single syllable sounds at 17.1 years (M18).

The remainder of the milestones focus on individual case studies due to the small sample sizes (n=0 to 2) of individuals affected. For example, most individuals can hold their head upright (M04*) or reach towards objects (M02). Other milestones with small sample sizes in regression of early developmental milestones or progression of other disease-related milestones were related to speech (M16, M19, M20), social (M11, M17), communication (M12), and optic atrophy (M27).

Interestingly, Cif et al. (2014) reported a singular case of hypothermia while Ma et al. (2019) and Toth-Bencsik et al. (2021) reported cases of autonomic nervous system dysfunction. In the PLANready study, a notable 13/16 study participants reported abnormal autonomic function. These abnormalities ranged from experiencing cold and/or hot extremities to a significant decrease in body temperature, necessitating hospitalization.

A noticeable uptrend in the frequency of individuals reporting regression at follow-up study visits was observed, particularly when reviewing the data in five-year intervals. Between 5.0 and 9.9 years, 13% reported regression, which then grew to 44% from 10.0 to 14.9 years, and further increased to 95% from 15.0 to 19.9 years. Notably, at 15.8 years and beyond, every

subject with follow-up visits at 6-month intervals reported regression, extending at least up to 21.4 years.

The inclusion of an open-text field for participants to describe their regression, captured additional features such as changes in behavior, cognition, dystonia, etc. These aspects will undergo further exploration in the subsequent in-depth retrospective analysis of medical records for eight of the 16 participants (a preliminary analysis was provided on page 45).

**4.4. Study Limitations**

While having 16 participants for an ultra-rare disorder is remarkable, a limitation lies in the relatively small sample size, compounded by the non-overlapping age ranges among participants. Each subject's engagement spanned between one and 11 study visits, with single visits offering static information and multiple visits enabling the measurement of disorder progression over time. The initial data serves as a foundation for future development. Ongoing participant enrollment and additional study visits will contribute to a more comprehensive dataset with overlapping ages, ultimately alleviating challenges in data interpretation.

**4.5. Conclusions**

The significance in the data derived from PLANready provides a better understanding of the frequency and age at onset of features, ensuring that families obtain an accurate diagnosis at an earlier stage. This information empowers families to gain insights into what to expect, connect with others affected by this ultra-rare disorder, and integrate this knowledge into their future family planning efforts. Furthermore, this data can be utilized when developing best practices in PLAN. Importantly, this information is critical to the success of future clinical trials

that aim to determine whether potential interventions of therapies, such as gene therapy, or

devices exhibit statistically significant effects.

# REFERENCES

Cheng, H. L., Chen, Y. J., Xue, Y. Y., Wu, Z. Y., Li, H. F., & Wang, N. (2022). Clinical Characterization and Founder Effect Analysis in Chinese Patients with Phospholipase A2-Associated Neurodegeneration. *Brain sciences*, *12*(5), 517. https://doi.org/10.3390/brainsci12050517

Cif, L., Kurian, M. A., Gonzalez, V., Garcia-Ptacek, S., Roujeau, T., Gelisse, P., Moura de Ribeiro, A. M., Crespel, A., MacPherson, L., & Coubes, P. (2014). Atypical *PLA2G6*-Associated Neurodegeneration: Social Communication Impairment, Dystonia and Response to Deep Brain Stimulation. *Movement disorders clinical practice*, *1*(2), 128–131. https://doi.org/10.1002/mdc3.12030

Dahlkamp, J. (2003). Tiefstehende Idioten. *Der Spiegel Oct*, *27*, 62.

Erro, R., Balint, B., Kurian, M. A., Brugger, F., Picillo, M., Barone, P., Bhatia, K. P., & Pellecchia, M. T. (2016). Early Ataxia and Subsequent Parkinsonism: PLA2G6 Mutations Cause a Continuum Rather Than Three Discrete Phenotypes. *Movement disorders clinical practice*, *4*(1), 125–128. https://doi.org/10.1002/mdc3.12319

Ferguson, R. P., & Thomas, D. (2014). Medical eponyms. *Journal of community hospital internal medicine perspectives*, *4*(3), 10.3402/jchimp.v4.25046. https://doi.org/10.3402/jchimp.v4.25046

Gregory A, Kurian MA, Maher ER, et al. *PLA2G6*-Associated Neurodegeneration. 2008a Jun 19 [Updated 2017 Mar 23]. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. *GeneReviews®* [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1675/

Gregory, A., Polster, B. J., & Hayflick, S. J. (2009). Clinical and genetic delineation of neurodegeneration with brain iron accumulation. *Journal of medical genetics, 46*(2), 73–80. https://doi.org/10.1136/jmg.2008.061929

Gregory, A., Westaway, S. K., Holm, I. E., Kotzbauer, P. T., Hogarth, P., Sonek, S., Coryell, J. C., Nguyen, T. M., Nardocci, N., Zorzi, G., Rodriguez, D., Desguerre, I., Bertini, E., Simonati,

A., Levinson, B., Dias, C., Barbot, C., Carrilho, I., Santos, M., Malik, I., … Hayflick, S. J. (2008b). Neurodegeneration associated with genetic defects in phospholipase A(2). *Neurology*, *71*(18), 1402–1409. https://doi.org/10.1212/01.wnl.0000327094.67726.28

Hallervorden, J., Spatz, H. Eigenartige Erkrankung im extrapyramidalen System mit besonderer Beteiligung des Globus pallidus und der Substantia nigra.: Ein Beitrag zu den Beziehungen zwischen diesen beiden Zentren. Z. Ges. Neurol. Psychiat. 79: 254-302, 1922.

Hayflick, S. J., Kurian, M. A., & Hogarth, P. (2018). Neurodegeneration with brain iron accumulation. Handbook of clinical neurology, 147, 293–305. https://doi.org/10.1016/B978-0-444-63233-3.00019-1

Hughes J. T. (2007). Neuropathology in Germany during World War II: Julius Hallervorden (1882-1965) and the Nazi programme of 'euthanasia'. *Journal of medical biography*, *15*(2), 116–122. https://doi.org/10.1258/j.jmb.2007.06-57

Illingworth, M. A., Meyer, E., Chong, W. K., Manzur, A. Y., Carr, L. J., Younis, R., Hardy, C., McDonald, F., Childs, A. M., Stewart, B., Warren, D., Kneen, R., King, M. D., Hayflick, S. J., & Kurian, M. A. (2014). PLA2G6-associated neurodegeneration (PLAN): further expansion of the clinical, radiological and mutation spectrum associated with infantile and atypical childhood-onset disease. *Molecular genetics and metabolism*, *112*(2), 183–189. https://doi.org/10.1016/j.ymgme.2014.03.008

Jain, S., Bhasin, H., Romani, M., Valente, E. M., & Sharma, S. (2019). Atypical Childhood-onset Neuroaxonal Dystrophy in an Indian Girl. Journal of pediatric neurosciences, 14(2), 90–93. https://doi.org/10.4103/jpn.JPN_91_18

Kim, Y. J., Lyoo, C. H., Hong, S., Kim, N. Y., & Lee, M. S. (2015). Neuroimaging studies and whole exome sequencing of PLA2G6-associated neurodegeneration in a family with intrafamilial phenotypic heterogeneity. *Parkinsonism & related disorders, 21*(4), 402–406. https://doi.org/10.1016/j.parkreldis.2015.01.010

Kondziella D. (2009). Thirty neurological eponyms associated with the nazi era. European neurology, 62(1), 56–64. https://doi.org/10.1159/000215880

Kurian, M. A., & Hayflick, S. J. (2013). Pantothenate kinase-associated neurodegeneration (PKAN) and PLA2G6-associated neurodegeneration (PLAN): review of two major neurodegeneration with brain iron accumulation (NBIA) phenotypes. *International review of neurobiology, 110*, 49–71. https://doi.org/10.1016/B978-0-12-410502-7.00003-X

Ma, L. M., Zhao, J., Shi, Y. Y., Chen, Z. Z., Ren, Z. X., & Zhang, J. W. (2019). Zhonghua yi xue za zhi, 99(5), 354–358. https://doi.org/10.3760/cma.j.issn.0376-2491.2019.05.007

Morgan, N. V., Westaway, S. K., Morton, J. E., Gregory, A., Gissen, P., Sonek, S., Cangul, H., Coryell, J., Canham, N., Nardocci, N., Zorzi, G., Pasha, S., Rodriguez, D., Desguerre, I., Mubaidin, A., Bertini, E., Trembath, R. C., Simonati, A., Schanen, C., Johnson, C. A., … Hayflick, S. J. (2006). PLA2G6, encoding a phospholipase A2, is mutated in neurodegenerative disorders with high brain iron. *Nature genetics, 38*(7), 752–754. https://doi.org/10.1038/ng1826

Mubaidin, A., Roberts, E., Hampshire, D., Dehyyat, M., Shurbaji, A., Mubaidien, M., Jamil, A., Al-Din, A., Kurdi, A., & Woods, C. G. (2003). Karak syndrome: a novel degenerative disorder of the basal ganglia and cerebellum. *Journal of medical genetics*, *40*(7), 543–546. https://doi.org/10.1136/jmg.40.7.543

Nardocci, N., Zorzi, G., Farina, L., Binelli, S., Scaioli, W., Ciano, C., Verga, L., Angelini, L., Savoiardo, M., & Bugiani, O. (1999). Infantile neuroaxonal dystrophy: clinical spectrum and diagnostic criteria. *Neurology*, *52*(7), 1472–1478. https://doi.org/10.1212/wnl.52.7.1472

Neugebauer, W., & Stacher, G. (1999). Nazi child 'euthanasia' in Vienna and the scientific exploitation of its victims before and after 1945. *Digestive diseases (Basel, Switzerland)*, *17*(5-6), 279–285. https://doi.org/10.1159/000016950

Pearce J. M. (2006). Neurodegeneration with brain iron accumulation: A cautionary tale. *European neurology*, *56*(1), 66–68. https://doi.org/10.1159/000095149

Romani, M., Kraoua, I., Micalizzi, A., Klaa, H., Benrhouma, H., Drissi, C., Turki, I., Castellana, S., Mazza, T., Valente, E. M., & Gouider-Khouja, N. (2015). Infantile and childhood onset

PLA2G6-associated neurodegeneration in a large North African cohort. *European journal of neurology*, *22*(1), 178–186. https://doi.org/10.1111/ene.12552

Salih, M. A., Mundwiller, E., Khan, A. O., AlDrees, A., Elmalik, S. A., Hassan, H. H., Al-Owain, M., Alkhalidi, H. M., Katona, I., Kabiraj, M. M., Chrast, R., Kentab, A. Y., Alzaidan, H., Rodenburg, R. J., Bosley, T. M., Weis, J., Koenig, M., Stevanin, G., & Azzedine, H. (2013). New findings in a global approach to dissect the whole phenotype of PLA2G6 gene mutations. *PloS one*, *8*(10), e76831. https://doi.org/10.1371/journal.pone.0076831

Seitelberger, F. (1952). Eine unbekannte Form von infantiler lipoid-Speicher Krankheit des Gehirns. In *Proceedings of First International Congress of Neuropathology, 1952* (Vol. 3, pp. 323-333). Rosenberg and Sellier.

Shevell M. (1992). Racial hygiene, active euthanasia, and Julius Hallervorden. *Neurology*, *42*(11), 2214–2219. https://doi.org/10.1212/wnl.42.11.2214

Shevell, M. I., & Peiffer, J. (2001). Julius Hallervorden's wartime activities: implications for science under dictatorship. *Pediatric neurology*, *25*(2), 162–165. https://doi.org/10.1016/s0887-8994(00)00243-5

Shevell M. (2012). The declining use of the Hallervorden-Spatz eponym. *Journal of child neurology*, *27*(10), 1308–1309. https://doi.org/10.1177/0883073812454575

Toth-Bencsik, R., Balicza, P., Varga, E. T., Lengyel, A., Rudas, G., Gal, A., & Molnar, M. J. (2021). New Insights of Phospholipase A2 Associated Neurodegeneration Phenotype Based on the Long-Term Follow-Up of a Large Hungarian Family. *Frontiers in genetics*, *12*, 628904. https://doi.org/10.3389/fgene.2021.628904

Voges, L., & Kupsch, A. (2021). Renaming of Hallervorden-Spatz disease: the second man behind the name of the disease. *Journal of neural transmission (Vienna, Austria : 1996)*, *128*(11), 1635–1640. https://doi.org/10.1007/s00702-021-02408-x

Wan, Y., Jiang, Y., Xie, Z., Ling, C., Du, K., Li, R., Yuan, Y., Wang, Z., Sun, W., & Jin, H. (2022). Novel *PLA2G6* Pathogenic Variants in Chinese Patients With *PLA2G6*-Associated

Neurodegeneration. *Frontiers in neurology*, *13*, 922528. https://doi.org/10.3389/fneur.2022.922528

# APPENDIX A: University of California, Irvine (UCI) Institutional Review Board (IRB) New Study Approval

**UCI** | University of California, Irvine

November 15, 2022

LEILA KARLENE RAHIM SCHWANEMANN
PEDIATRICS

RE: UCI IRB #1097    Phenotypic Characterization of Juvenile PLA2G6-Associated Neurodegeneration (PLAN)

The above-referenced human-subjects research project has been approved by the University of California, Irvine Institutional Review Board (UCI IRB).  This approval is limited to the activities described in the approved protocol and extends to the performance of these activities at each respective site identified. In accordance with this approval, the specific conditions for the conduct of this research are listed below, and informed consent from subjects must be obtained unless otherwise indicated below. Additional conditions for the general conduct of human-subjects research are detailed on the attached sheet.

NOTE: Approval by the Institutional Review Board does not, in and of itself, constitute approval for the implementation of this research.  Other institutional clearances and approvals may be required. Research undertaken in conjunction with outside entities, such as drug or device companies, are typically contractual in nature and require an agreement between the University and the entity.  Such agreements must be executed by an institutional official in Sponsored Projects, a division in the UCI Office of Research.  The University is not obligated to legally defend or indemnify an employee who individually enters into these agreements and investigators are personally liable for contracts they sign. **Accordingly, the project should not begin until all required approvals have been obtained.**

Questions concerning the approval of this research project may be directed to the Office of Research, 160 Aldrich Hall, Irvine, CA 92697-7600; 949-824-6068, 949-824-2125, or 949-824-0665 (biomedical committee) or 949-824-6662 (social-behavioral committee).

**Important Reminder:** UCI is in Research Phase 4 as of June 22, 2021. UCI's research activities will increase over time in parallel with the stages in California's Pandemic Roadmap and other public health and higher education guidance.  Refer to the Office of Research webpage on Research Continuity for more details.

Minimal Risk (Expedited) Review Categories: 5,7

Cristobal Barrios, MD
Vice Chair, Institutional Review Board

Approval Issued: November 10, 2022
Expiration Date: November 9, 2025
UCI (FWA) 00004071, Approved: January 31, 2003

**IRB Determinations as Conditions of Approval:**

UNIVERSITY OF CALIFORNIA

# APPENDIX B: UCI IRB Amendment Approval Letter

**UCI** University of California, Irvine

December 22, 2022

LEILA KARLENE RAHIM SCHWANEMANN
PEDIATRICS

RE: UCI IRB #1097    Phenotypic Characterization of Juvenile PLA2G6-Associated Neurodegeneration (PLAN)

**The amendment(s) for the above-referenced human-subjects research project has been approved by the University of California, Irvine Institutional Review Board (UCI IRB). Specific changes approved by the IRB are noted below.**

The IRB may not have approved all changes proposed in the amendment application. Review the above summary of approved changes and any revised documents provided with this letter. If a requested change does not appear in the summary or in the revised documents, the IRB did not approve that change. Please consult with Human Research Protections (HRP) Staff for further information.

If the approved amendment(s) includes changes to the informed consent document, the approved stamped consent form is enclosed. Please discontinue use of any previous versions of the informed consent document and use only the most updated version for enrollment of all new subjects.

Changes in approved research, during the period for which IRB approval has already been given, may not be initiated without IRB review and approval except where necessary to eliminate apparent immediate hazards to the human subjects [21 CFR Part 56.108 (a)(4), and if applicable 45 CFR 4.108 (a)(3)(iii)].

**Below is a summary of the approved changes requested:**

**Request: Consent**
**Rationale: A Certificate of Confidentiality approval letter has been added to the Attachments section. This update also required a minor change to the consent form text from "we are in the process of obtaining" to "we have obtained" a CoC from the NIH (changes are tracked in the updated consent form, please see Attachments section).**

**Request: Other Change(s)**
**Rationale: Updated recruitment methods so that the IRB-approved flyer/email can be shared more broadly.**

Questions concerning approval of this study may be directed to the UC Irvine Office of Research, 160 Aldrich Hall, Irvine, CA 92697-7600; 949-824-6068 or 949-824-2125 (biomedical committee) or 949-824-6662 (social-behavioral committee).

**Important Reminder: UCI is in Research Phase 4 as of June 22, 2021. UCI's research activities will increase over time in parallel with the stages in California's Pandemic Roadmap and other public health and higher education guidance. Refer to the Office of Research webpage on Research Continuity for more details.**

Level of Review of Amendment: Minimal Risk (Expedited) Review

Tahseen Mozaffar, MD
Chair, Institutional Review Board
Approval Issued: December 22, 2022
Expiration Date: November 09, 2025
UCI (FWA) 00004071, Approved: January 31, 2003

UNIVERSITY OF CALIFORNIA

# APPENDIX C: PLANready Milestones (1-31)

Please answer whether your child is CURRENTLY able to do the skills listed below

1. Grasps a toy or other object when it is touched to the backs or tips of the fingers

    1.1. Yes

    1.1. No

2. Reaches toward objects placed in front of him/her and tries to grab them

    2.1. Yes

    2.2. No

3. Picks up small objects, like a Cheerio or raisin

    3.1. Yes

        3.1.1. How does your child pick up small objects?

            3.1.1.1. With a pincer grasp (brings together any part of thumb and 1 or more

                fingers)

            3.1.1.2. With a rake grasp (raking motions with entire hand)

    3.2. No

4. Holds head upright & steady for several seconds when held in sitting position, no bobbing action

    4.1. Yes

    4.2. No

5. Rolls over independently

    5.1. Yes

5.2. No

6. Sits up alone for at least 5 seconds without help from another person

    6.1. Yes

    6.2. No

7. Pulls self to stand without assistance

    7.1. Yes

    7.2. No

8. Get into a sitting position by him/herself (from lying down or standing with support)

    8.1. Yes

    8.2. No

9. Walks 5 steps independently (without support)

    9.1. Yes

    9.2. No

10. Stands balanced on one foot for at least one second

    10.1. Yes

    10.2. No

11. Smiles back in response to being smiled at or spoken to (but not touched)

    11.1. Yes

    11.2. No

12. Can child indicate a want to parent without crying? Can include pointing, reaching, making

    sounds, moving arms, pulling, saying a word, others

    12.1. Yes

12.2. No

13. Waves 'bye-bye' when someone waves to him/her

13.1. Yes

13.2. No

14. Feeds him/herself finger foods WITHOUT help

14.1. Yes

14.2. No

15. Feeds him/herself with fork or spoon without help and gets most of the food into his/her

mouth

15.1. Yes

15.2. No

16. Vocalizes - small throaty sounds or short vowel sounds (crying does not count)

16.1. Yes

16.2. No

17. Laughs out loud

17.1. Yes

17.2. No

18. Makes single syllable sounds with consonant and vowel like 'ga', 'ma', or 'ba'

18.1. Yes

18.2. No

19. Uses 2 words other than mama, dada, or the names of other family or pets

19.1. Yes

19.2. No

20. Combines at least 2 words to make a meaningful phrase that indicates an action, like 'play ball' or 'want drink' or 'go bye-bye'

20.1. Yes

20.2. No

21. Uses wheelchair/stroller?

21.1. Yes

21.2. No

22. Falls frequently while walking (at least once/day)?

22.1. Yes

22.2. No

23. Has seizures (or treated for them)?

23.1. Yes

23.2. No

24. Requires diet modifications (soft diet, blending, dicing, thickening liquids, etc.)?

24.1. Yes

24.2. No

25. Has swallowing difficulty / coughing with foods or liquids?

25.1. Yes

25.2. No

26. Has a gastrostomy tube (feeding tube)?

26.1. Yes

26.2. No

27. Has optic nerve atrophy or 'pale' optic nerve?

27.1. Yes

27.2. No

28. Has constipation (or uses treatments for it)?

28.1. Yes

28.2. No

29. Has gastroesophageal reflux (or uses treatments for it)?

29.1. Yes

29.2. No

30. Has a tracheostomy?

30.1. Yes

30.2. No

31. Has abnormal autonomic function (such as cold hands/feet or sudden changes in core body temperature)?

31.1. Yes

31.1.1. Please describe the symptoms of abnormal autonomic function:

31.2. No

- Has the individual with PLAN had regression since the last online study visit?

  o Yes

    ▪ Please describe the regression:

  o No

# APPENDIX D: Recruitment Flyer

Lead Researcher

Leila Schwanemann, Genetic Counseling Graduate Student

Department of Pediatrics, Division of Genetic & Genomic Medicine

Study Title: Phenotypic Characterization of Juvenile PLA2G6-Associated Neurodegeneration (PLAN)

University of California, Irvine

The purpose of this research study is to better describe Juvenile PLAN (also called aNAD) by performing a detailed review of medical records to establish early signs, symptoms, and disease progression. This way affected individuals receive the appropriate diagnosis and services as early as possible.

You can participate in this study if you can provide information to confirm your genetic and clinical diagnosis of Juvenile PLAN.

The study will take place virtually (Zoom or phone). Participation will include two study visits to collect information about your doctors and sign medical records release forms for your pediatrician, neurologist, geneticist, and other healthcare professionals.

If you participate, there is no anticipated direct benefit, however, you may help us learn how to benefit patients in the future.

Questions? Please contact Leila Schwanemann at (714) 456-3987 or lschwane@hs.uci.edu.

# DECISION-MAKING CAPACITY ASSESSMENT TOOL

Potential Subject: <u>***</u>
Date: <u>***</u>

The Phenotypic Characterization of Juvenile *PLA2G6*-Associated Neurodegeneration (PLAN)

Protocol IRB #1097

There are four elements of decision-making capacity that will be assessed for this specific research protocol:

1. **<u>Understanding:</u>**
   What is the purpose of the research study? <u>***</u>

   What will happen to you in this research study? <u>***</u>

2. **<u>Appreciation:</u>**
   What are the potential risks of this research study? <u>***</u>

   What are the potential benefits of this research study? <u>***</u>

3. **<u>Reasoning:</u>**
   What alternative is there if you choose not to participate in this study? <u>***</u>

4. **<u>Expressing a Choice:</u>**
   Does the individual express a choice about whether or not to participate? <u>***</u>

5. **Does the individual have the decision-making capacity to give informed consent for this study?**
   ☐ Yes
   ☐ No

_____                    _____
 Printed Name of Evaluator                              Signature of Evaluator

# APPENDIX F

**Investigator Certification of Surrogate Decision Makers for Potential Subject's Participation in University of California Research**

**Section 1: Research Information**

Name of Participant: _____ _____

Title of Research Project: _____ _____

IRB #:_____ _____

**Section 2: IRB Application Information**

For use of surrogate consent in research, investigators must follow their IRB-approved application for use of a surrogate decision maker: *(Check that each criterion has been met)*:

☐ The protocol-specific plan for assessment of the decision-making capacity by the investigator of any research participants who may require the consent of a legally authorized representative, including the below, has been followed.

☐ The research participant has been determined to lack capacity to consent, and the investigator has made a reasonable effort to describe the research to the participant in a manner consistent with the standard consent process and indicate the intent to obtain surrogate consent.

☐ The research participant has not expressed resistance or dissent to being in the research or to the use of the surrogate consent by word or gesture.

☐ The surrogate decision maker identified when this form is completed is the highest level of surrogate, except in the case that the research is taking place in an emergency room environment.

**Section 3: Category of Potential Surrogate**

Check the category that best describes the relationship between the study participant and the surrogate decision maker.

☐ 1. Agent named in the potential subject's advanced health care directive.

☐ 2. Conservator or guardian of the potential subject, with authority to make health care decisions for the potential subject.

☐ 3. Spouse of the potential subject.

☐ 4. Registered domestic partner of the potential subject.

☐ 5. Adult child of potential subject.

☐ 6. Custodial parent of the potential subject.

☐ 7. Adult sibling of the potential subject.

***The remaining selections may only be utilized in non-emergency room settings, as specified in California Health & Safety Code Section 24178:***

☐ 8. Adult grandchild of the potential subject.

☐ 9. An available adult relative with the closest degree of kinship to the potential subject, whose relationship to the potential subject does not fall within one of the above listed categories, and which relationship can best be described as (e.g., aunt; uncle; cousin; etc.):
_____

**Section 4: Potential Surrogate's Contact Information**

Check one of the applicable boxes:
☐ I have a way to reach the surrogate decision maker.
☐ Should follow up information be needed or should a surrogate need to be re-consented, any of the following contact information may be used:

Name:_____   Phone Number: _____
Address:_____   Email: _____
_____

# APPENDIX G: National Institutes of Health Certificate of Confidentiality

**DEPARTMENT OF HEALTH & HUMAN SERVICES**                    Public Health Service

National Institutes of Health
Bethesda, Maryland 20892
www.nih.gov

11/17/2022

Leila Karlene Rahim Schwanemann, BS in Biochemistry; MS in Genetic Counseling (2023)
University of California, Irvine (UCI Health)
Office of Research 160 Aldrich Hall
Irvine, CA, 92697

Dear Leila Karlene Rahim Schwanemann, BS in Biochemistry; MS in Genetic Counseling (2023)

Enclosed is the Confidentiality Certificate, protecting the identity of research participants in your project entitled, "Phenotypic Characterization of Juvenile PLA2G6-Associated Neurodegeneration (PLAN)".

You and your institution's responsibilities related to this Certificate are outlined on the NIH Certificate of Confidentiality website and in the Institutional Assurance Statement. You and your institution's responsibilities include that you cannot release a participant's identifiable, sensitive information to any other person not connected with the research OR in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding.

Protections against disclosure under the Certificate of Confidentiality are limited in certain circumstances. Disclosure of information, physical documents, or biospecimens is not protected by the Certificate when this information or material is associated with mandatory reporting by Federal, State, or local laws, including reporting of child and elder abuse and specific infectious diseases.

Research participants must be informed about the Certificate protections under the applicable regulations at Title 42 CFR Part 2a.4. In addition, participants must be informed about any exceptions to those protections, such as the mandatory reporting described above. Sample informed consent language can be found at https://grants.nih.gov/policy/humansubjects/coc/helpful-resources/suggested-consent.htm that may be used or adapted as appropriate.

If you make a significant change to the research project for this study (e.g., change of principal investigator or institution, change in the scope or direction of the research), you will need to obtain a new Certificate of Confidentiality to continue protections for new data collection. You may make a request through the NIH online CoC system.

If legal action is brought to release personally identifying information protected by a Certificate of Confidentiality you should immediately seek legal counsel from your institution.

As a reminder, your institution has attested that the research activities will be conducted in accordance with all applicable Federal, State, and local laws and regulations.

Please contact the NIH Certificate of Confidentiality Coordinator if you have any questions about your Certificate of Confidentiality at NIH-CoC-Coordinator@mail.nih.gov.

Sincerely,

ANGELA Chambers
NIH Certificates of Confidentiality Coordinator
Approval Date: 11/17/2022        Office of Extramural Research
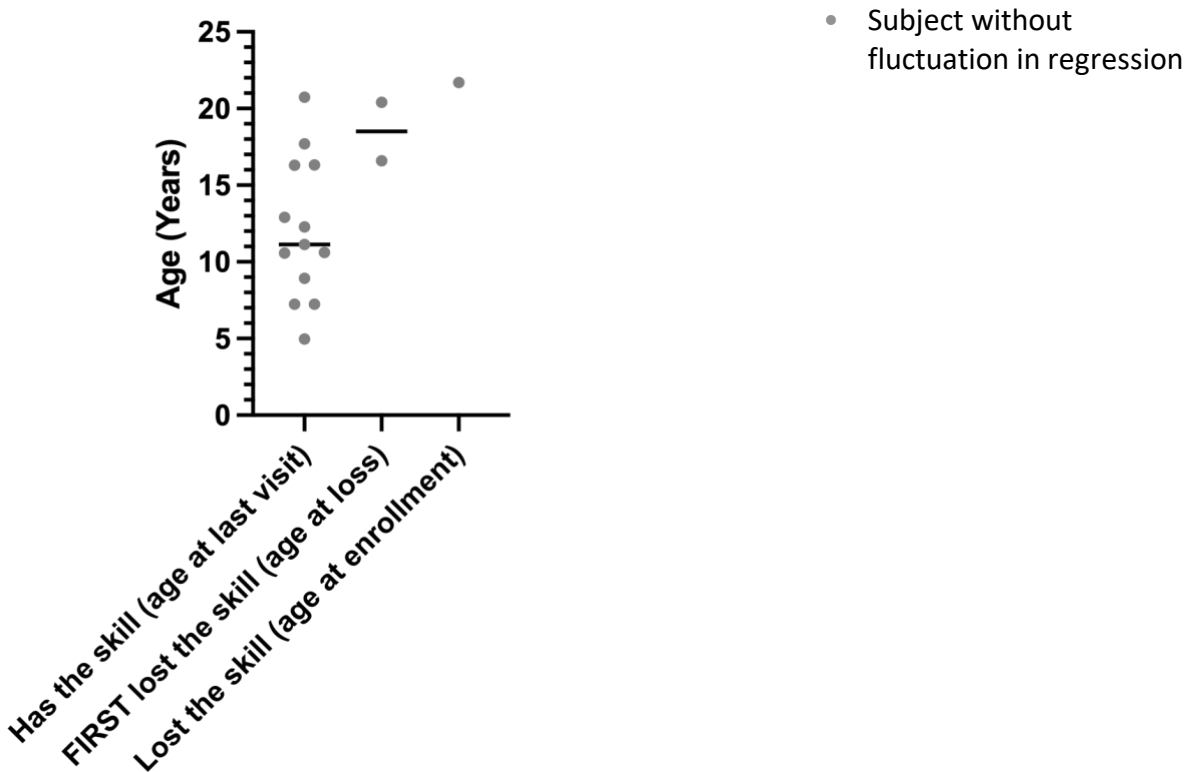National Institutes of Health

# APPENDIX H: Supplementary Figures and Tables for PLANready Milestones

**Early Developmental Milestone 2**

*Reaches toward objects placed in front of him/her and tries to grab them*
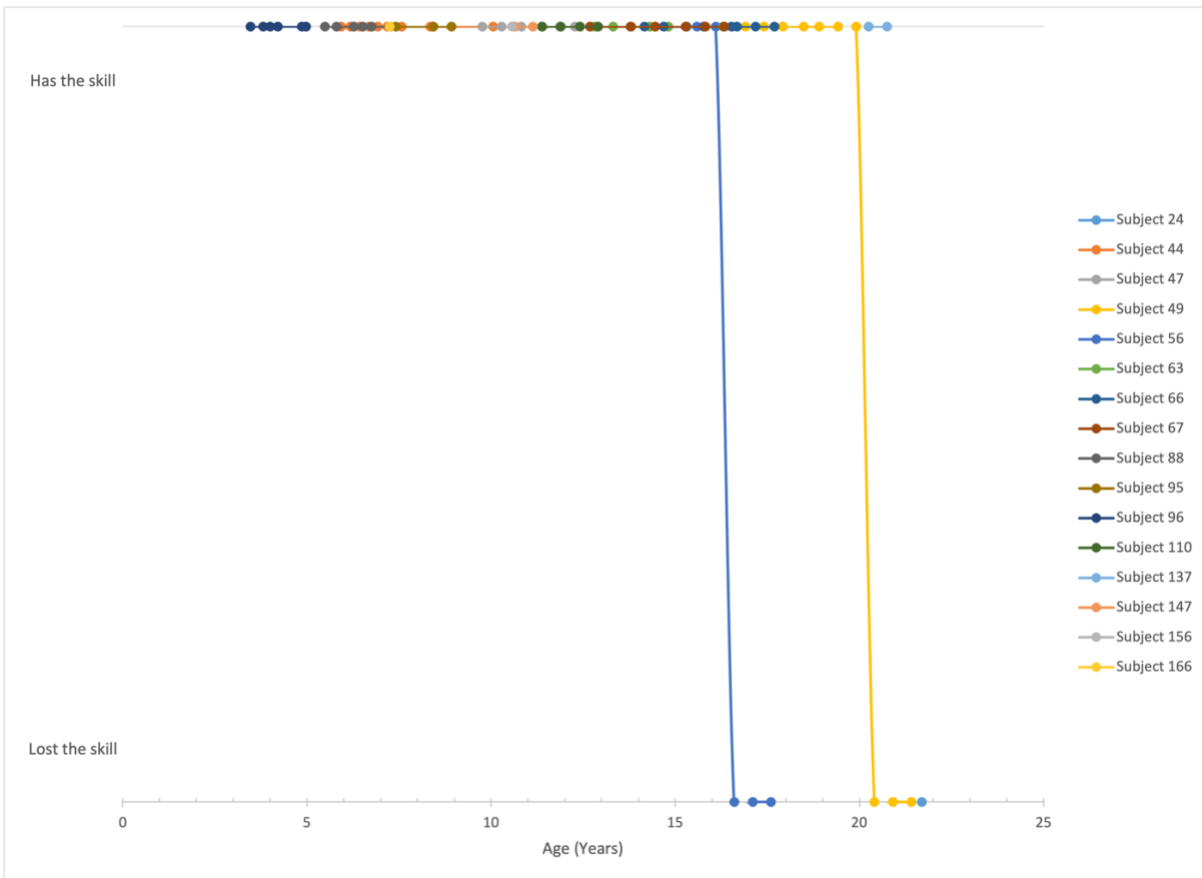
**Appendix Figure 2A**

*Scatter Plot—Regression of Milestone 2*



*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 2B**

*Longitudinal Plot—Regression of Milestone 2*



*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 2, and a positive slope indicates fluctuations in regression.
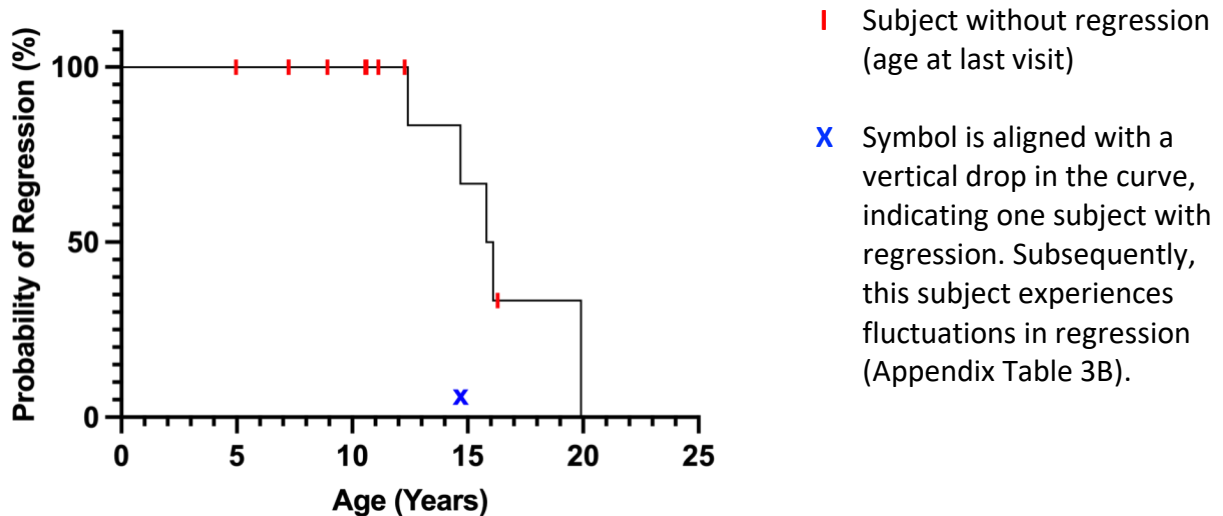
**Appendix Table 2**

*Kaplan-Meier Curve Analysis—Regression of Milestone 2*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 16.6 | 100 |
| 16.6 | to | 20.4 | 75 |
| 20.4 | to | 20.7 | 38 |

**Appendix Figure 2C**

*Kaplan-Meier Curve—Regression of Milestone 2*
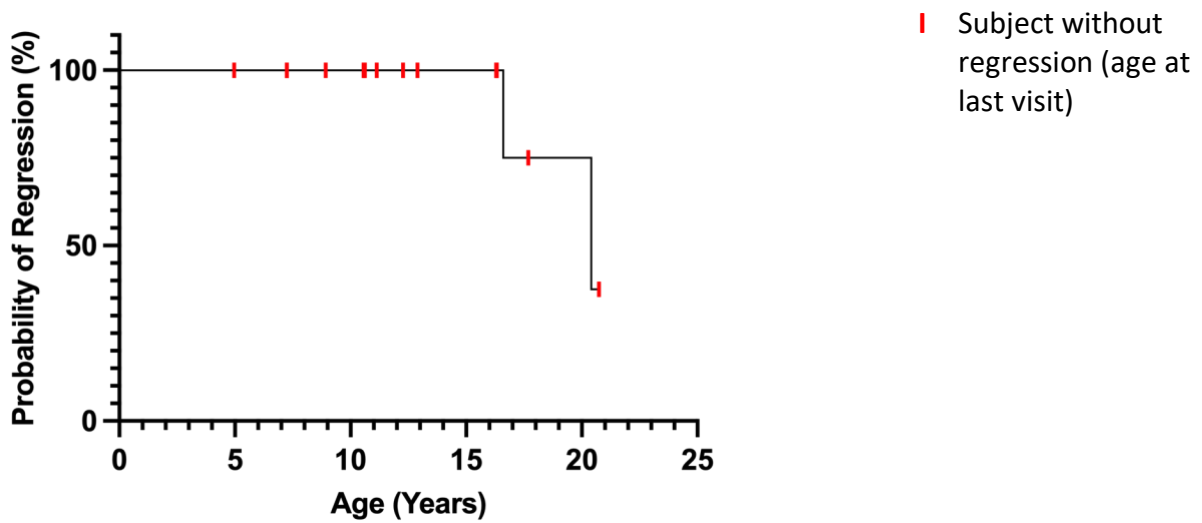


I Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 2 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 2 and Table 6.

**Appendix Figure 2D**

*Grouped Columns—Regression of Milestone 2*
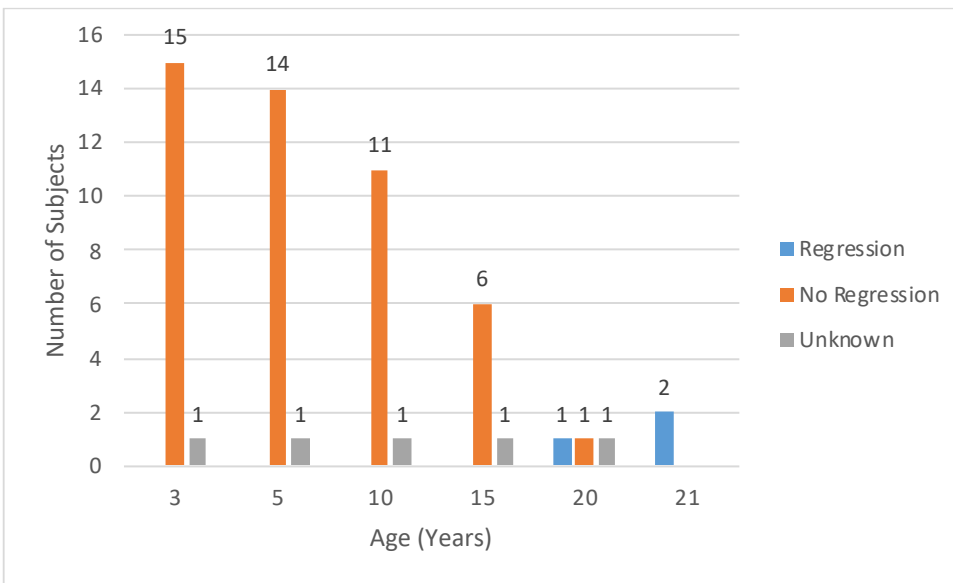


*Note.* At visit 1, if a subject reported no regression to milestone 2, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Early Developmental Milestone 3**

*Picks up small objects, like a Cheerio or raisin*

**Appendix Figure 3A**

*Scatter Plot: Regression of Milestone 3*
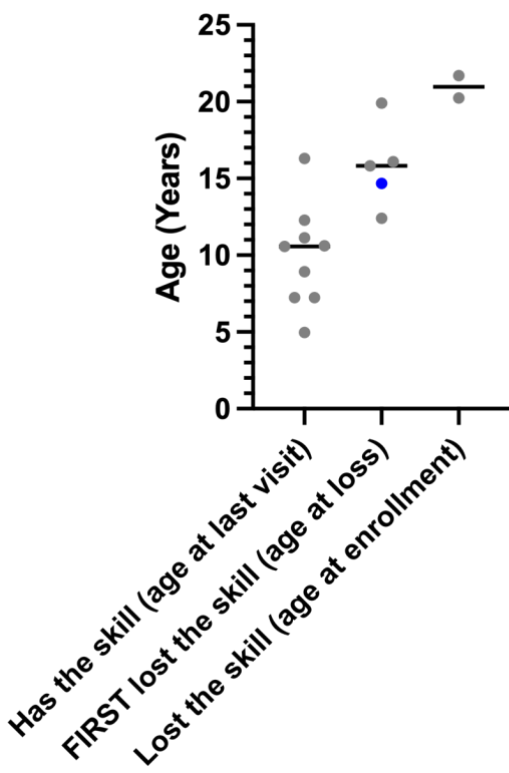


- Subject without fluctuation in regression
- Subject with fluctuation in regression (Appendix Table 3B)

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 3B**

*Longitudinal Plot—Regression of Milestone 3*
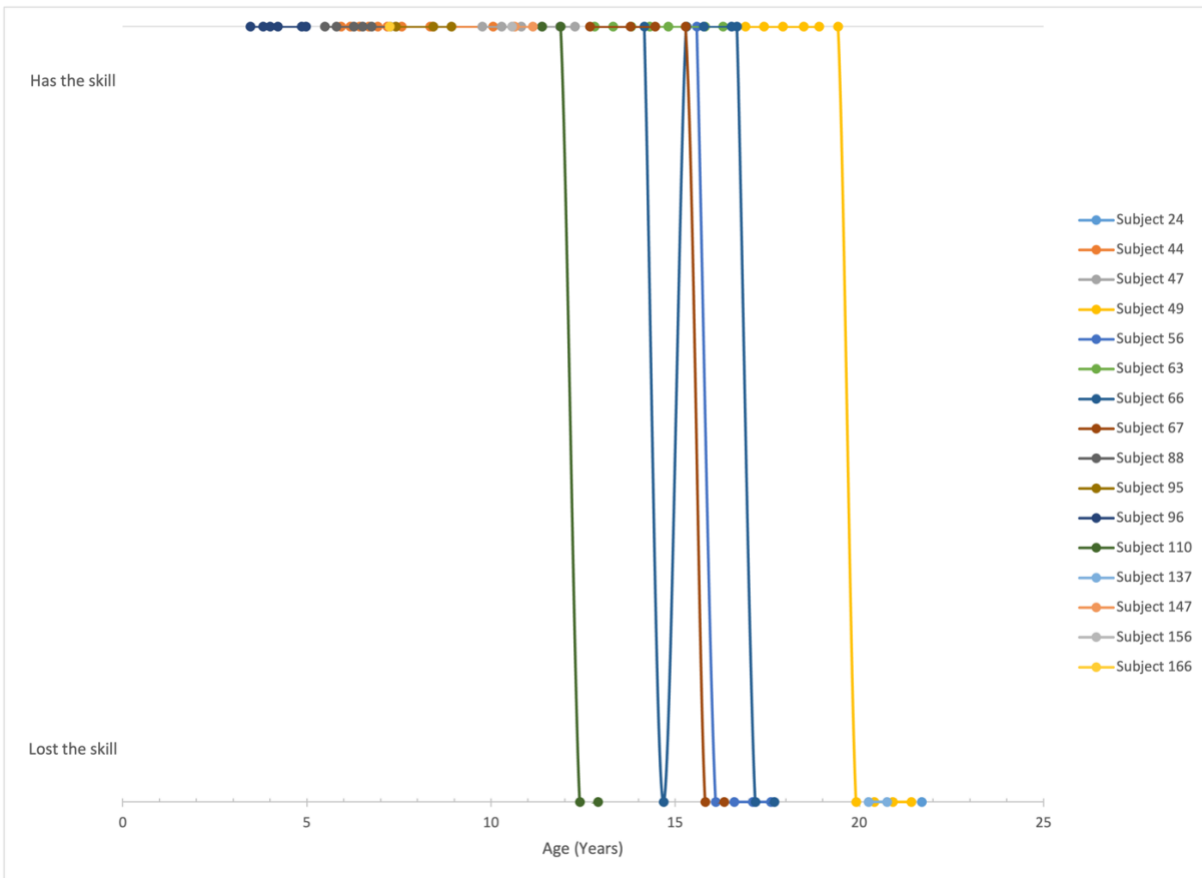


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 3, and a positive slope indicates fluctuations in regression (Appendix Table 3B).

**Appendix Table 3A**

*Kaplan-Meier Curve Analysis—Regression of Milestone 3*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 12.4 | 100 |
| 12.4 | to | 14.7 | 83 |
| 14.7 | to | 15.8 | 67 |
| 15.8 | to | 16.1 | 50 |
| 16.1 | to | 19.9 | 33 |
| 19.9 | to | - | 0 |

**Appendix Figure 3C**

*Kaplan-Meier Curve—Regression of Milestone 3*



**I**  Subject without regression (age at last visit)

**X**  Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 3B).

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 3 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 3A and Table 6.

**Appendix Figure 3D**

*Grouped Columns—Regression of Milestone 3*
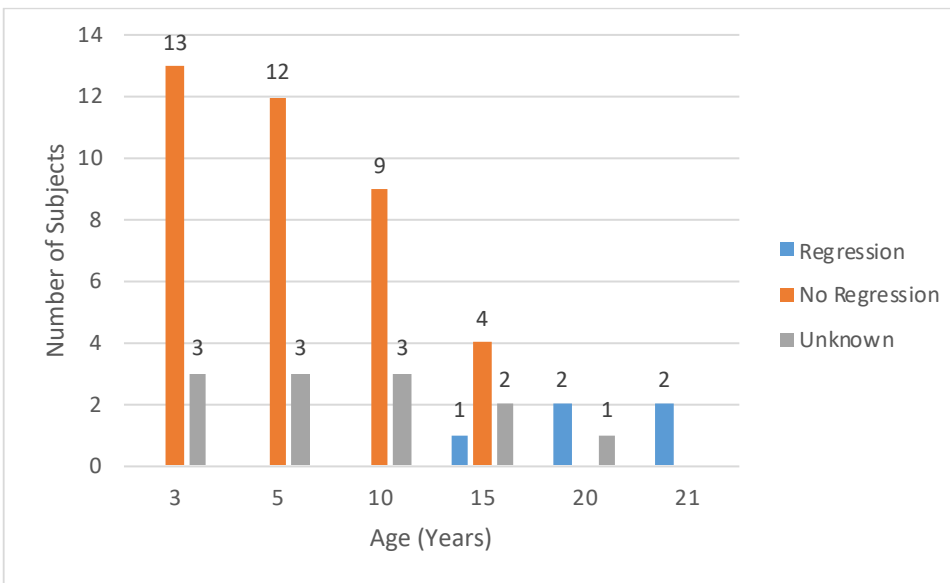


*Note.* At visit 1, if a subject reported no regression to milestone 3, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 3B**

*Fluctuations—Regression of Milestone 3*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 66 | 1 | 14.2 | No |
| | 2 | 14.7 | Yes |
| | 3 | 15.3 | No |
| | 4 | 15.8 | No |
| | 5 | 16.5 | No |
| | 6 | 16.7 | No |
| | 7 | 17.2 | Yes |
| | 8 | 17.7 | Yes |

**Early Developmental Sub-Milestone 3**

*How does your child pick up small objects?*

  *With a pincer grasp (brings together any part of thumb and 1 or more fingers)*

  *With a rake grasp (raking motions with entire hand)*


**Appendix Figure 3E**

*Scatter Plot—Regression of Sub-Milestone 3*



- Subject without fluctuation in regression

- Subject with fluctuation in regression
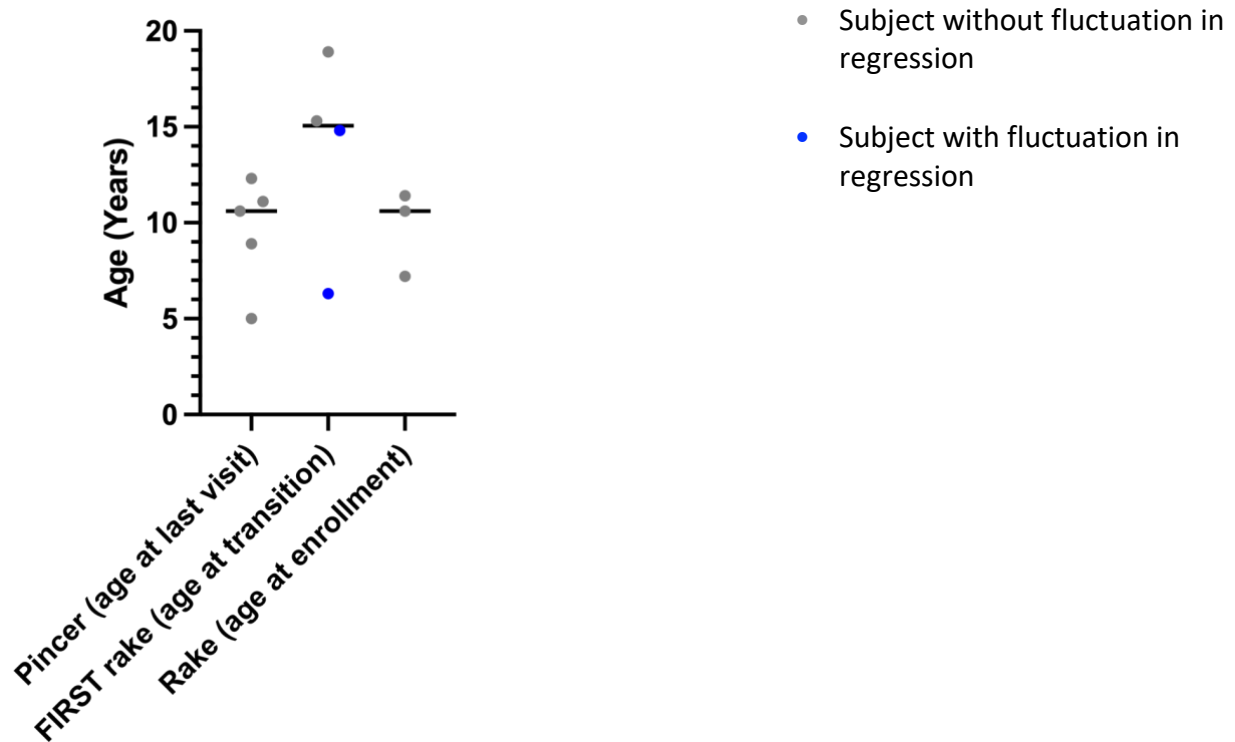
*Note.* Participants were categorized into one of three groups: 'Pincer' denotes those with an unchanged pincer grasp. 'First rake' represents regression captured in real-time from a pincer to a rake grasp. 'Rake' indicates participants who experienced regression before enrolling in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 3.

**Appendix Figure 3F**

*Longitudinal Plot—Regression of Sub-Milestone 3*
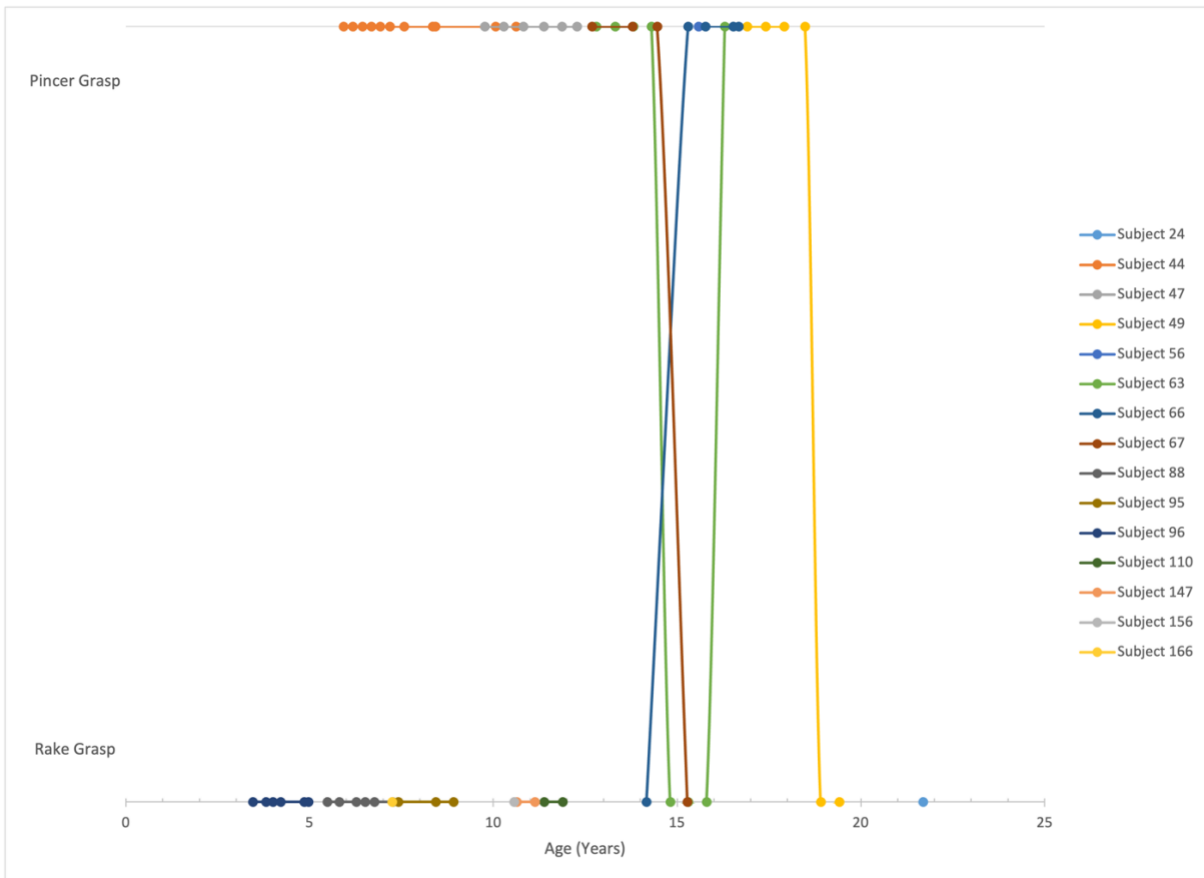


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of sub-milestone 3, and a positive slope indicates fluctuations in regression.

**Early Developmental Milestone 4**

*Holds head upright & steady for several seconds when held in sitting position, no bobbing action*

**Appendix Figure 4A**

*Scatter Plot—Regression of Milestone 4*
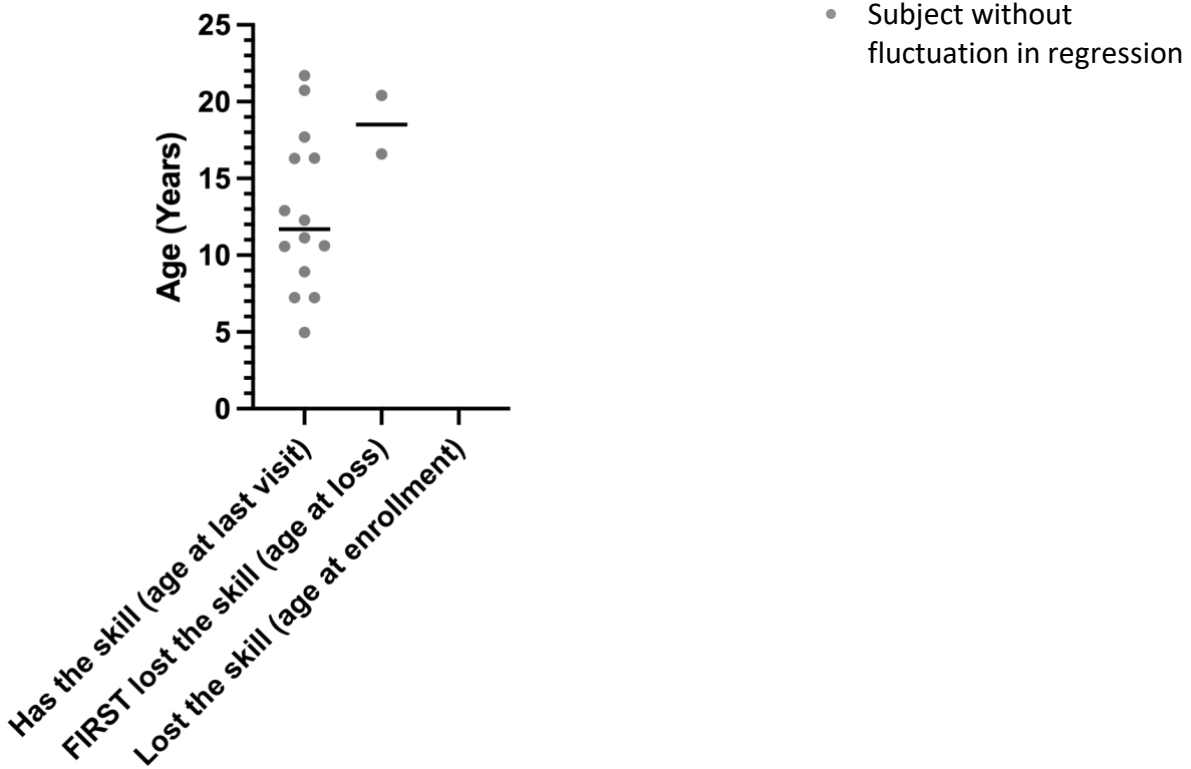


• Subject without fluctuation in regression

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 4B**

*Longitudinal Plot—Regression of Milestone 4*
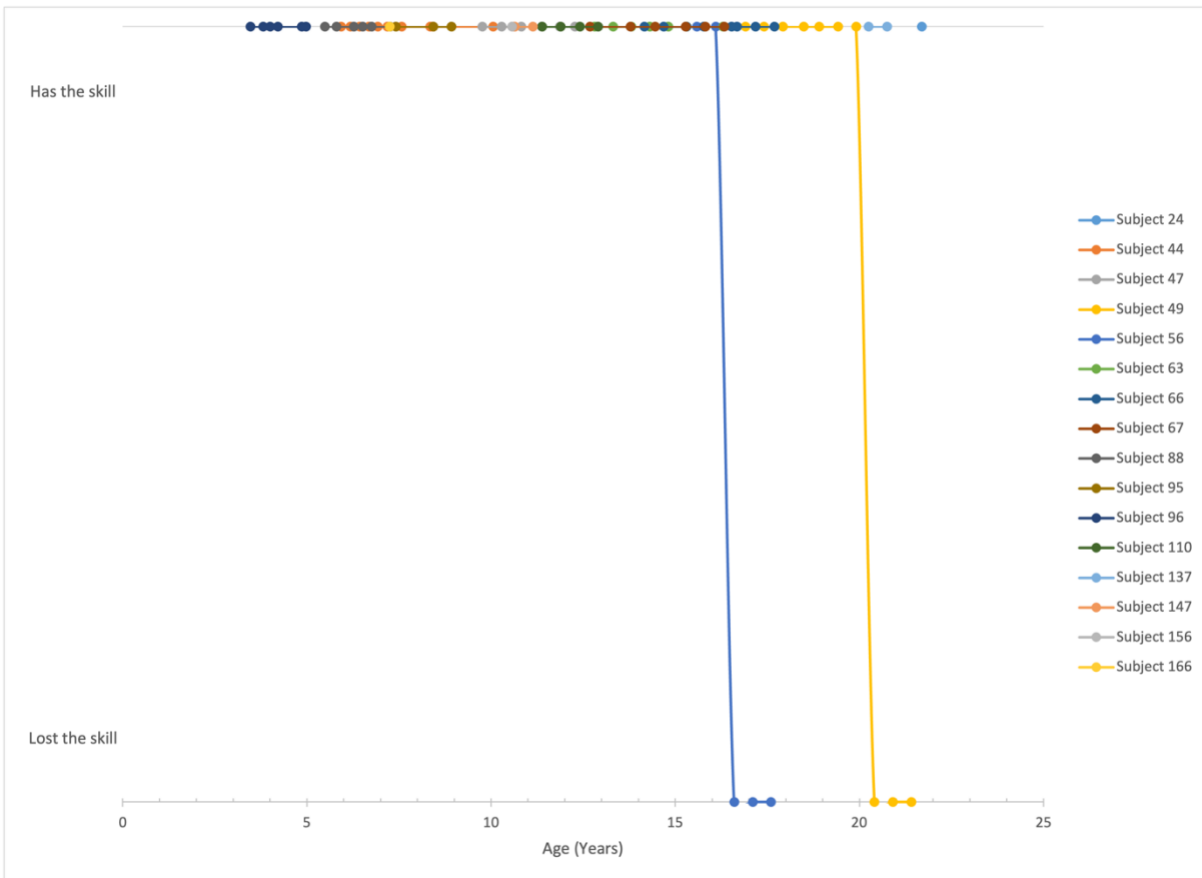


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 4, and a positive slope indicates fluctuations in regression.

**Appendix Table 4**

*Kaplan-Meier Curve Analysis—Regression of Milestone 4*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 16.6 | 100 |
| 16.6 | to | 20.4 | 80 |
| 20.4 | to | 21.7 | 53 |

**Appendix Figure 4C**

*Kaplan-Meier Curve—Regression of Milestone 4*
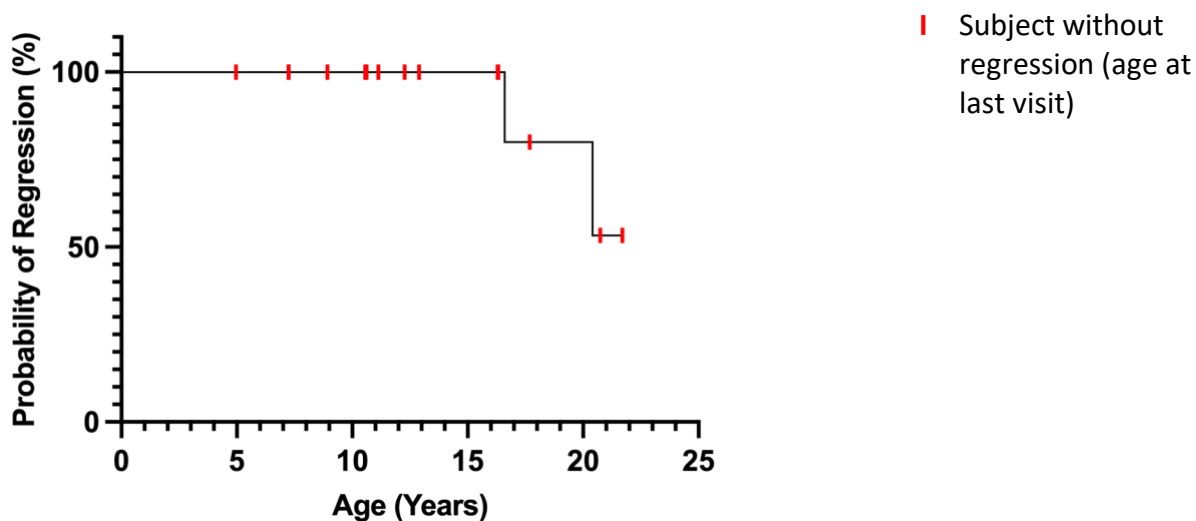


Ⅰ Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 4 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 4 and Table 6.

**Appendix Figure 4D**

*Grouped Columns—Regression of Milestone 4*
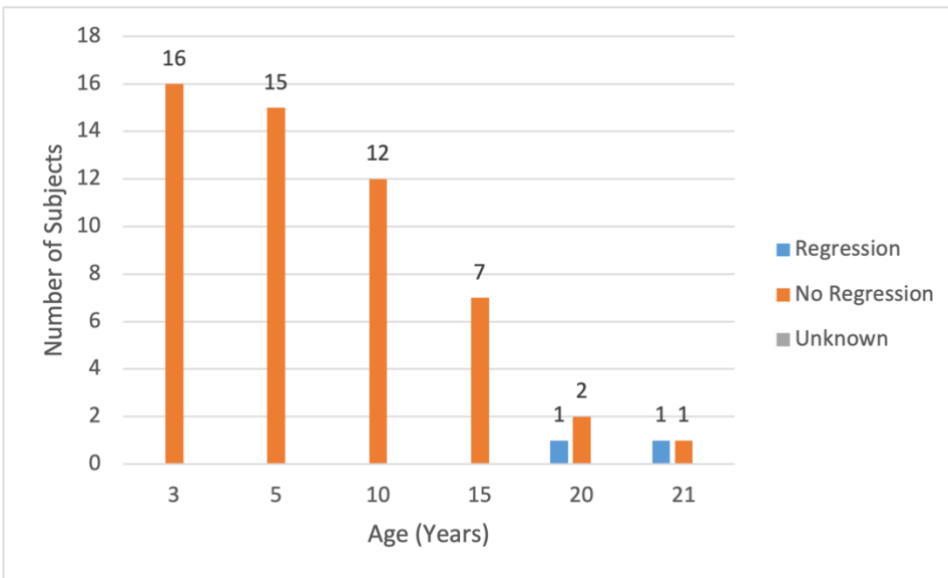


*Note.* At visit 1, if a subject reported no regression to milestone 4, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Early Developmental Milestone 5**

*Rolls over independently*

**Appendix Figure 5A**

*Scatter Plot—Regression of Milestone 5*
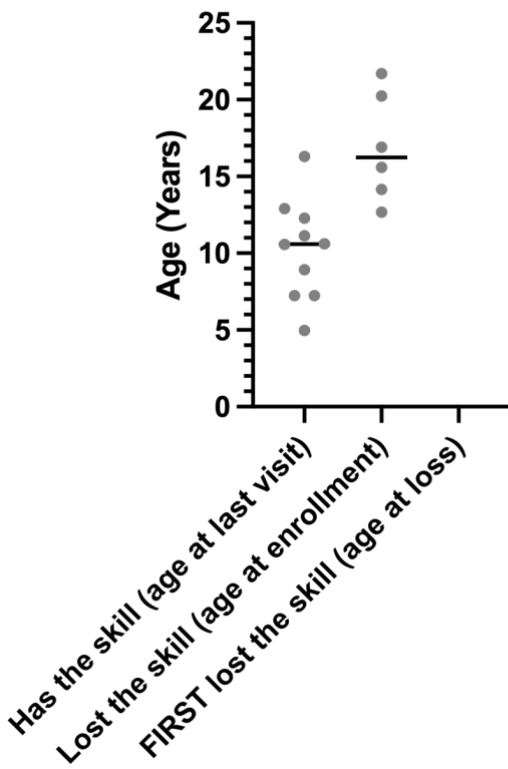


- Subject without fluctuation in regression

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 5B**

*Longitudinal Plot—Regression of Milestone 5*
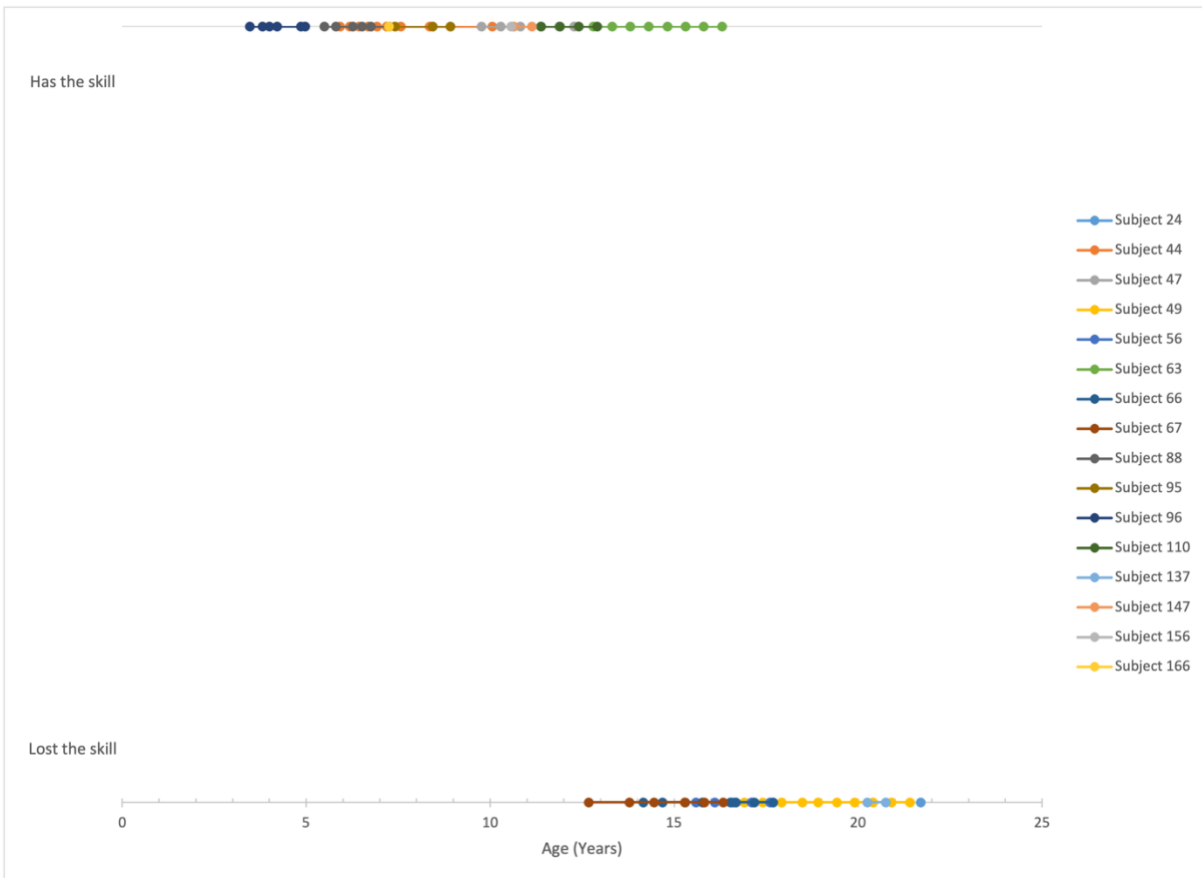


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 5, and a positive slope indicates fluctuations in regression.

**Appendix Table 5**

*Kaplan-Meier Curve Analysis—Regression of Milestone 5*

| Age Range for Regression (Years) | No Regression (%) |
|---|---|
| 0.0 to 16.3 | 100 |

**Appendix Figure 5C**

*Kaplan-Meier Curve—Regression of Milestone 5*
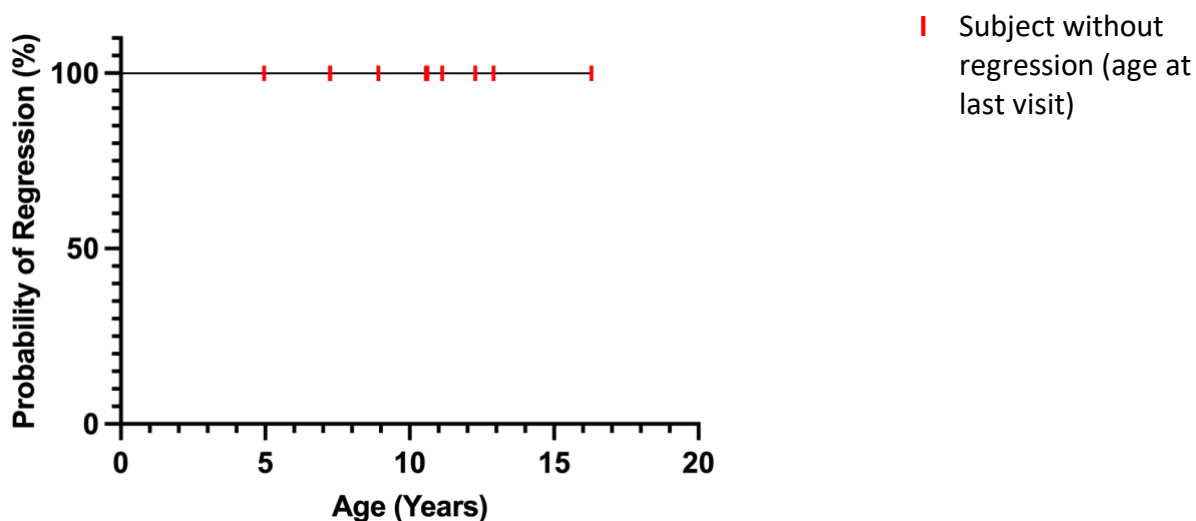


*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 5 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 5 and Table 6.

**Appendix Figure 5D**

*Grouped Columns—Regression of Milestone 5*
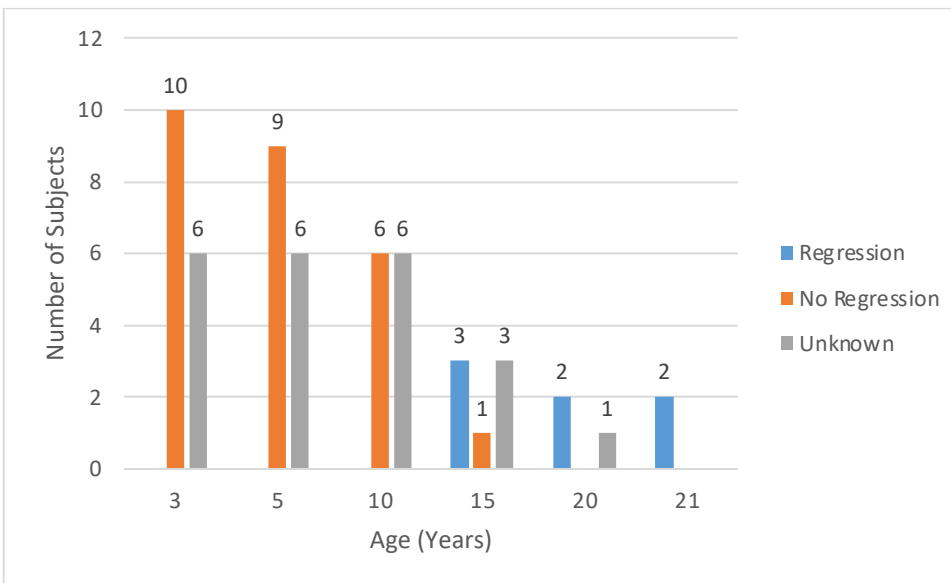


*Note.* At visit 1, if a subject reported no regression to milestone 5, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Early Developmental Milestone 6**

*Sits up alone for at least 5 seconds without help from another person*

**Appendix Figure 6A**

*Scatter Plot—Regression of Milestone 6*
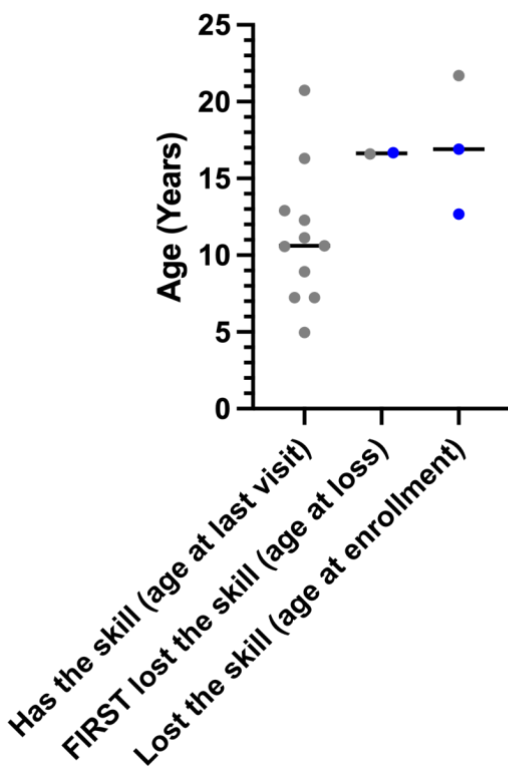


- Subject without fluctuation in regression
- Subject with fluctuation in regression (Appendix Figure 6B)

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 6B**

*Longitudinal Plot—Regression of Milestone 6*
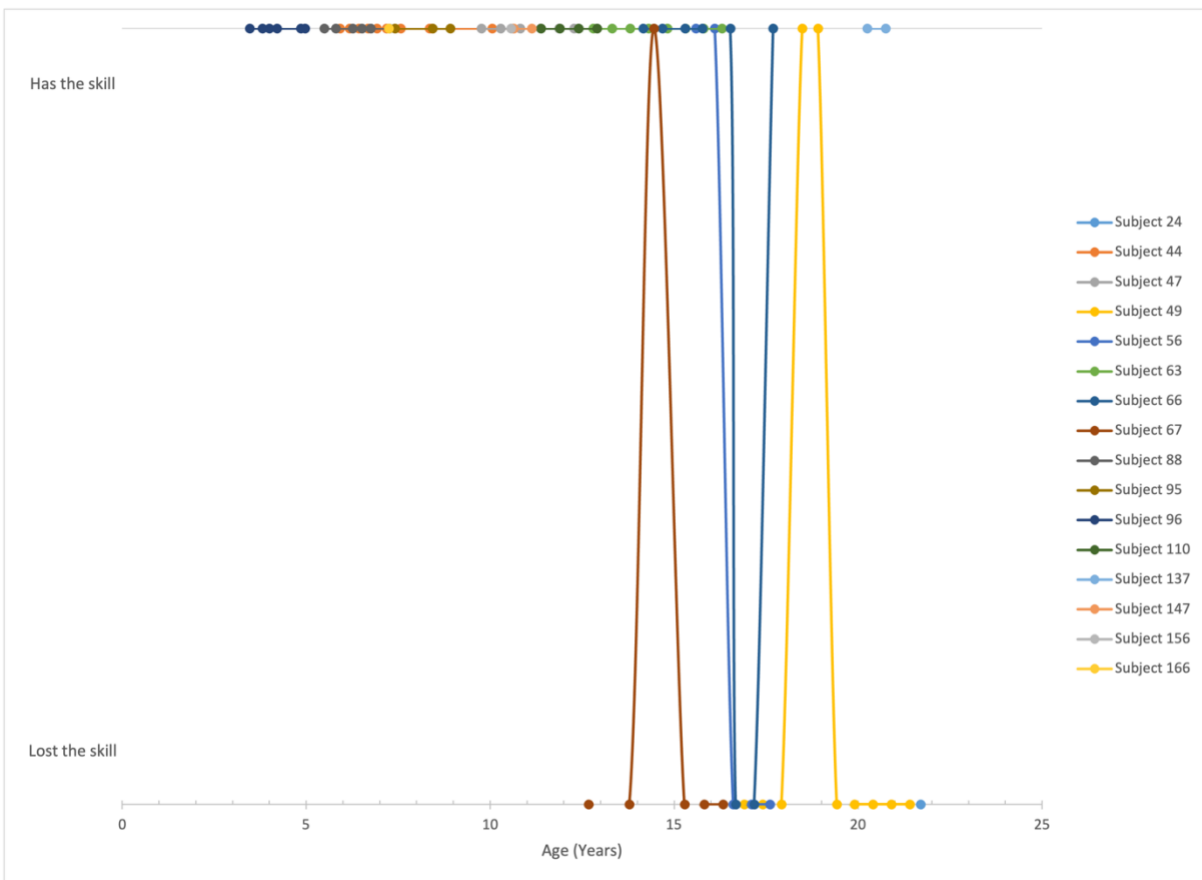


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 6, and a positive slope indicates fluctuations in regression (Appendix Figure 6B).
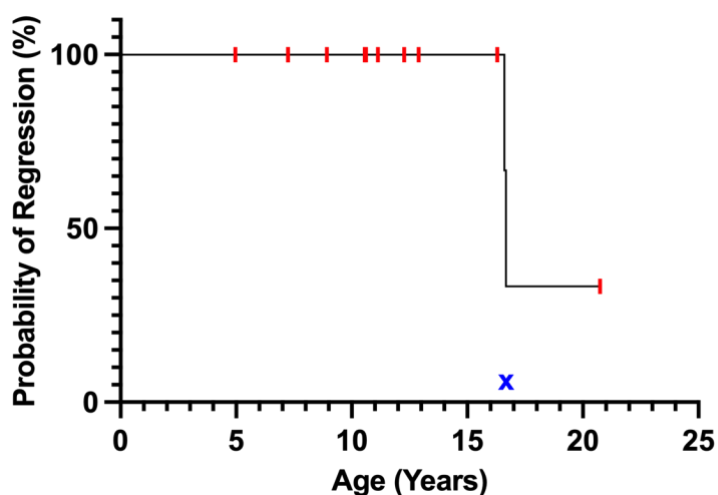
**Appendix Table 6A**

*Kaplan-Meier Curve Analysis—Regression of Milestone 6*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 16.6 | 100 |
| 16.6 | to | 16.7 | 67 |
| 16.7 | to | 20.7 | 33 |

**Appendix Figure 6C**

*Kaplan-Meier Curve—Regression of Milestone 6*



**I** Subject without regression (age at last visit)

**X** Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Figure 6B).

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 6 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 6A and Table 6.

**Appendix Figure 6D**

*Grouped Columns—Regression of Milestone 6*
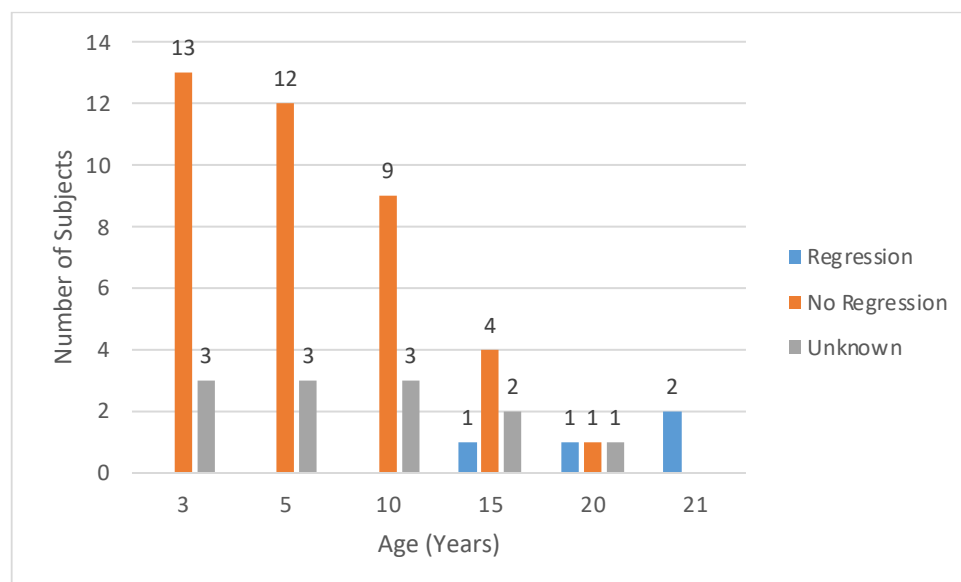


*Note.* At visit 1, if a subject reported no regression to milestone 6, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 6B**

*Fluctuations—Regression of Milestone 6*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 49      | 1     | 16.9        | Yes        |
|         | 2     | 17.4        | Yes        |
|         | 3     | 17.9        | Yes        |
|         | 4     | 18.5        | No         |
|         | 5     | 18.9        | No         |
|         | 6     | 19.4        | Yes        |
|         | 7     | 19.9        | Yes        |
|         | 8     | 20.4        | Yes        |
|         | 9     | 20.9        | Yes        |
|         | 10    | 21.4        | Yes        |

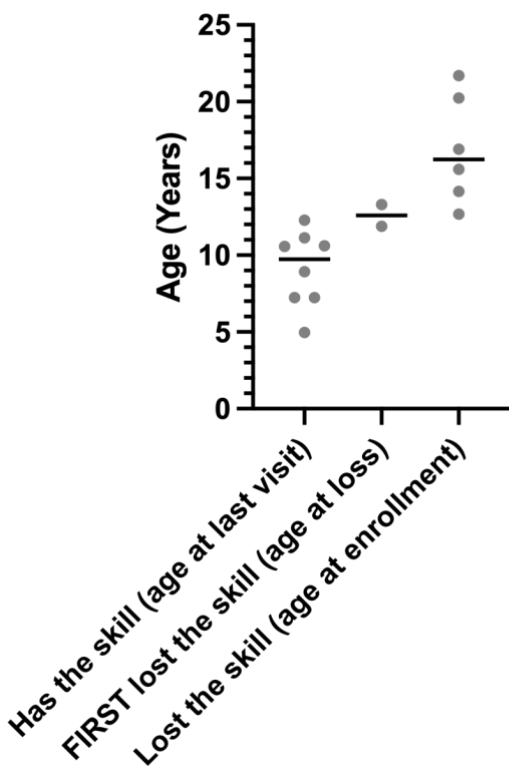**Appendix Table 6B Continued**

*Fluctuations—Regression of Milestone 6*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 66 | 1 | 14.2 | No |
|    | 2 | 14.7 | No |
|    | 3 | 15.3 | No |
|    | 4 | 15.8 | No |
|    | 5 | 16.5 | No |
|    | 6 | 16.7 | Yes |
|    | 7 | 17.2 | Yes |
|    | 8 | 17.7 | No |
|    |   |      |     |
| 67 | 1 | 12.7 | Yes |
|    | 2 | 13.8 | Yes |
|    | 3 | 14.5 | No |
|    | 4 | 15.3 | Yes |
|    | 5 | 15.8 | Yes |
|    | 6 | 16.3 | Yes |

**Early Developmental Milestone 7**

*Pulls self to stand without assistance*

**Appendix Figure 7A**

*Scatter Plot—Regression of Milestone 7*



*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 7B**

*Longitudinal Plot—Regression of Milestone 7*
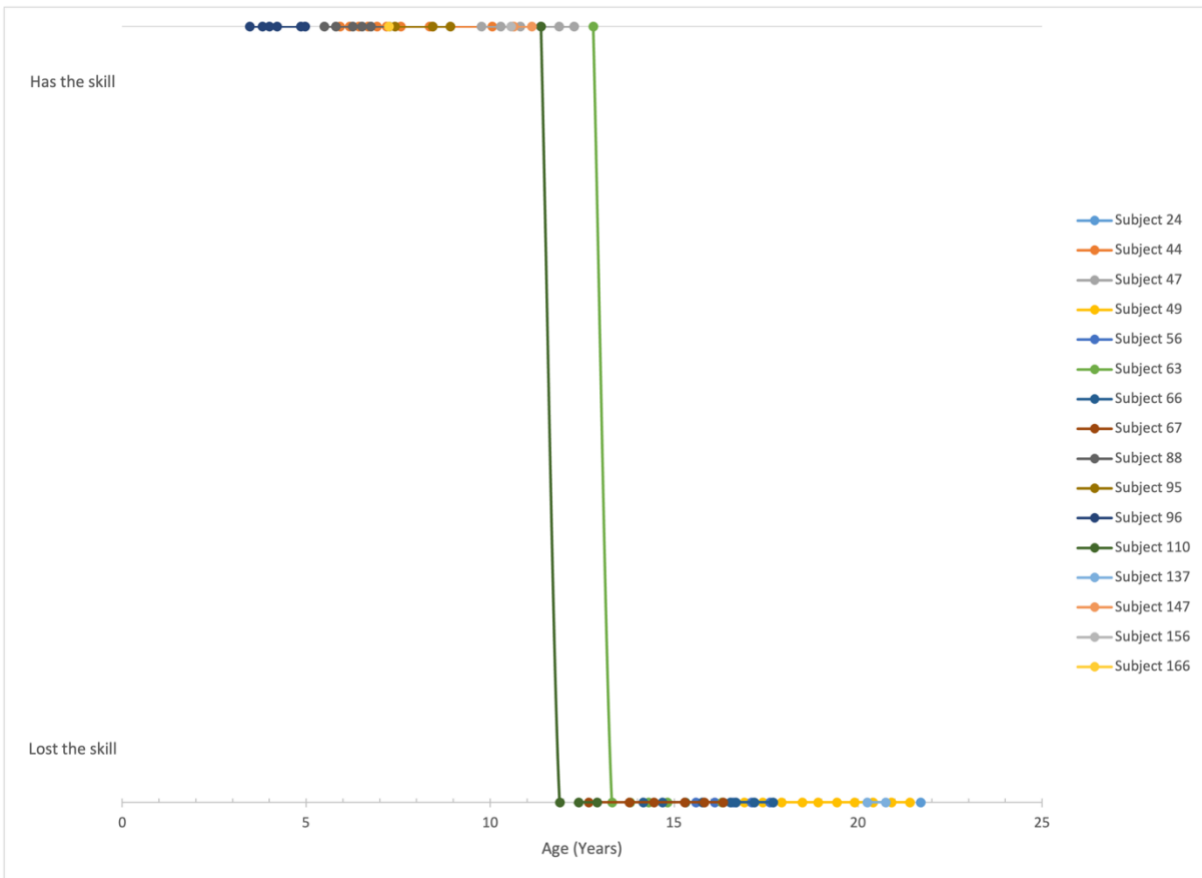


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 7, and a positive slope indicates fluctuations in regression.

**Appendix Table 7**

*Kaplan-Meier Curve Analysis—Regression of Milestone 7*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 11.9 | 100 |
| 11.9 | to | 13.3 | 67 |
| 13.3 | to | - | 0 |

**Appendix Figure 7C**

*Kaplan-Meier Curve—Regression of Milestone 7*
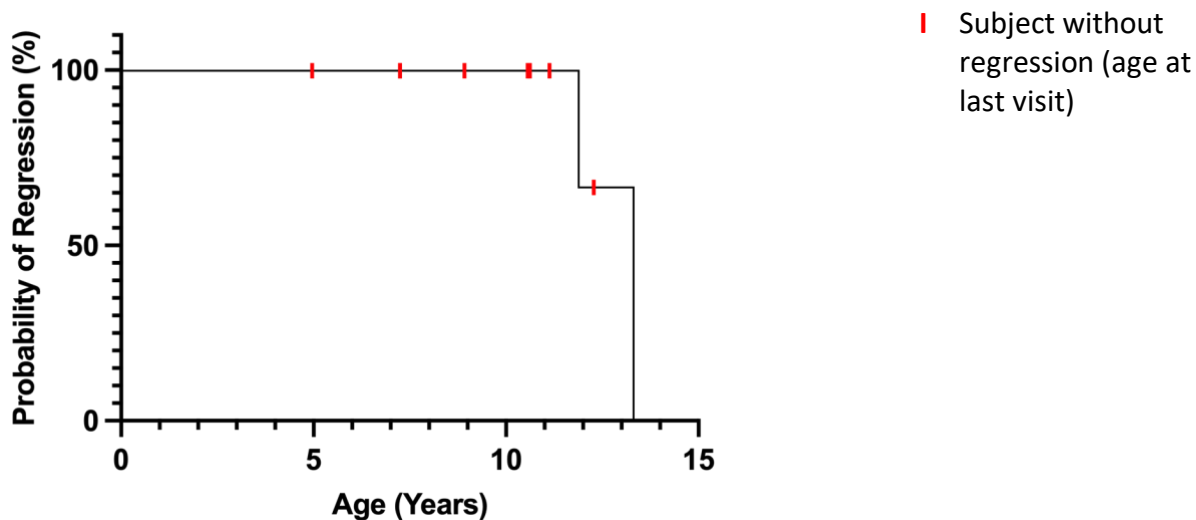


I Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 7 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 7 and Table 6.

**Appendix Figure 7D**

*Grouped Columns—Regression of Milestone 7*
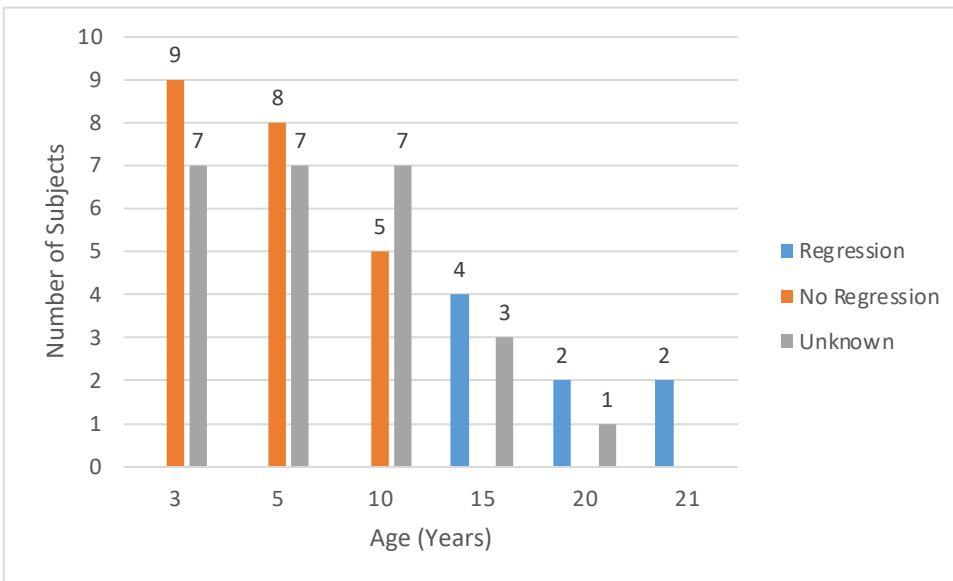


*Note.* At visit 1, if a subject reported no regression to milestone 7, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Early Developmental Milestone 8**

*Get into a sitting position by him/herself (from lying down or standing with support)*

**Appendix Figure 8A**

*Scatter Plot—Regression of Milestone 8*
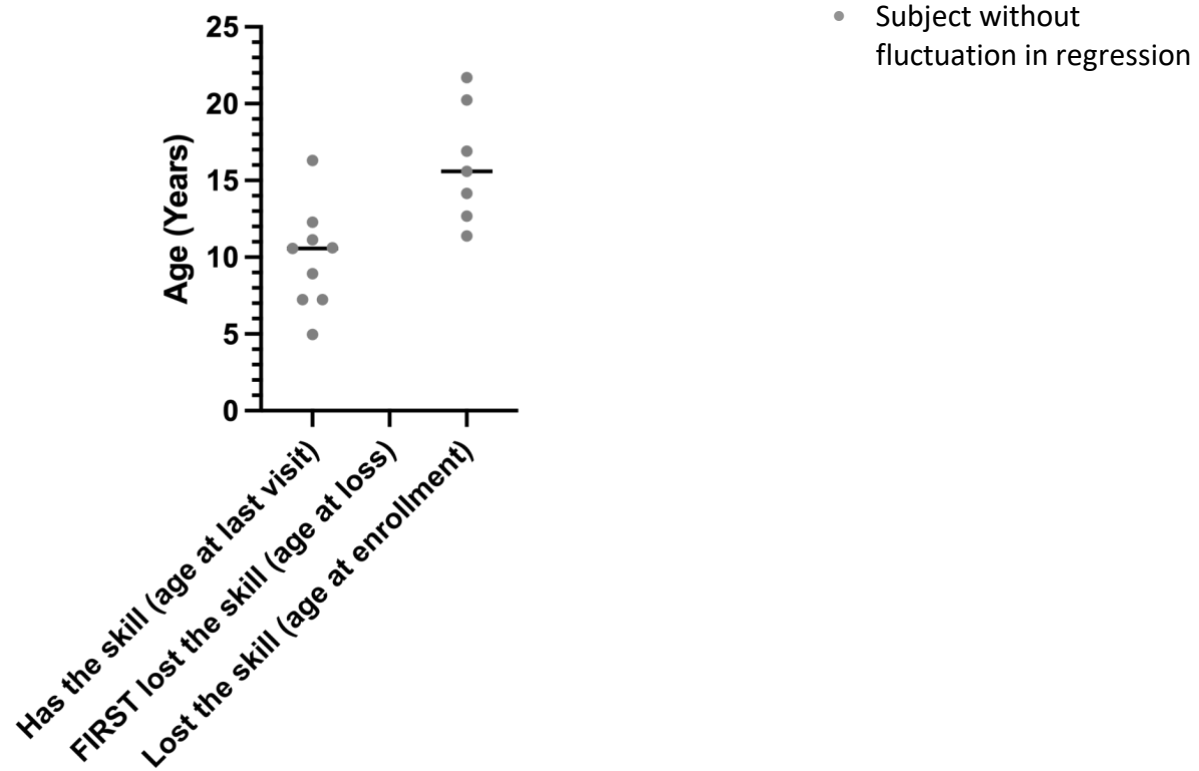


- Subject without fluctuation in regression

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 8B**

*Longitudinal Plot—Regression of Milestone 8*
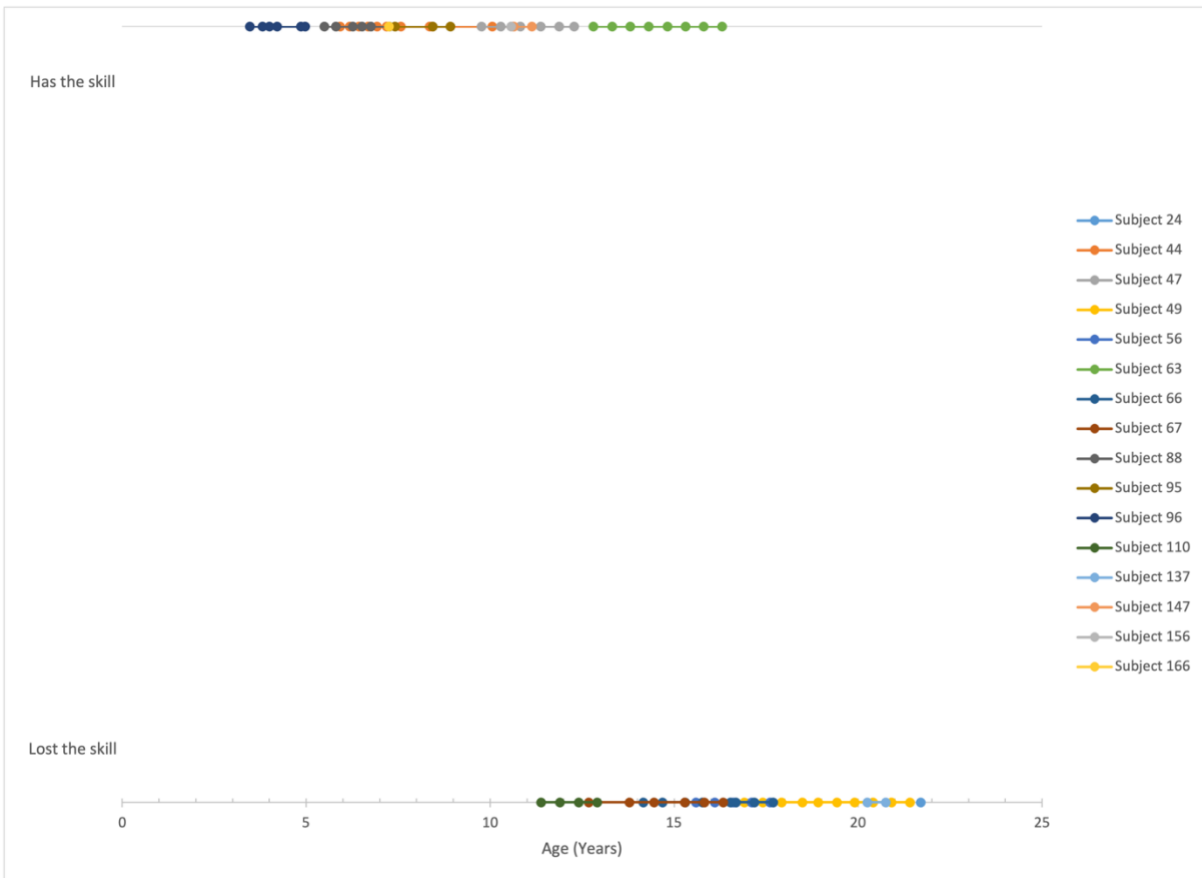


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 8, and a positive slope indicates fluctuations in regression.
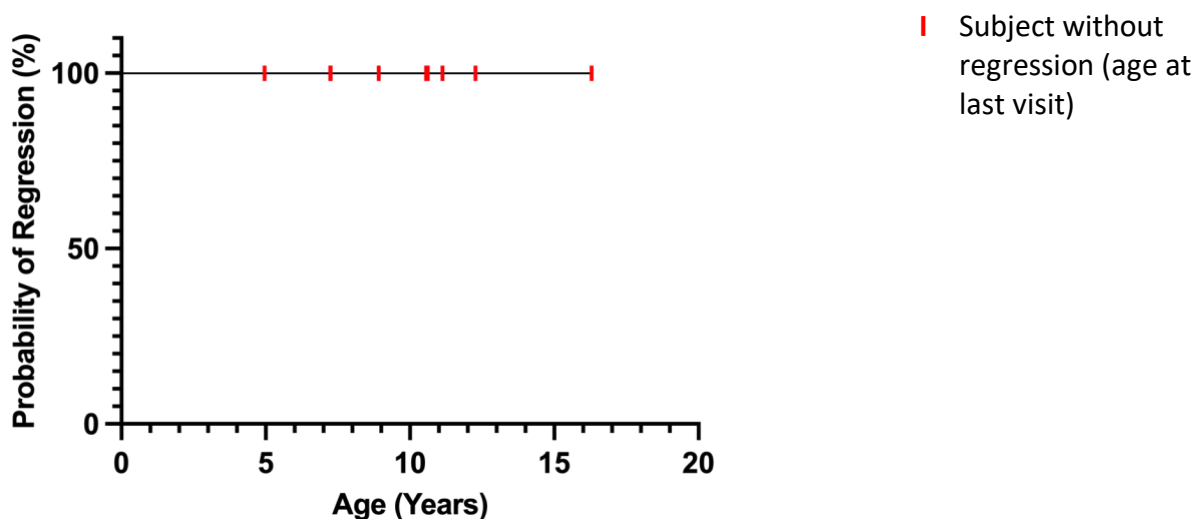
**Appendix Table 8**

*Kaplan-Meier Curve Analysis—Regression of Milestone 8*

| Age Range for Regression (Years) | No Regression (%) |
|---|---|
| 0.0  to  16.3 | 100 |

**Appendix Figure 8C**

*Kaplan-Meier Curve—Regression of Milestone 8*



❚ Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 8 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 8 and Table 6.

**Appendix Figure 8D**

*Grouped Columns—Regression of Milestone 8*
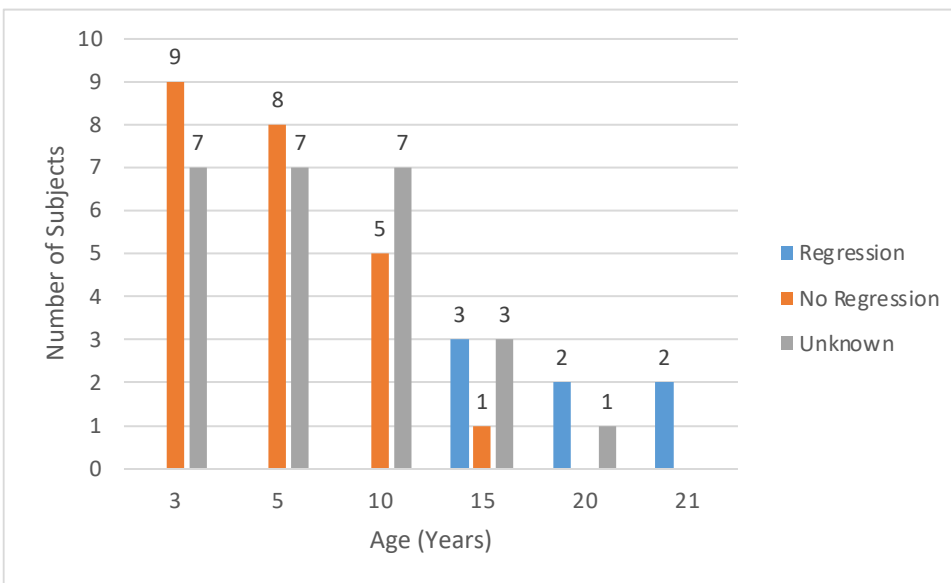


*Note.* At visit 1, if a subject reported no regression to milestone 8, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Early Developmental Milestone 9**

*Walks 5 steps independently (without support)*

**Appendix Figure 9A**

*Scatter Plot—Regression of Milestone 9*
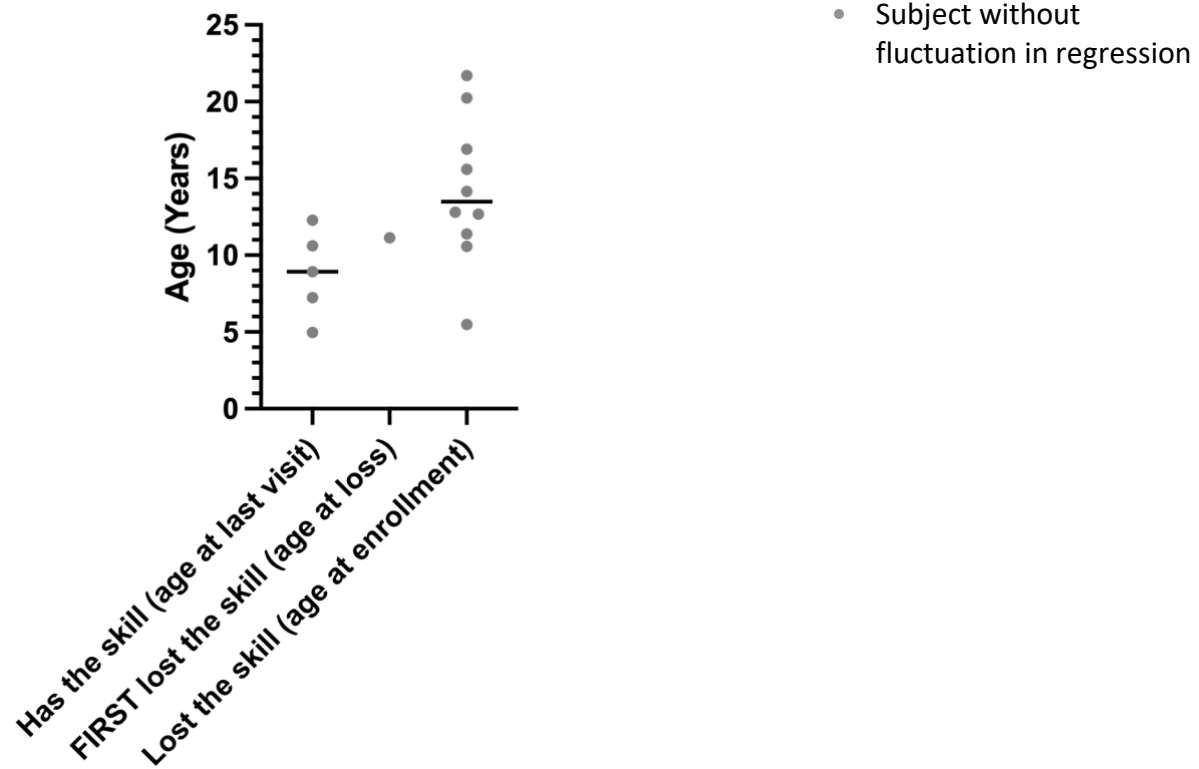


- Subject without fluctuation in regression

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 9B**

*Longitudinal Plot—Regression of Milestone 9*
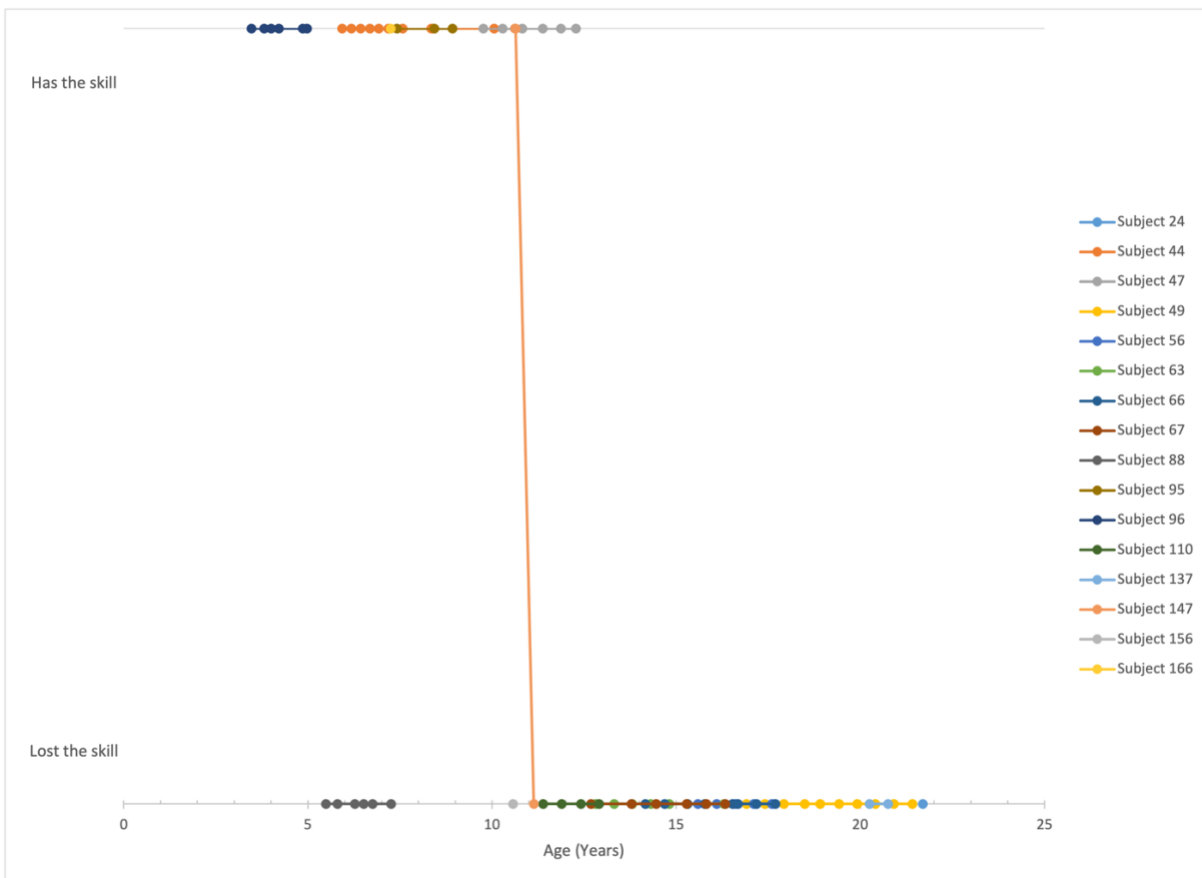


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 9, and a positive slope indicates fluctuations in regression.
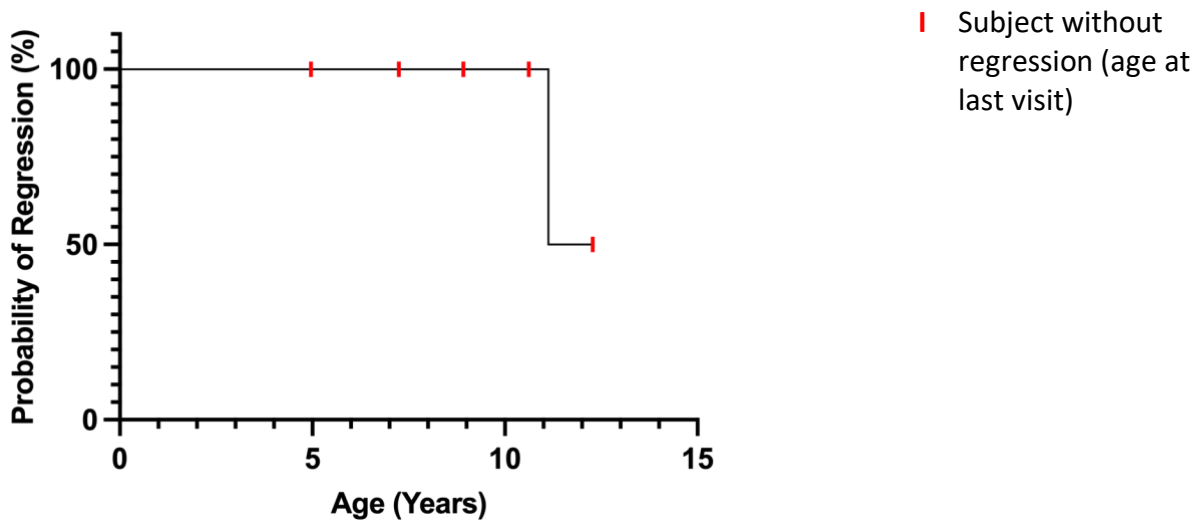
**Appendix Table 9**

*Kaplan-Meier Curve Analysis—Regression of Milestone 9*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 11.1 | 100 |
| 11.1 | to | 12.3 | 50 |

**Appendix Figure 9C**

*Kaplan-Meier Curve—Regression of Milestone 9*



❙ Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 9 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 9 and Table 6.

**Appendix Figure 9D**

*Grouped Columns—Regression of Milestone 9*
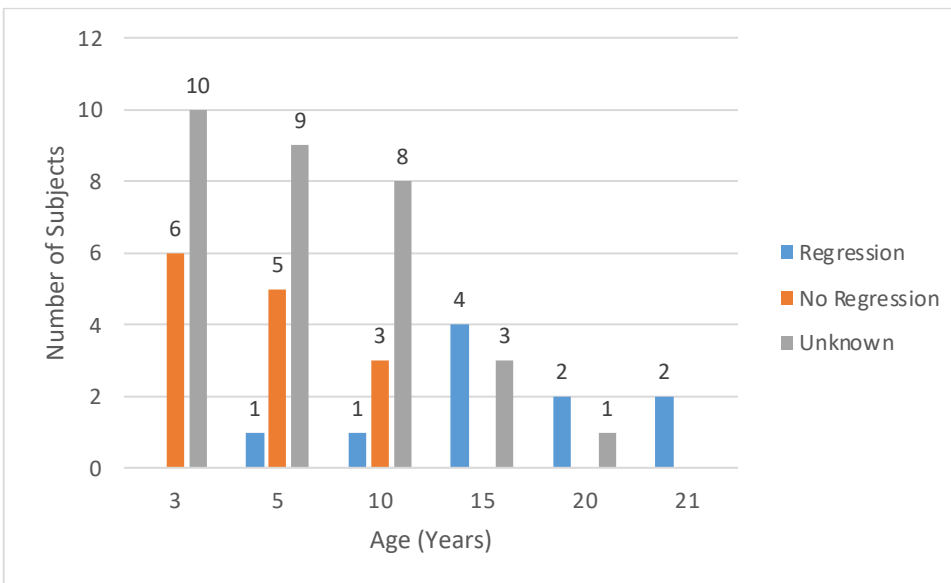


*Note.* At visit 1, if a subject reported no regression to milestone 9, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Early Developmental Milestone 10**

*Stands balanced on one foot for at least one second*

**Appendix Figure 10A**
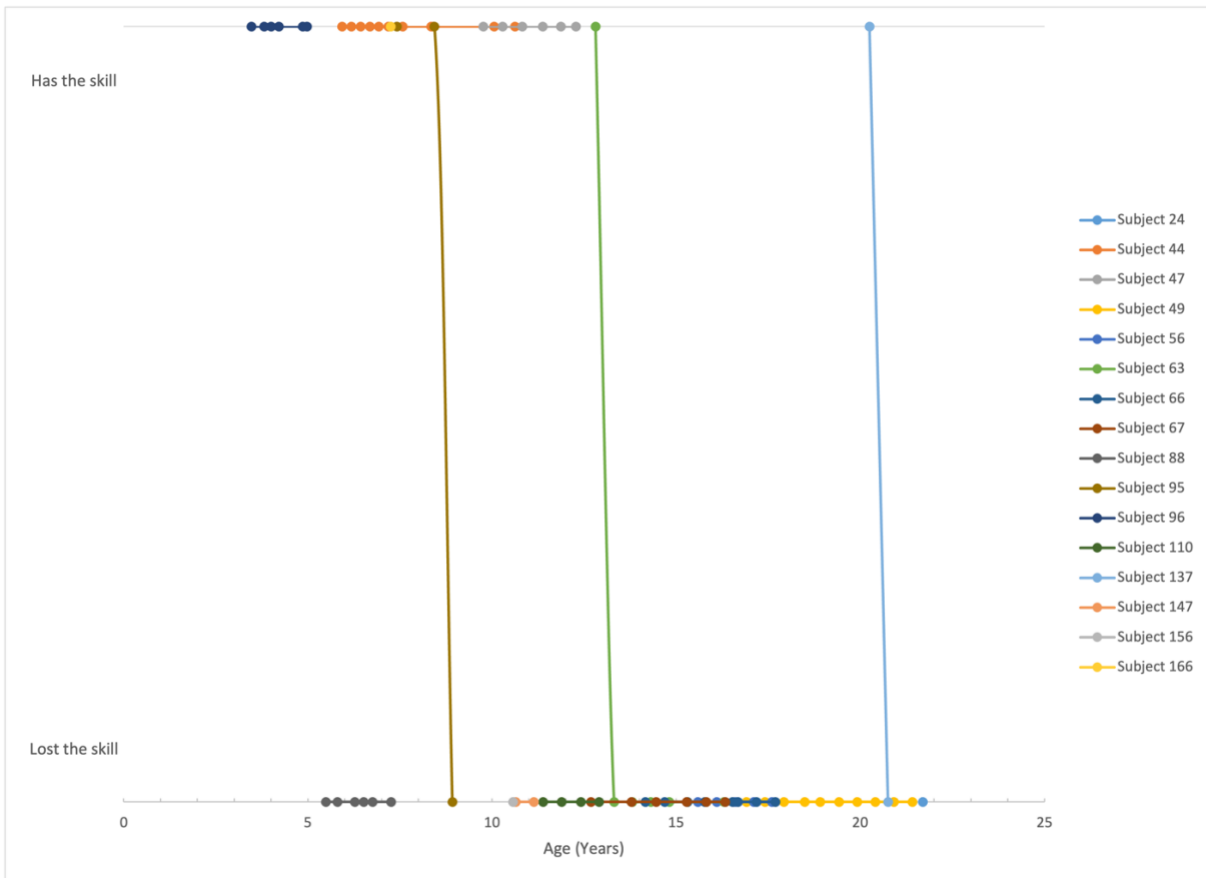
*Scatter Plot—Regression of Milestone 10*
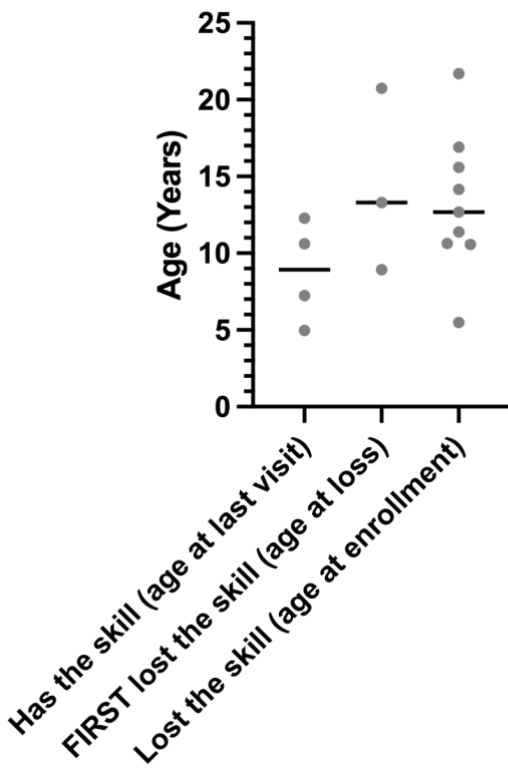


- Subject without fluctuation in regression

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 10B**

*Longitudinal Plot—Regression of Milestone 10*



*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 10, and a positive slope indicates fluctuations in regression.
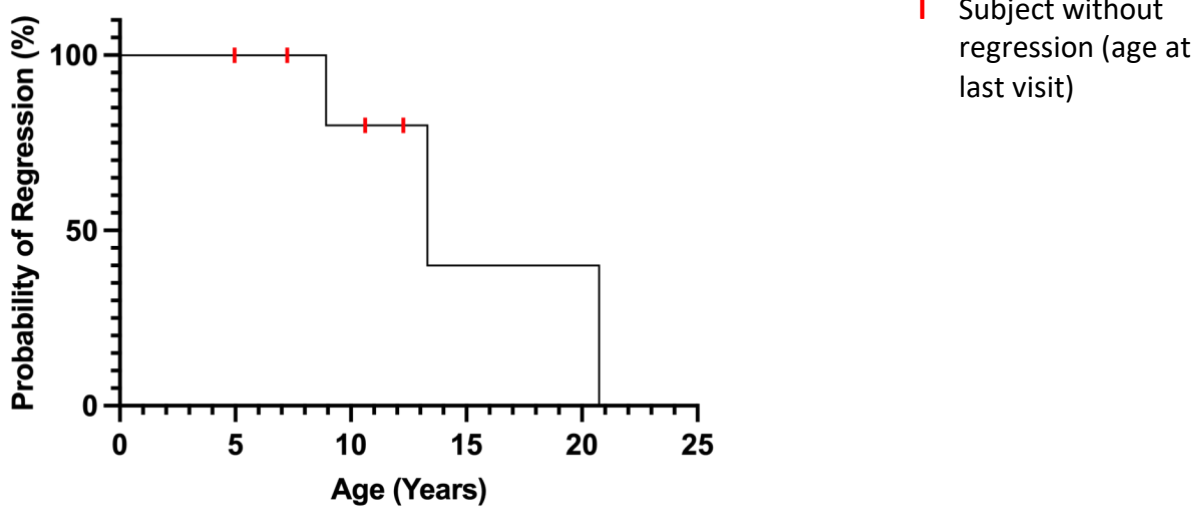
**Appendix Table 10**

*Kaplan-Meier Curve Analysis—Regression of Milestone 10*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 8.9 | 100 |
| 8.9 | to | 13.3 | 80 |
| 13.3 | to | 20.7 | 40 |
| 20.7 | to | - | 0 |

**Appendix Figure 10C**

*Kaplan-Meier Curve—Regression of Milestone 10*



I  Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 10 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 10 and Table 6.

**Appendix Figure 10D**

*Grouped Columns—Regression of Milestone 10*
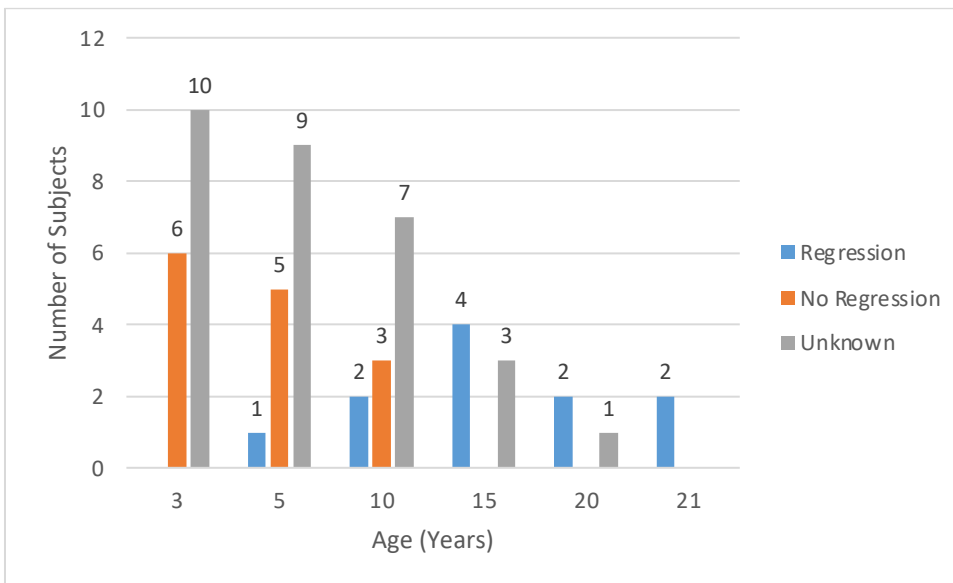


*Note.* At visit 1, if a subject reported no regression to milestone 10, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Early Developmental Milestone 11**

*Smiles back in response to being smiled at or spoken to (but not touched)*

**Appendix Figure 11A**

*Scatter Plot—Regression of Milestone 11*
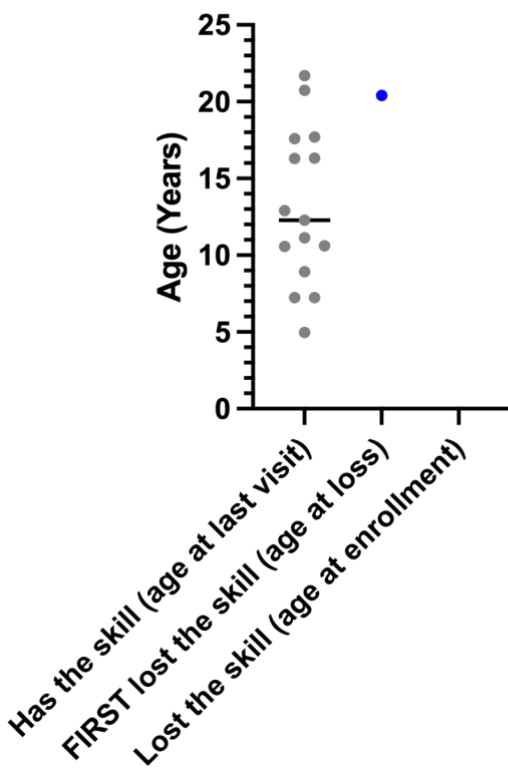


- Subject without fluctuation in regression

- Subject with fluctuation in regression (Appendix Table 11B)

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 11B**

*Longitudinal Plot—Regression of Milestone 11*
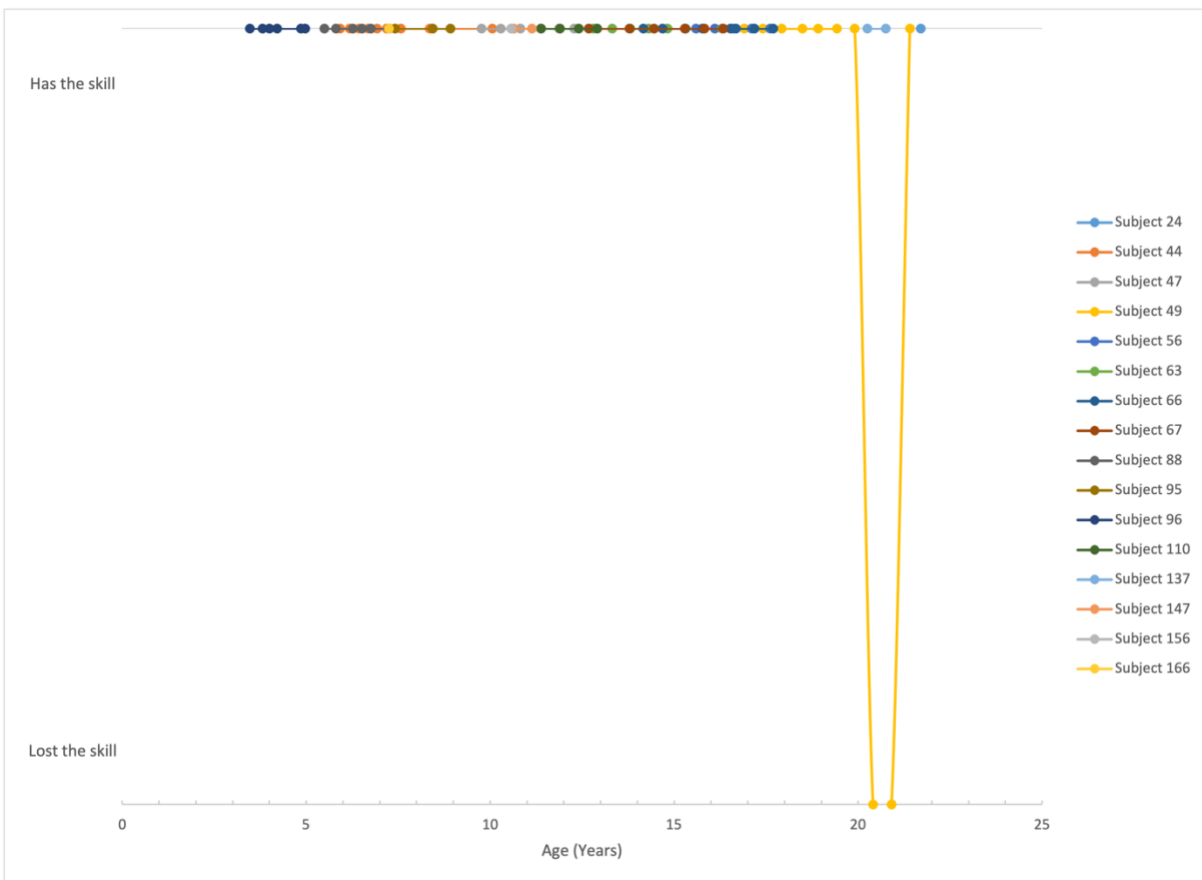


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 11, and a positive slope indicates fluctuations in regression (Appendix Table 11B).
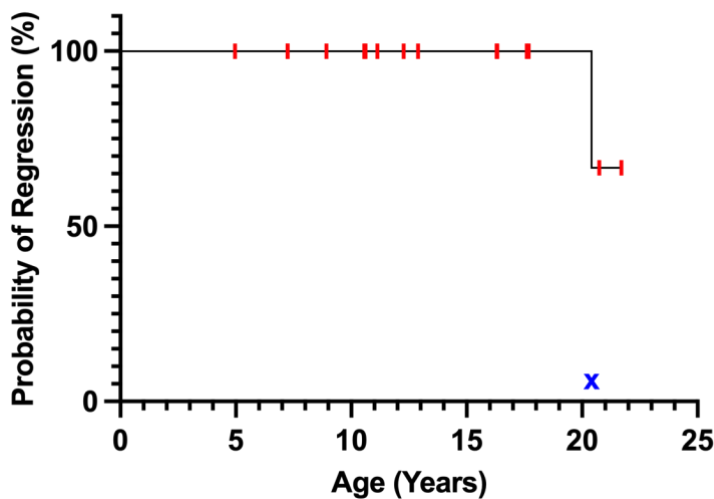
**Appendix Table 11A**

*Kaplan-Meier Curve Analysis—Regression of Milestone 11*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 20.4 | 100 |
| 20.4 | to | 21.7 | 67 |

**Appendix Figure 11C**

*Kaplan-Meier Curve—Regression of Milestone 11*



**I** Subject without regression (age at last visit)

**X** Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 11B).

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 11 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 11B and Table 6.

**Appendix Figure 11D**

*Grouped Columns—Regression of Milestone 11*
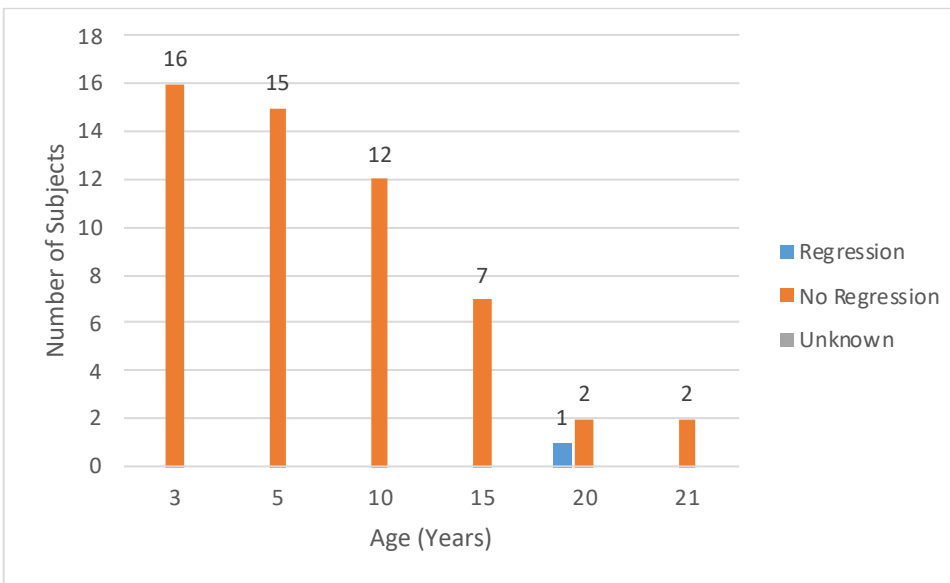


*Note.* At visit 1, if a subject reported no regression to milestone 11, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 11B**

*Fluctuations—Regression of Milestone 11*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 49      | 1     | 16.9        | No         |
|         | 2     | 17.4        | No         |
|         | 3     | 17.9        | No         |
|         | 4     | 18.5        | No         |
|         | 5     | 18.9        | No         |
|         | 6     | 19.4        | No         |
|         | 7     | 19.9        | No         |
|         | 8     | 20.4        | Yes        |
|         | 9     | 20.9        | Yes        |
|         | 10    | 21.4        | No         |

123

**Early Developmental Milestone 12**

*Can child indicate a want to parent without crying? Can include pointing, reaching, making sounds, moving arms, pulling, saying a word, others*

**Appendix Figure 12A**

*Scatter Plot—Regression of Milestone 12*
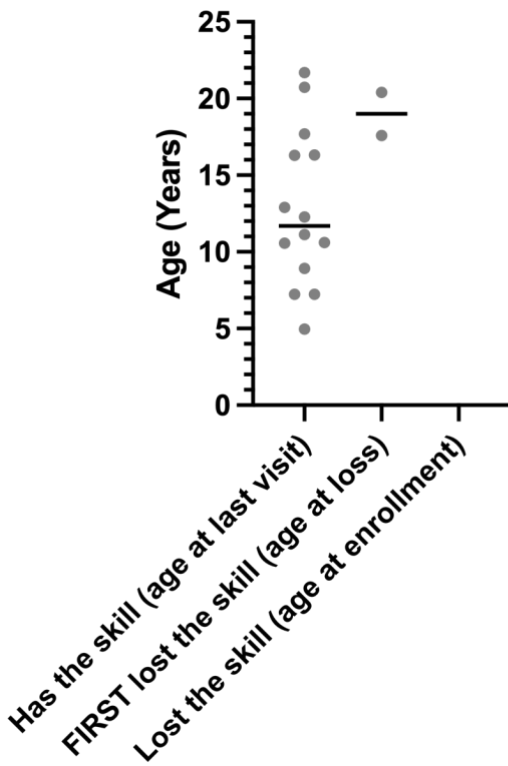


• Subject without fluctuation in regression

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 12B**

*Longitudinal Plot—Regression of Milestone 12*
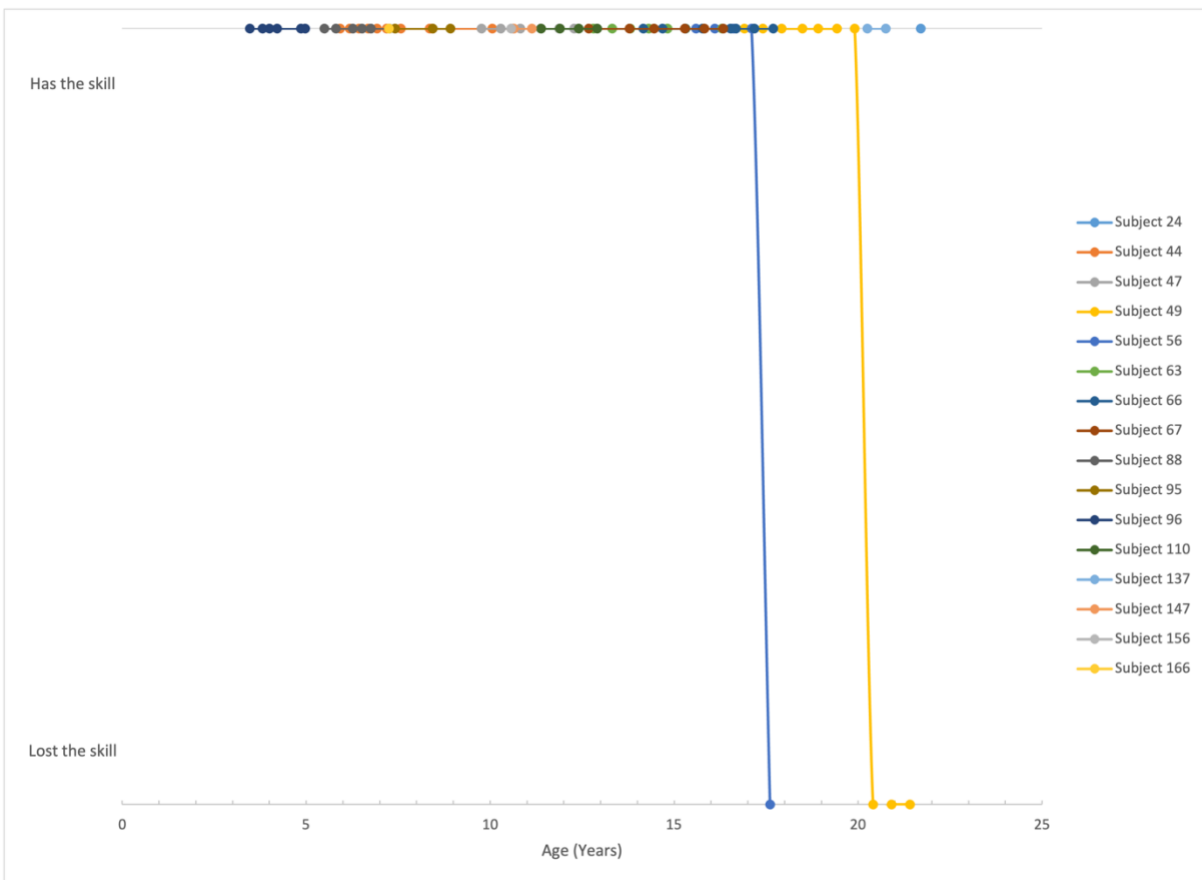


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 12, and a positive slope indicates fluctuations in regression.

**Appendix Table 12**

*Kaplan-Meier Curve Analysis—Regression of Milestone 12*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 17.6 | 100 |
| 17.6 | to | 20.4 | 80 |
| 20.4 | to | 21.7 | 53 |

**Appendix Figure 12C**

*Kaplan-Meier Curve—Regression of Milestone 12*
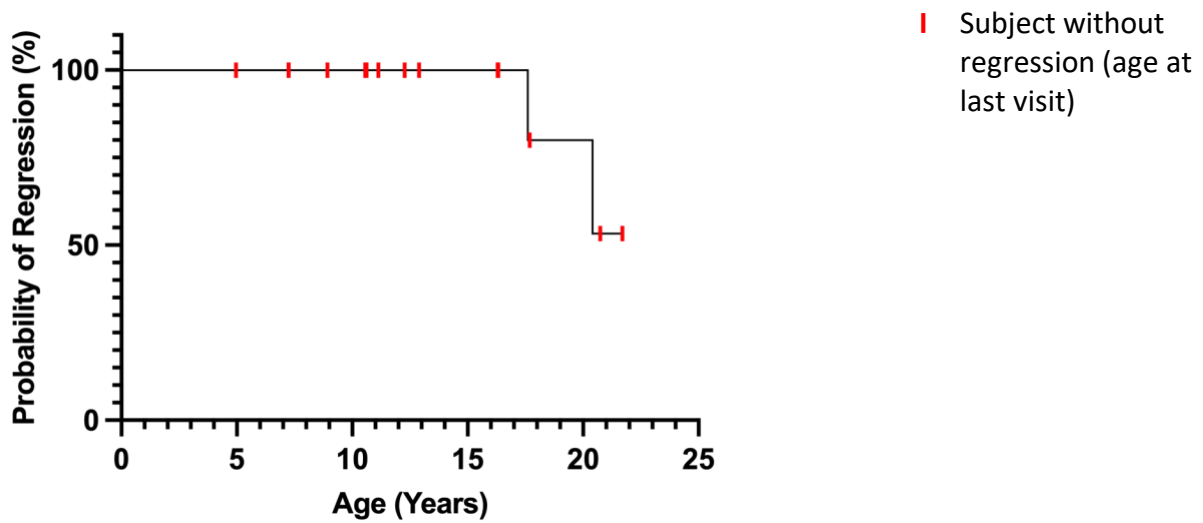


| Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 12 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 12 and Table 6.

**Appendix Figure 12D**

*Grouped Columns—Regression of Milestone 12*
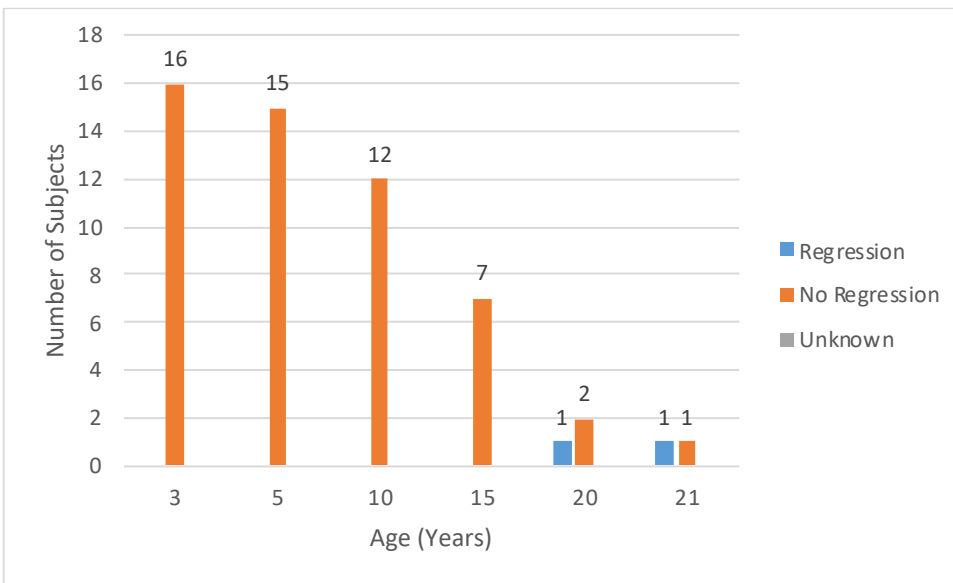


*Note.* At visit 1, if a subject reported no regression to milestone 12, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Early Developmental Milestone 13**

*Waves 'bye-bye' when someone waves to him/her*

**Appendix Figure 13A**

*Scatter Plot—Regression of Milestone 13*
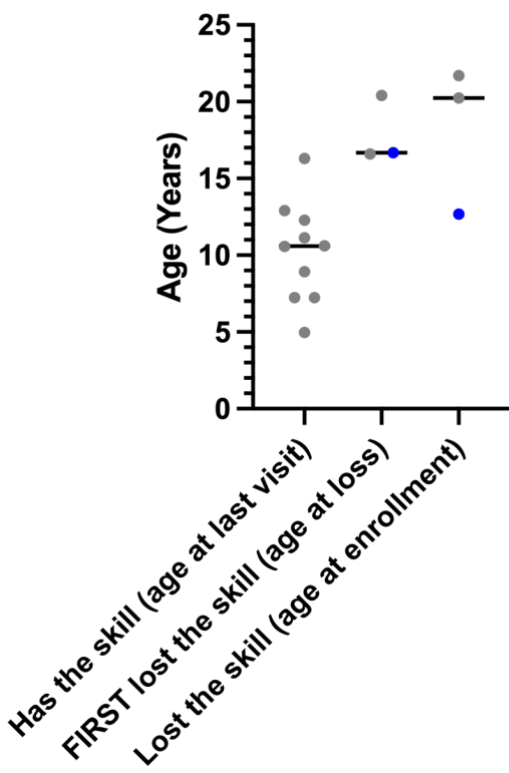


- Subject without fluctuation in regression

- Subject with fluctuation in regression (Appendix Table 13B)

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 13B**

*Longitudinal Plot—Regression of Milestone 13*
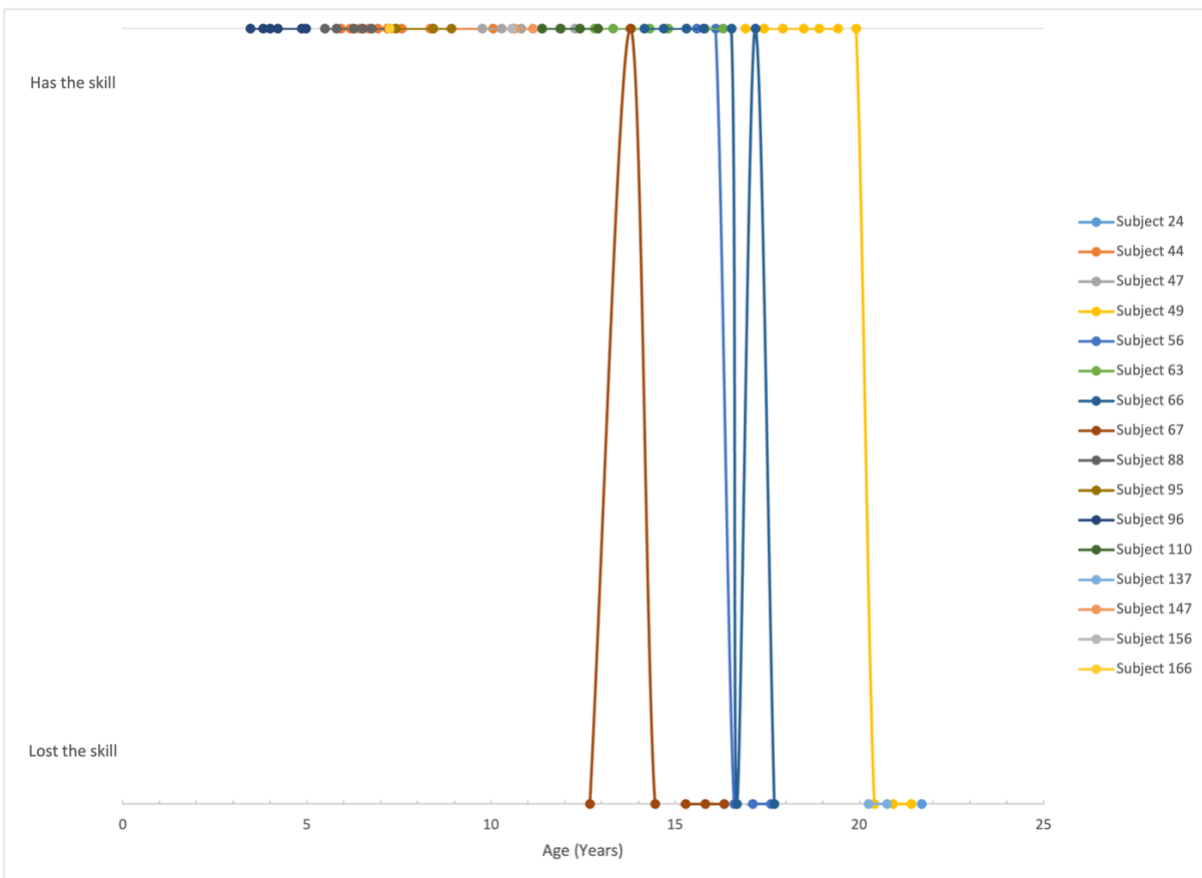


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 13, and a positive slope indicates fluctuations in regression (Appendix Table 13B).

**Appendix Table 13A**

*Kaplan-Meier Curve Analysis—Regression of Milestone 13*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 16.6 | 100 |
| 16.6 | to | 16.7 | 67 |
| 16.7 | to | 20.4 | 33 |
| 20.4 | to | - | 0 |

**Appendix Figure 13C**

*Kaplan-Meier Curve—Regression of Milestone 13*
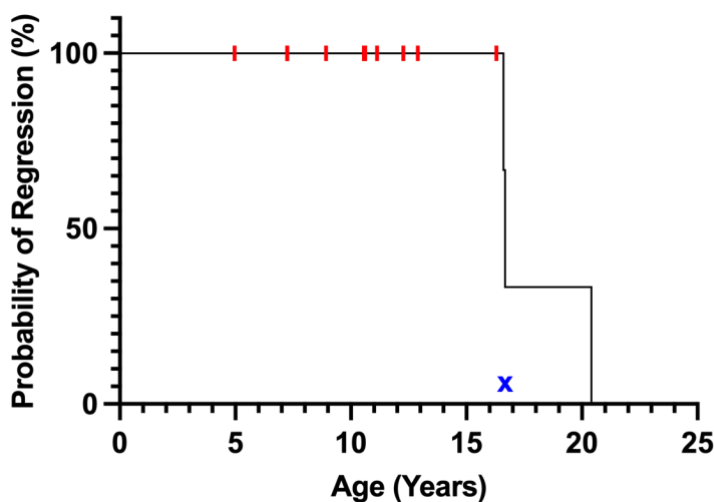


**I** Subject without regression (age at last visit)

**X** Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 13B).

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 13 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 13A and Table 6.

**Appendix Figure 13D**

*Grouped Columns—Regression of Milestone 13*
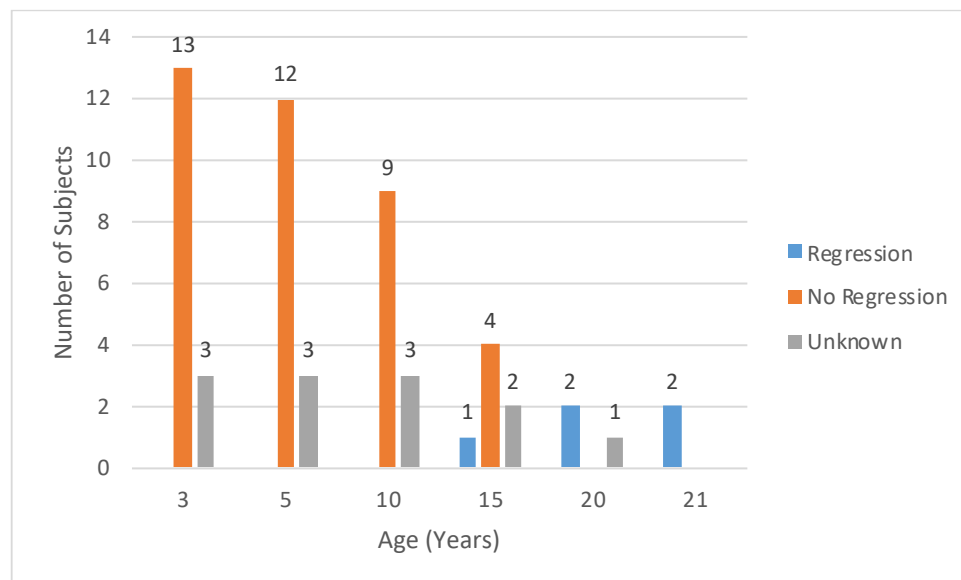


*Note.* At visit 1, if a subject reported no regression to milestone 13, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 13B**

*Fluctuations—Regression of Milestone 13*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 66      | 1     | 14.2        | No         |
|         | 2     | 14.7        | No         |
|         | 3     | 15.3        | No         |
|         | 4     | 15.8        | No         |
|         | 5     | 16.5        | No         |
|         | 6     | 16.7        | Yes        |
|         | 7     | 17.2        | No         |
|         | 8     | 17.7        | Yes        |
|         |       |             |            |
| 67      | 1     | 12.7        | Yes        |

**Appendix Table 13B Continued**

*Fluctuations—Regression of Milestone 13*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
|         | 2     | 13.8        | No         |
|         | 3     | 14.5        | Yes        |
|         | 4     | 15.3        | Yes        |
|         | 5     | 15.8        | Yes        |
|         | 6     | 16.3        | Yes        |

**Early Developmental Milestone 14**

*Feeds him/herself finger foods WITHOUT help*

**Appendix Figure 14A**

*Scatter Plot—Regression of Milestone 14*
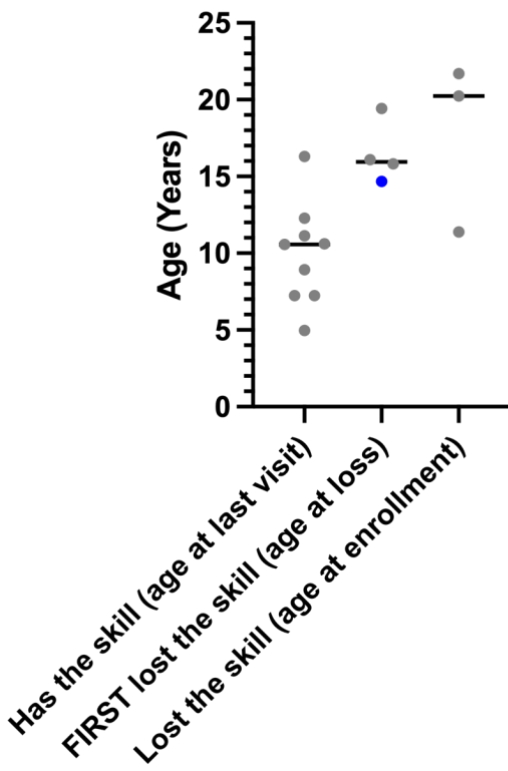


- Subject without fluctuation in regression

- Subject with fluctuation in regression (Appendix Table 14B)

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 14B**

*Longitudinal Plot—Regression of Milestone 14*
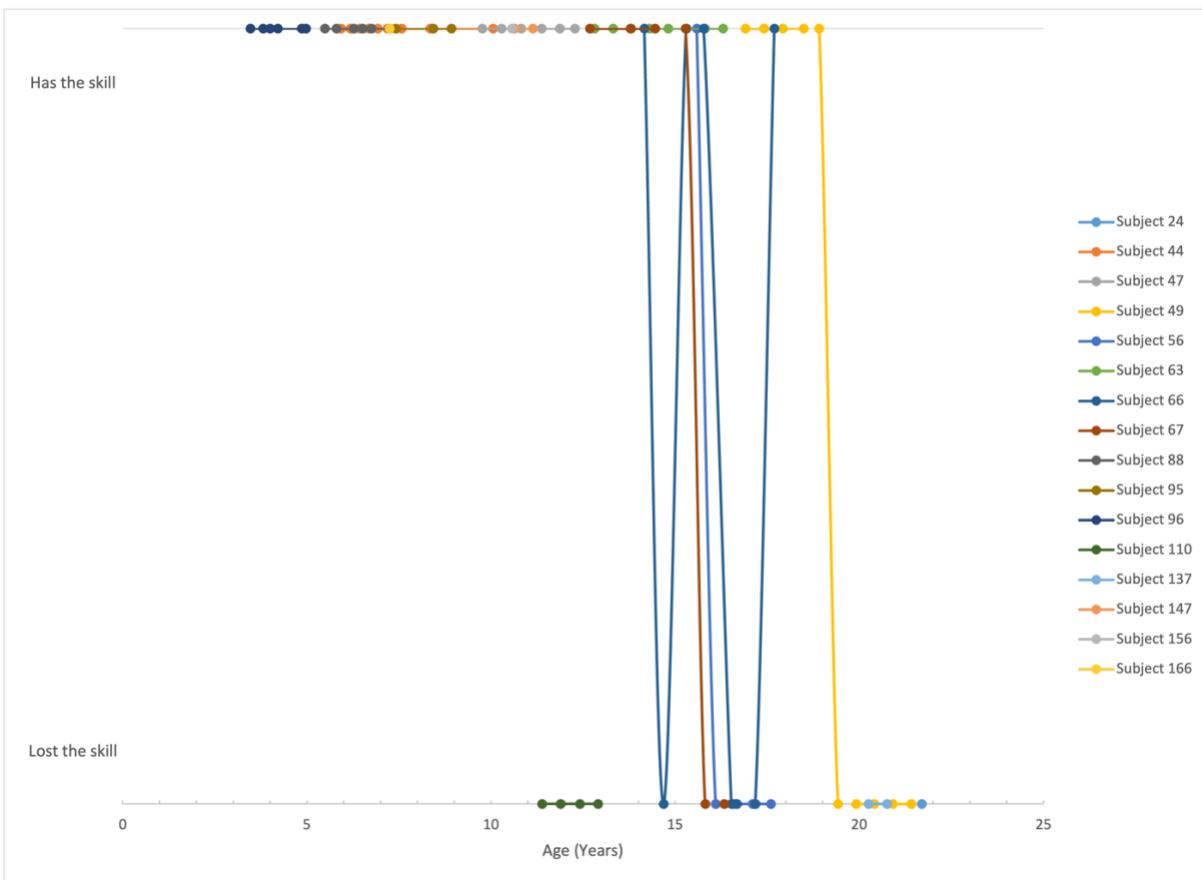


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 14, and a positive slope indicates fluctuations in regression (Appendix Table 14B).

**Appendix Table 14A**

*Kaplan-Meier Curve Analysis—Regression of Milestone 14*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 14.7 | 100 |
| 14.7 | to | 15.8 | 80 |
| 15.8 | to | 16.1 | 60 |
| 16.1 | to | 19.4 | 40 |
| 19.4 | to | - | 0 |

**Appendix Figure 14C**

*Kaplan-Meier Curve—Regression of Milestone 14*
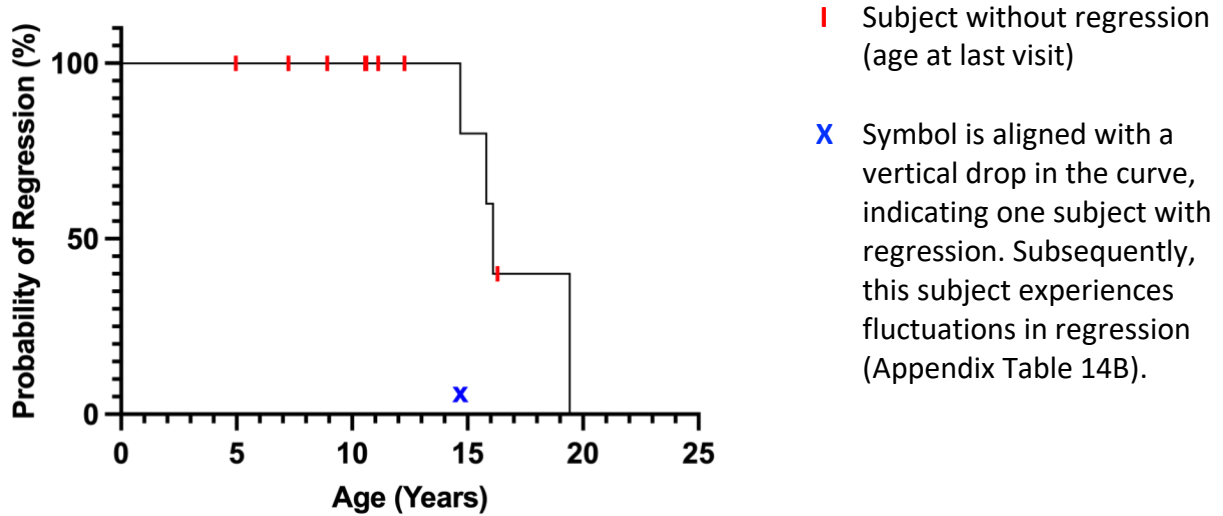


**I**    Subject without regression (age at last visit)

**X**    Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 14B).

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 14 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 14A and Table 6.

**Appendix Figure 14D**

*Grouped Columns—Regression of Milestone 14*



*Note.* At visit 1, if a subject reported no regression to milestone 14, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ag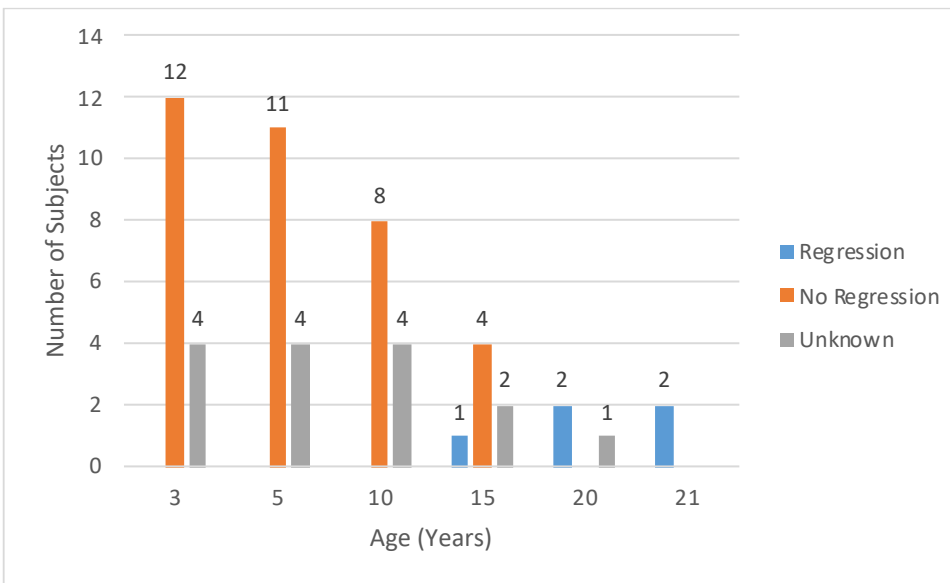es 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 14B**

*Fluctuations—Regression of Milestone 14*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 66      | 1     | 14.2        | No         |
|         | 2     | 14.7        | Yes        |
|         | 3     | 15.3        | No         |
|         | 4     | 15.8        | No         |
|         | 5     | 16.5        | Yes        |
|         | 6     | 16.7        | Yes        |
|         | 7     | 17.2        | Yes        |
|         | 8     | 17.7        | No         |

**Early Developmental Milestone 15**

*Feeds him/herself with fork or spoon without help and gets most of the food into his/her mouth*

**Appendix Figure 15A**

*Scatter Plot—Regression of Milestone 15*
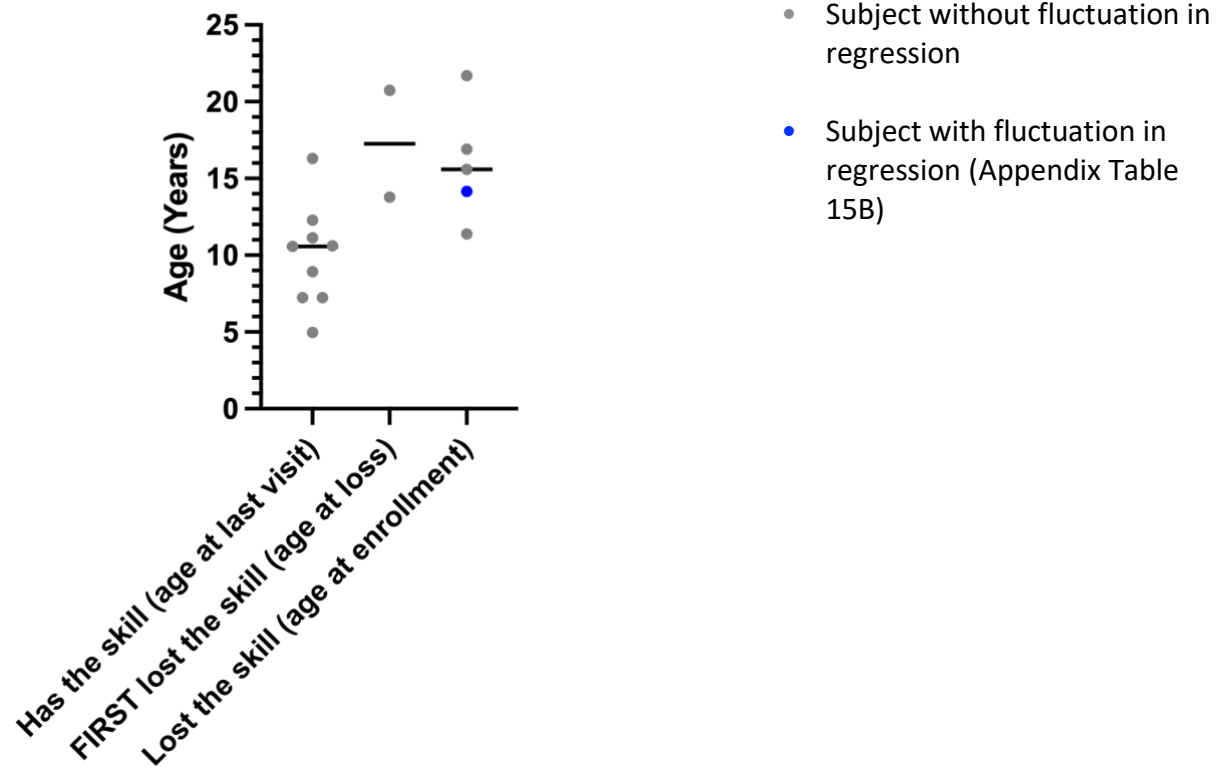


- Subject without fluctuation in regression

- Subject with fluctuation in regression (Appendix Table 15B)

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 15B**

*Longitudinal Plot—Regression of Milestone 15*
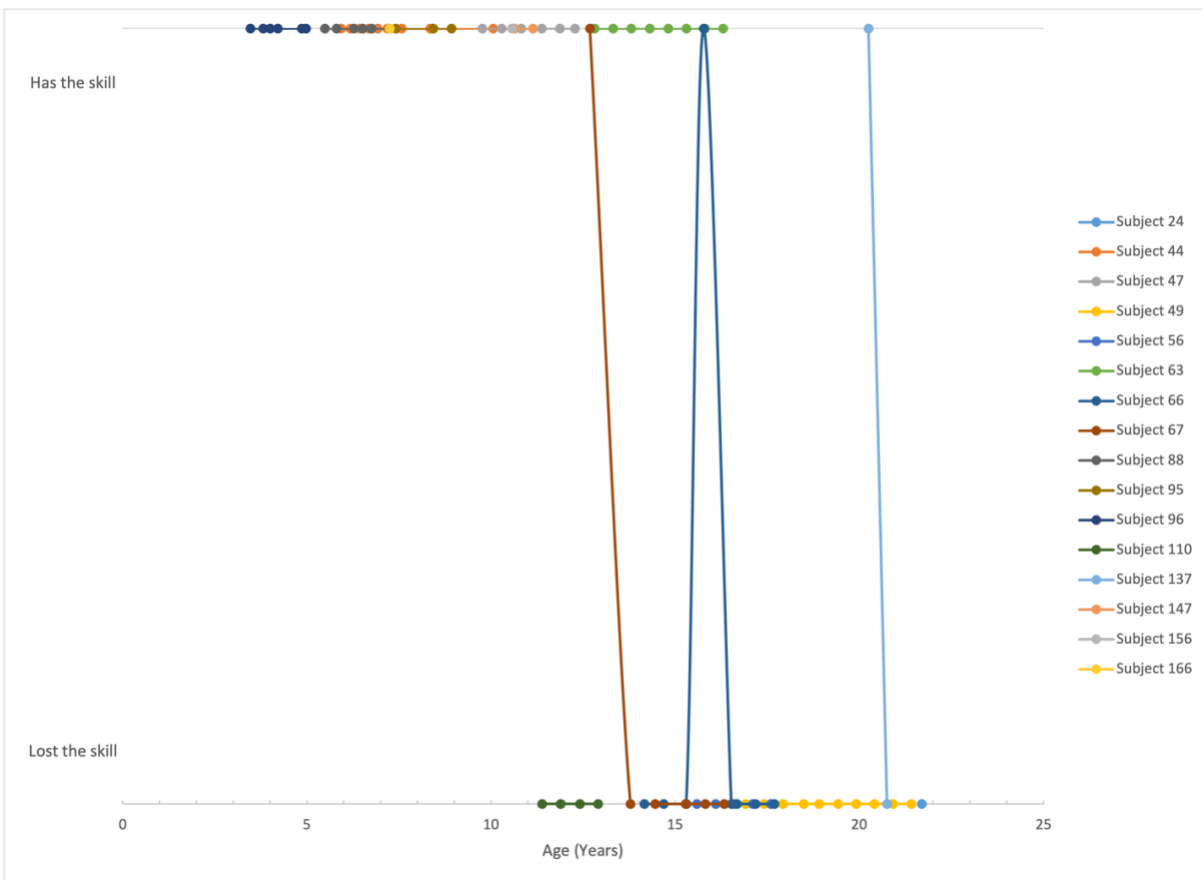


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 15, and a positive slope indicates fluctuations in regression (Appendix Table 15B).

**Appendix Table 15A**

*Kaplan-Meier Curve Analysis—Regression of Milestone 15*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 13.8 | 100 |
| 13.8 | to | 20.7 | 67 |
| 20.7 | to | - | 0 |

**Appendix Figure 15C**

*Kaplan-Meier Curve—Regression of Milestone 15*
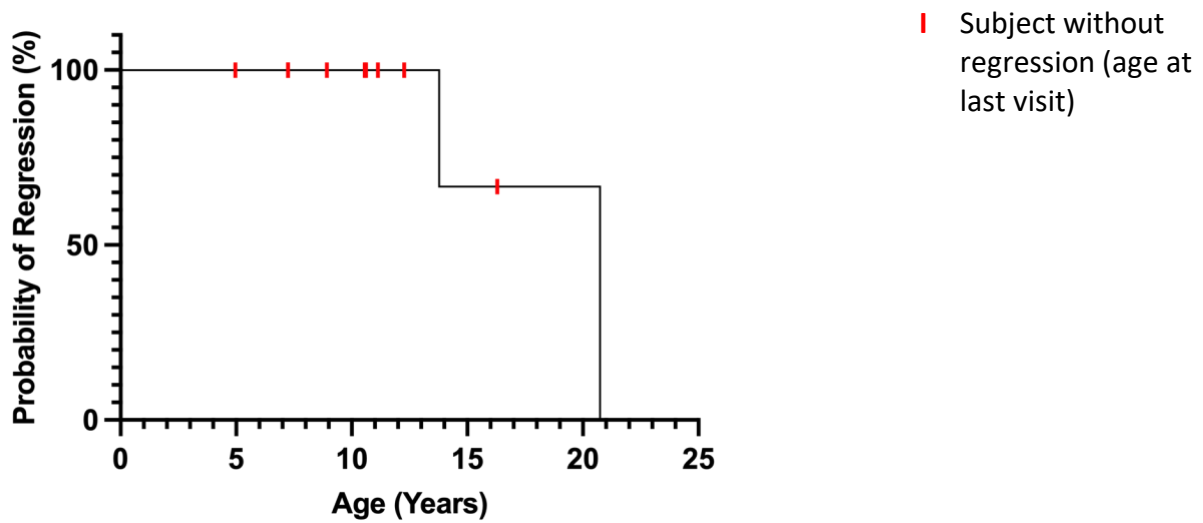


I Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 15 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 15A and Table 6.

**Appendix Figure 15D**

*Grouped Columns—Regression of Milestone 15*
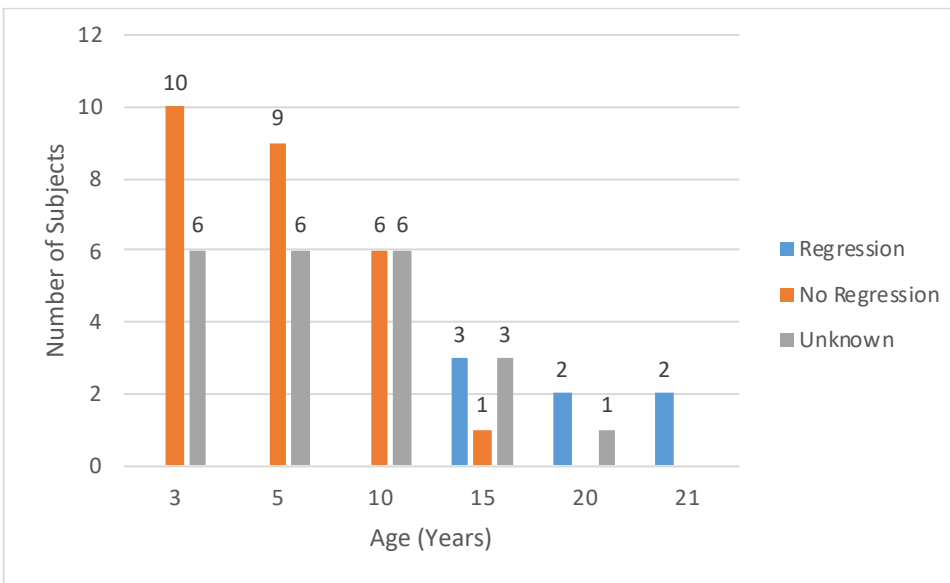


*Note.* At visit 1, if a subject reported no regression to milestone 15, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 15B**

*Fluctuations—Regression of Milestone 15*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 66      | 1     | 14.2        | Yes        |
|         | 2     | 14.7        | Yes        |
|         | 3     | 15.3        | Yes        |
|         | 4     | 15.8        | No         |
|         | 5     | 16.5        | Yes        |
|         | 6     | 16.7        | Yes        |
|         | 7     | 17.2        | Yes        |
|         | 8     | 17.7        | Yes        |

**Early Developmental Milestone 16**

*Vocalizes - small throaty sounds or short vowel sounds (crying does not count)*

**Appendix Figure 16A**

*Scatter Plot: Regression of Milestone 16*
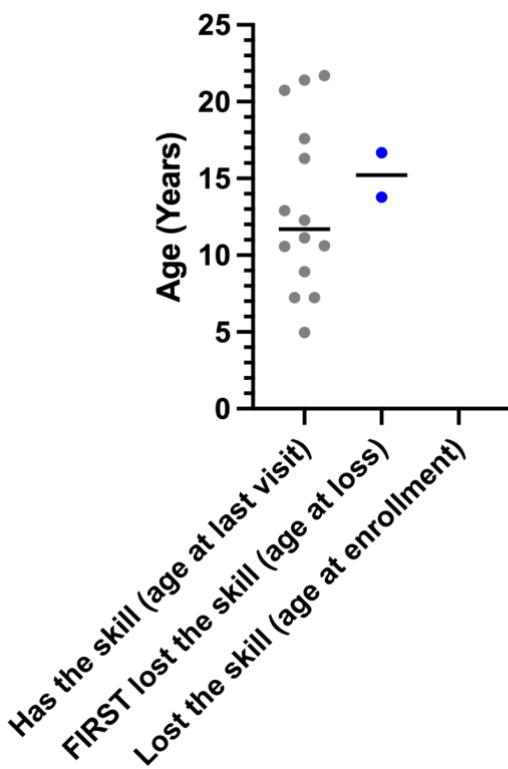


- Subject without fluctuation in regression

- Subject with fluctuation in regression (Appendix Table 16B)

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 16B**

*Longitudinal Plot—Regression of Milestone 16*
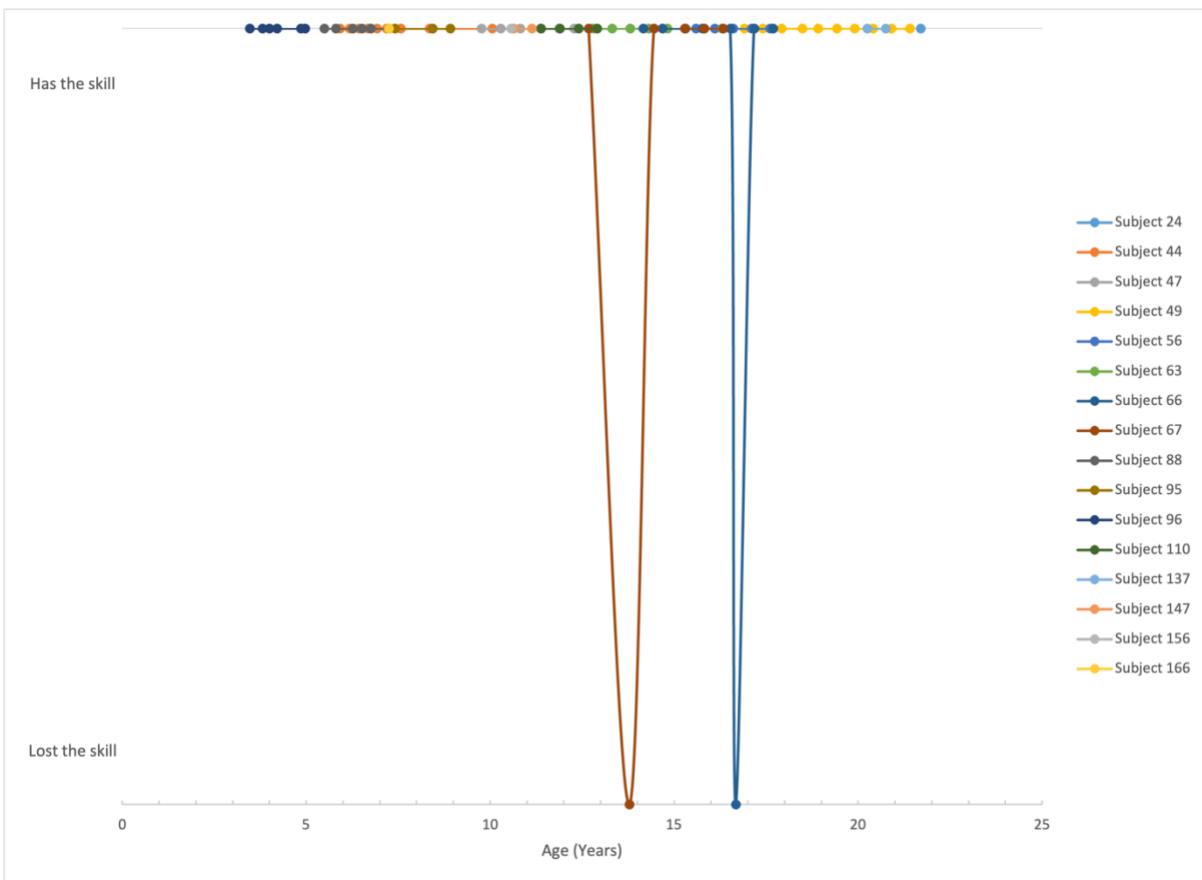


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 16, and a positive slope indicates fluctuations in regression (Appendix Table 16B).

**Appendix Table 16A**

*Kaplan-Meier Curve Analysis—Regression of Milestone 16*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 13.8 | 100 |
| 13.8 | to | 16.7 | 86 |
| 16.7 | to | 21.7 | 69 |

**Appendix Figure 16C**

*Kaplan-Meier Curve—Regression of Milestone 16*
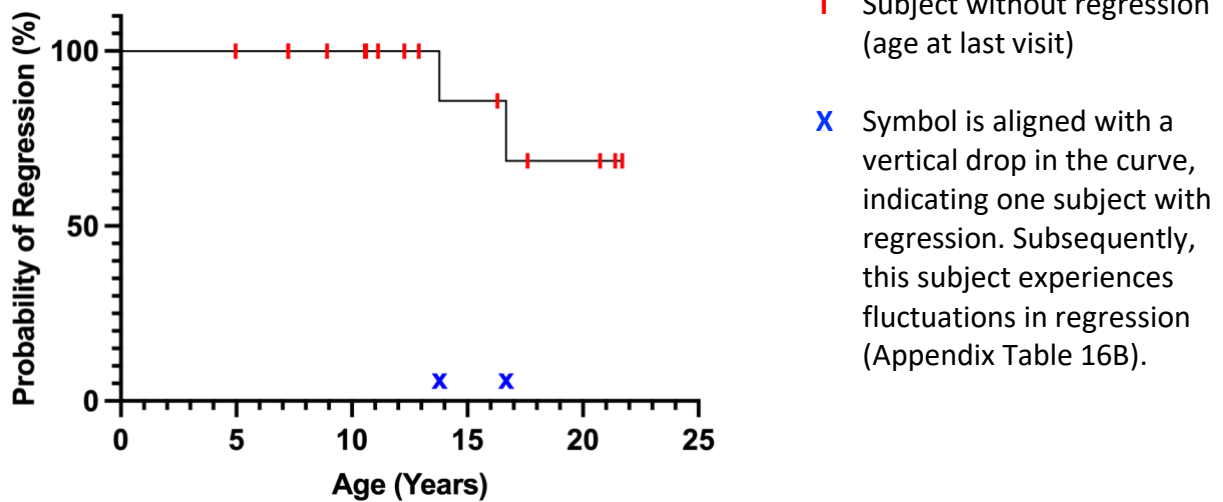


**I** Subject without regression (age at last visit)

**X** Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 16B).

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 16 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 16B and Table 6.

**Appendix Figure 16D**

*Grouped Columns—Regression of Milestone 16*



*Note.* At visit 1, if a subject reported no regression to milestone 16, they were assumed to have had no prior regression and were marked ac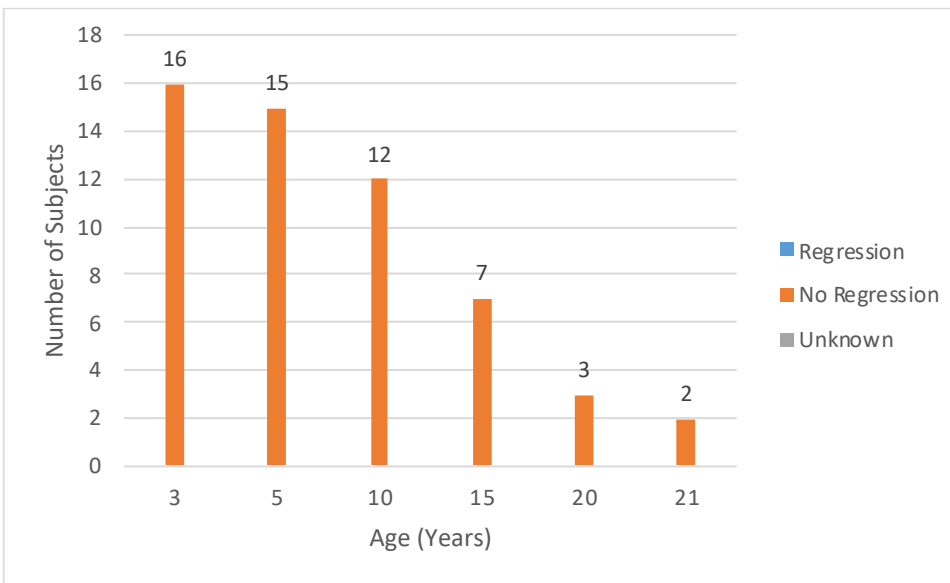cordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 16B**

*Fluctuations—Regression of Milestone 16*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 66      | 1     | 14.2        | No         |
|         | 2     | 14.7        | No         |
|         | 3     | 15.3        | No         |
|         | 4     | 15.8        | No         |
|         | 5     | 16.5        | No         |
|         | 6     | 16.7        | Yes        |
|         | 7     | 17.2        | No         |
|         | 8     | 17.7        | No         |
| 67      | 1     | 12.7        | No         |

**Appendix Table 16B Continued**

*Fluctuations—Regression of Milestone 16*

| Subject | Visit | Age (Years) | Regression |
|---|---|---|---|
| | 2 | 13.8 | Yes |
| | 3 | 14.5 | No |
| | 4 | 15.3 | No |
| | 5 | 15.8 | No |
| | 6 | 16.3 | No |

**Early Developmental Milestone 17**

*Laughs out loud*

**Appendix Figure 17A**

*Scatter Plot: Regression of Milestone 17*
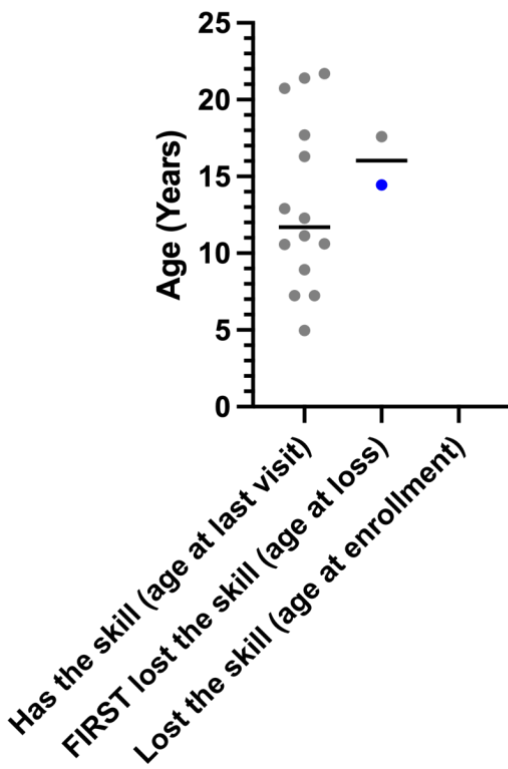


*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 17B**

*Longitudinal Plot—Regression of Milestone 17*
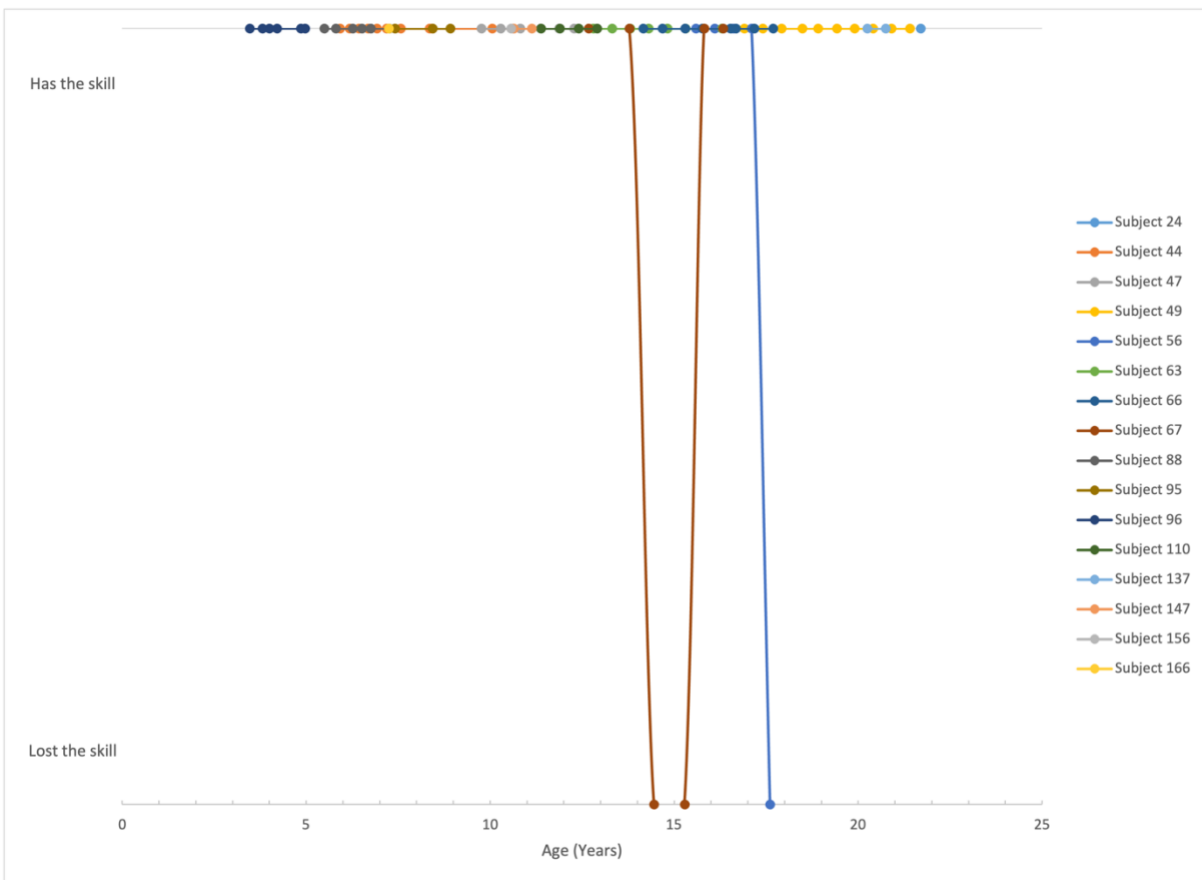


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 17, and a positive slope indicates fluctuations in regression (Appendix Table 17B).

**Appendix Table 17A**

*Kaplan-Meier Curve Analysis—Regression of Milestone 17*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 14.5 | 100 |
| 14.5 | to | 17.6 | 86 |
| 17.6 | to | 21.7 | 69 |

**Appendix Figure 17C**

*Kaplan-Meier Curve—Regression of Milestone 17*
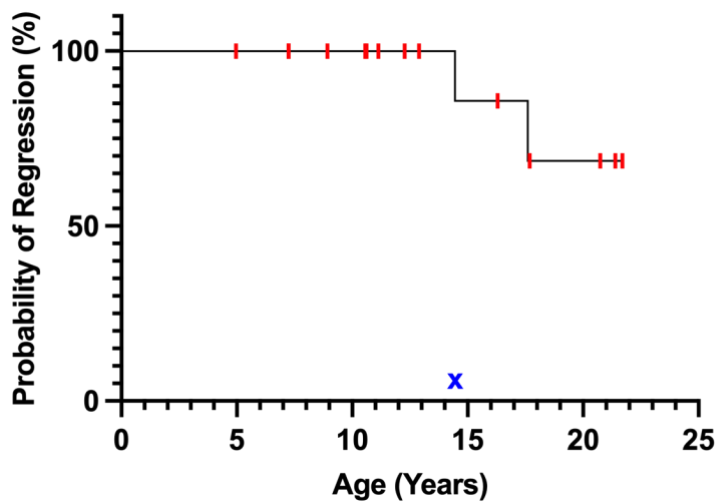


**I**  Subject without regression (age at last visit)

**X**  Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 17B).

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 17 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 17B and Table 6.

**Appendix Figure 17D**

*Grouped Columns—Regression of Milestone 17*
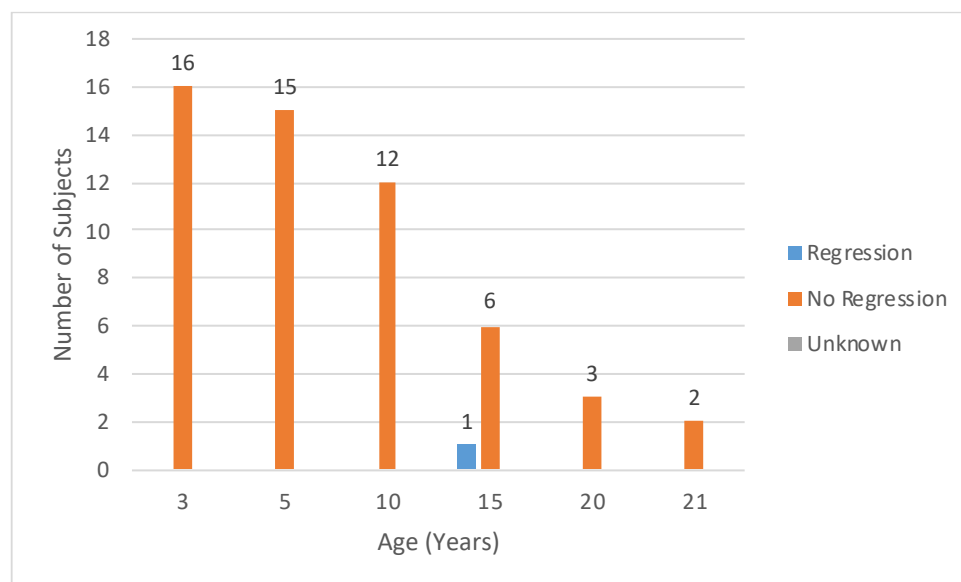


*Note.* At visit 1, if a subject reported no regression to milestone 17, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 17B**

*Fluctuations—Regression of Milestone 17*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 67      | 1     | 12.7        | No         |
|         | 2     | 13.8        | No         |
|         | 3     | 14.5        | Yes        |
|         | 4     | 15.3        | Yes        |
|         | 5     | 15.8        | No         |
|         | 6     | 16.3        | No         |

**Early Developmental Milestone 18**

*Makes single syllable sounds with consonant and vowel like 'ga', 'ma', or 'ba'*

**Appendix Figure 18A**

*Scatter Plot: Regression of Milestone 18*
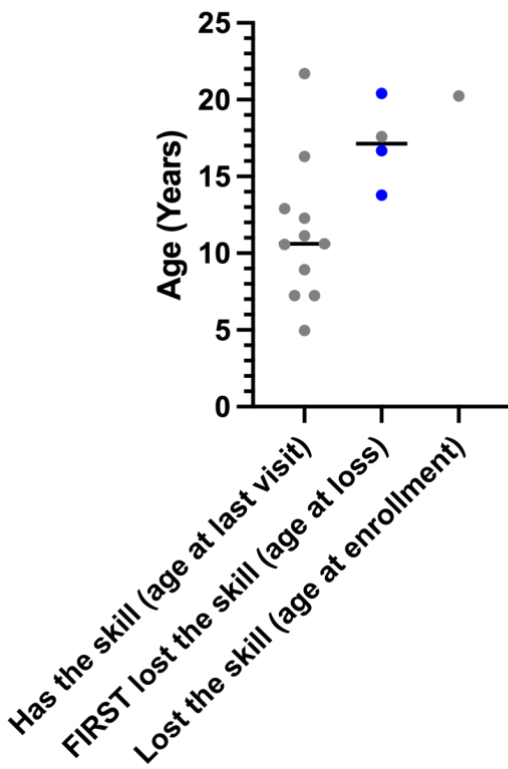


- Subject without fluctuation in regression

- Subject with fluctuation in regression (Appendix Table 18B)

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 18B**

*Longitudinal Plot—Regression of Milestone 18*
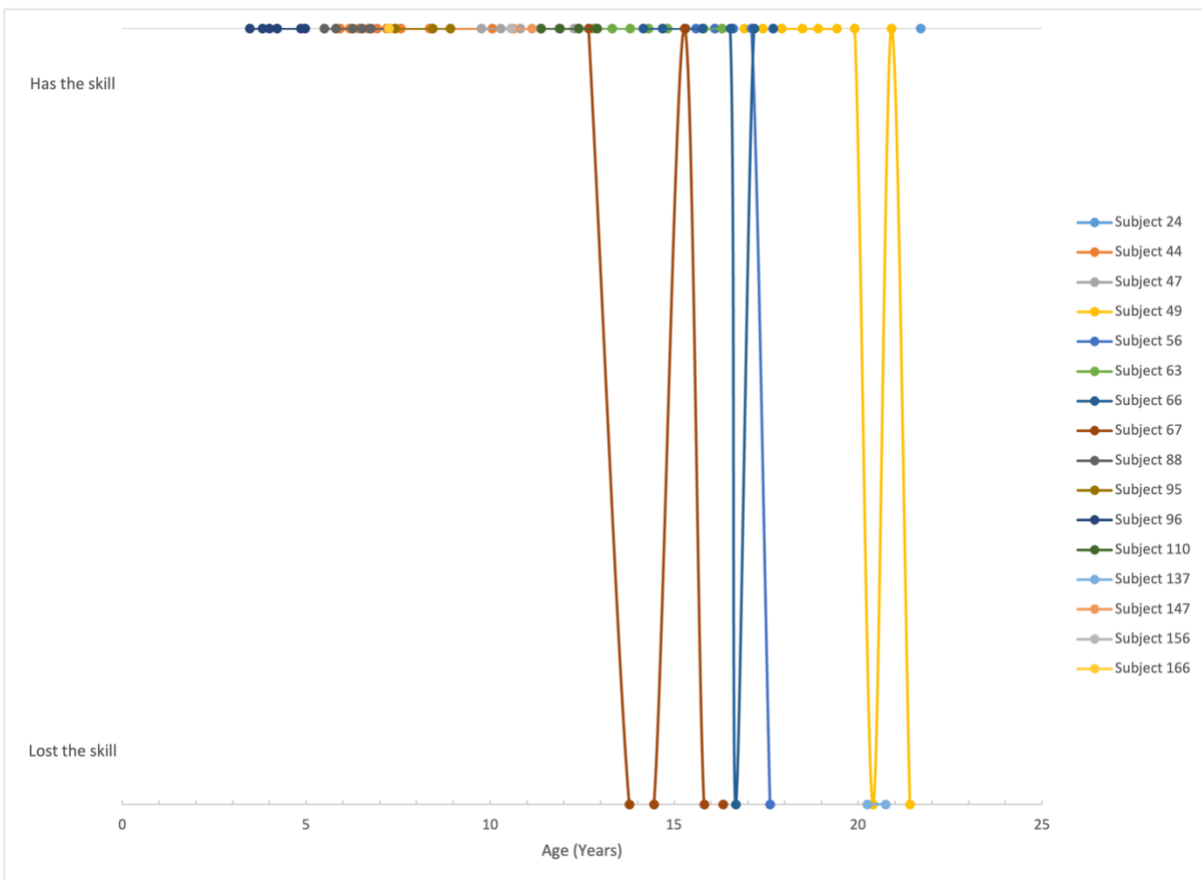


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 18, and a positive slope indicates fluctuations in regression (Appendix Table 18B).

**Appendix Table 18A**

*Kaplan-Meier Curve Analysis—Regression of Milestone 18*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 13.8 | 100 |
| 13.8 | to | 16.7 | 83 |
| 16.7 | to | 17.6 | 63 |
| 17.6 | to | 20.4 | 42 |
| 20.4 | to | 21.7 | 21 |

**Appendix Figure 18C**

*Kaplan-Meier Curve—Regression of Milestone 18*
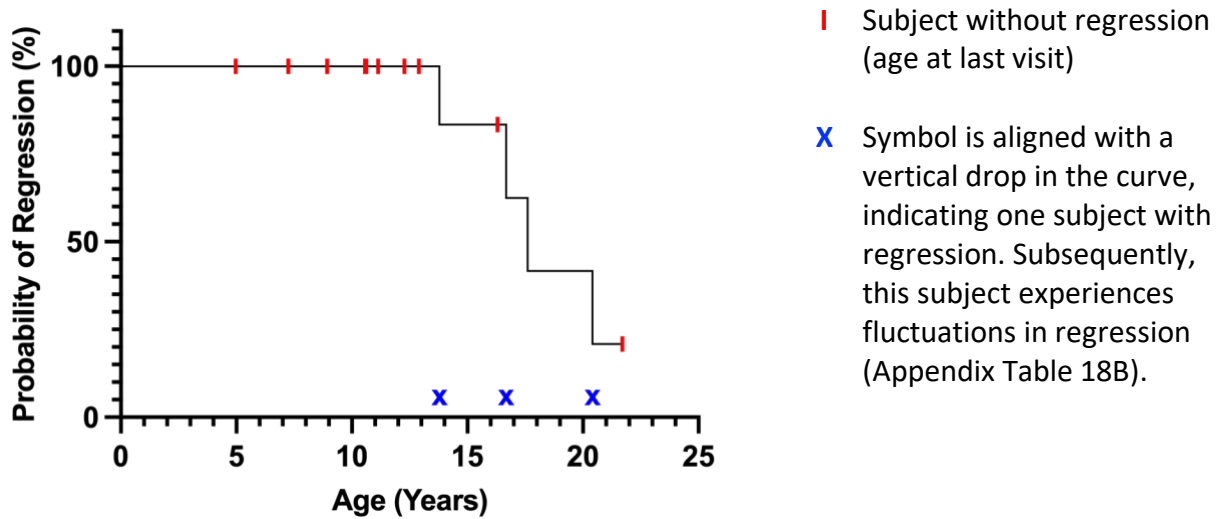


**I** Subject without regression (age at last visit)

**X** Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 18B).

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 18 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 18B and Table 6.

**Appendix Figure 18D**

*Grouped Columns—Regression of Milestone 18*



*Note.* At visit 1, if a subject reported no regression to milestone 18, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no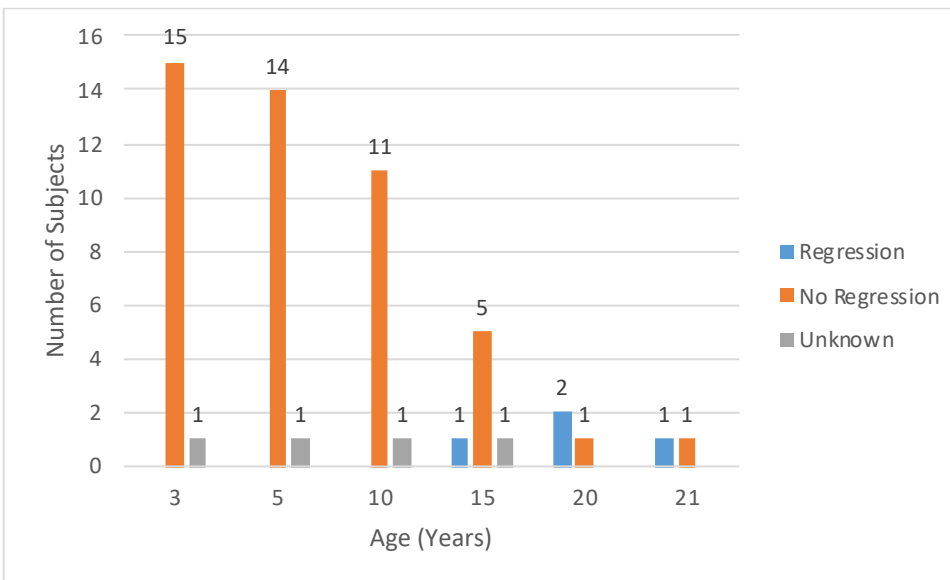 regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 18B**

*Fluctuations—Regression of Milestone 18*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 49      | 1     | 16.9        | No         |
|         | 2     | 17.4        | No         |
|         | 3     | 17.9        | No         |
|         | 4     | 18.5        | No         |
|         | 5     | 18.9        | No         |
|         | 6     | 19.4        | No         |
|         | 7     | 19.9        | No         |
|         | 8     | 20.4        | Yes        |
|         | 9     | 20.9        | No         |
|         | 10    | 21.4        | Yes        |

**Appendix Table 18B Continued**

*Fluctuations—Regression of Milestone 18*

| Subject | Visit | Age (Years) | Regression |
|---------|-------|-------------|------------|
| 66      | 1     | 14.2        | No         |
|         | 2     | 14.7        | No         |
|         | 3     | 15.3        | No         |
|         | 4     | 15.8        | No         |
|         | 5     | 16.5        | No         |
|         | 6     | 16.7        | Yes        |
|         | 7     | 17.2        | No         |
|         | 8     | 17.7        | No         |
|         |       |             |            |
| 67      | 1     | 12.7        | No         |
|         | 2     | 13.8        | Yes        |
|         | 3     | 14.5        | Yes        |
|         | 4     | 15.3        | No         |
|         | 5     | 15.8        | Yes        |
|         | 6     | 16.3        | Yes        |

**Early Developmental Milestone 19**

*Uses 2 words other than mama, dada, or the names of other family or pets*

**Appendix Figure 19A**

*Scatter Plot: Regression of Milestone 19*
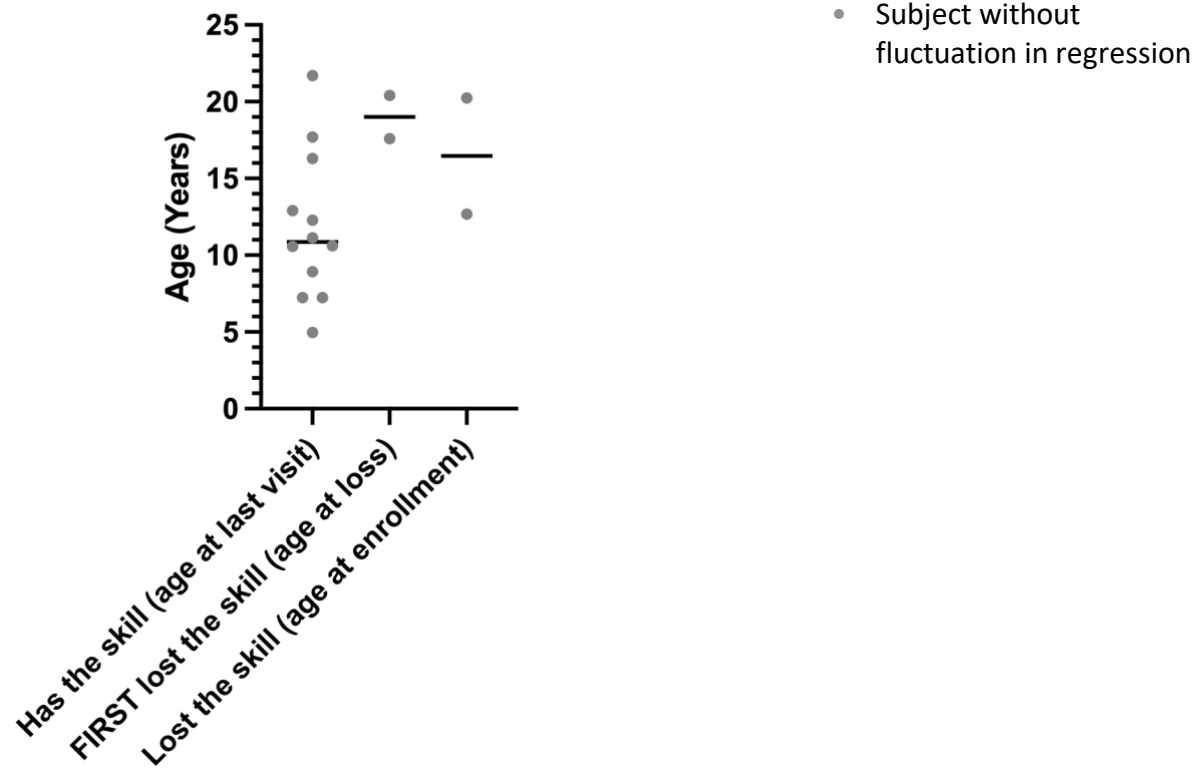


- Subject without fluctuation in regression

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 19B**

*Longitudinal Plot—Regression of Milestone 19*
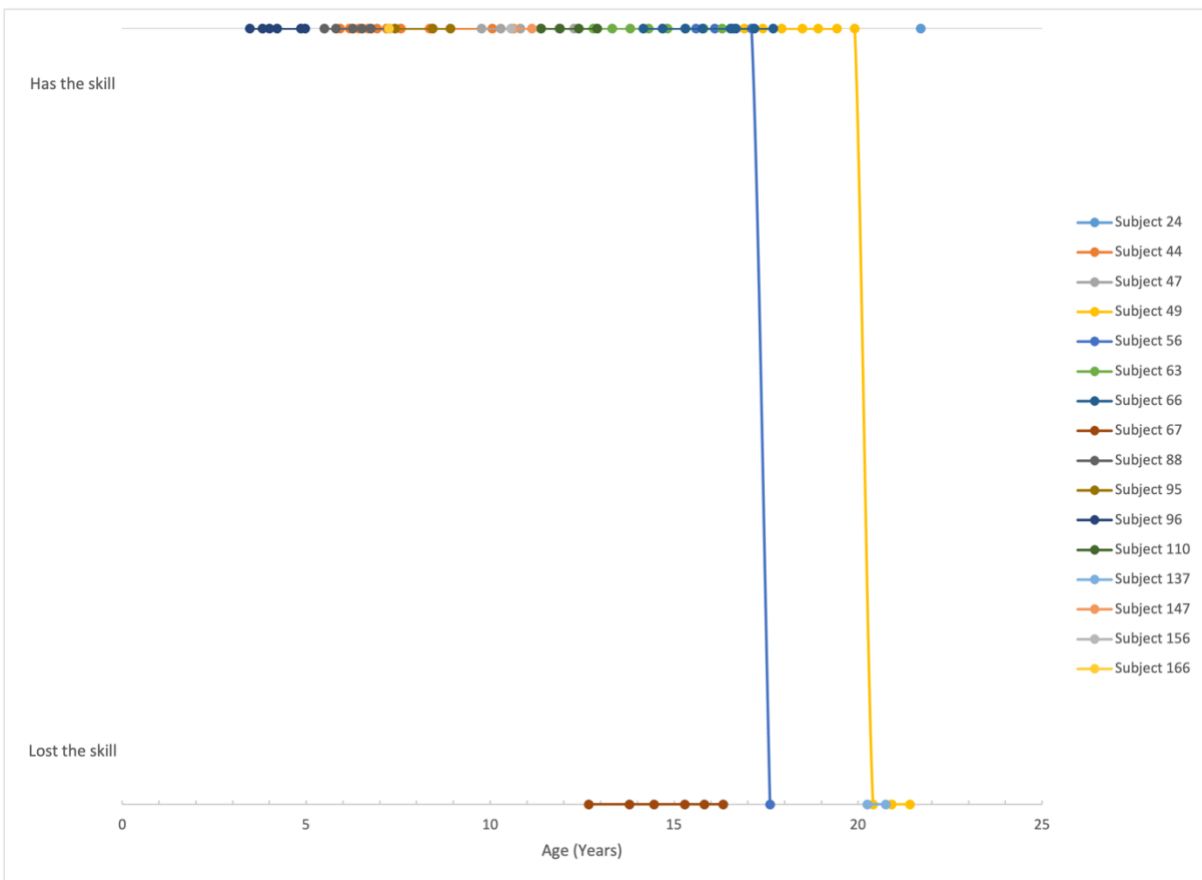


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 19, and a positive slope indicates fluctuations in regression.
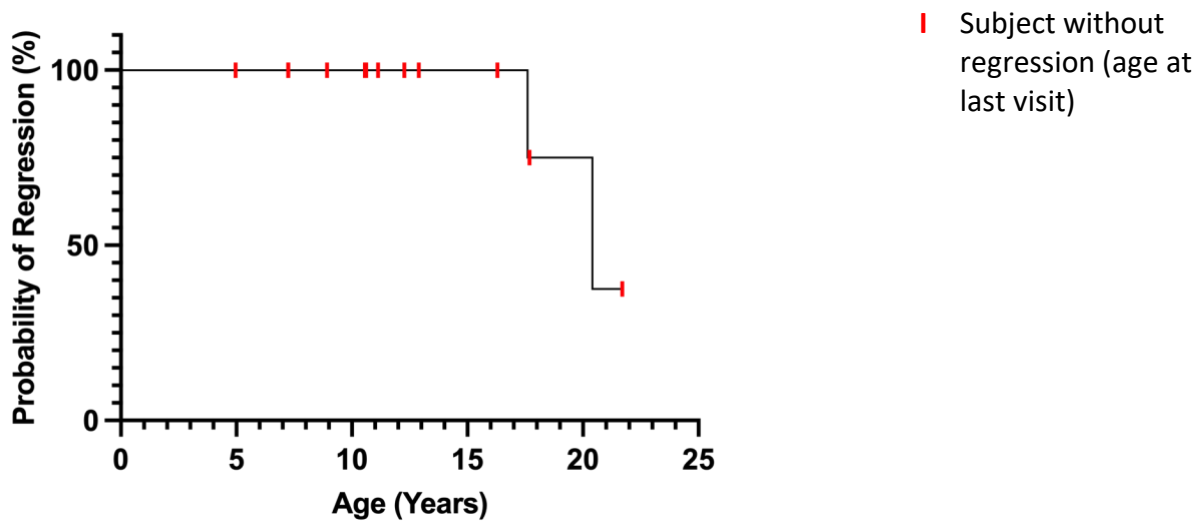
**Appendix Table 19**

*Kaplan-Meier Curve Analysis—Regression of Milestone 19*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 17.6 | 100 |
| 17.6 | to | 20.4 | 75 |
| 20.4 | to | 21.7 | 38 |

**Appendix Figure 19C**

*Kaplan-Meier Curve—Regression of Milestone 19*



**|** Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 19 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 19 and Table 6.

**Appendix Figure 19D**

*Grouped Columns—Regression of Milestone 19*
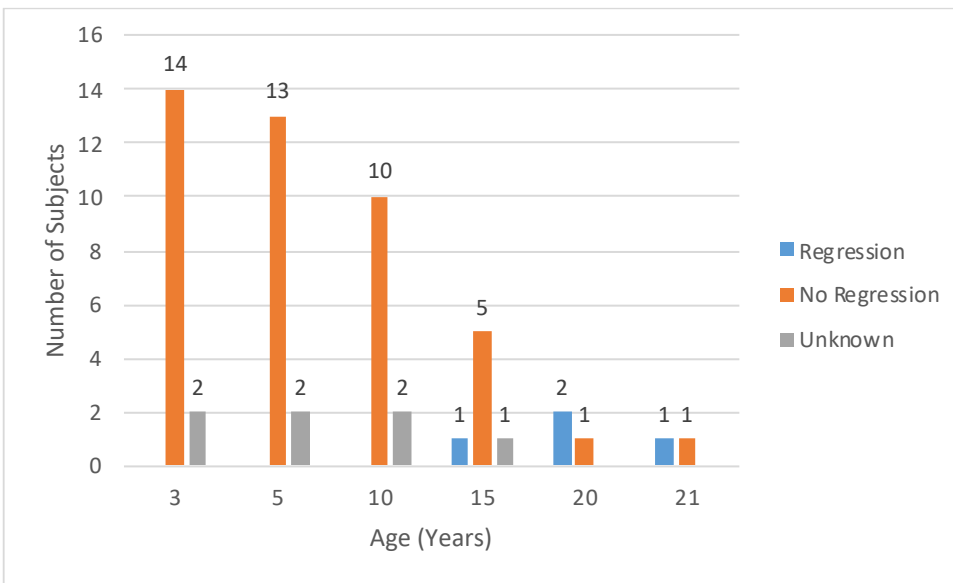


*Note.* At visit 1, if a subject reported no regression to milestone 19, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Early Developmental Milestone 20**

*Combines at least 2 words to make a meaningful phrase that indicates an action, like 'play ball' or 'want drink' or 'go bye-bye'*

**Appendix Figure 20A**

*Scatter Plot: Regression of Milestone 20*
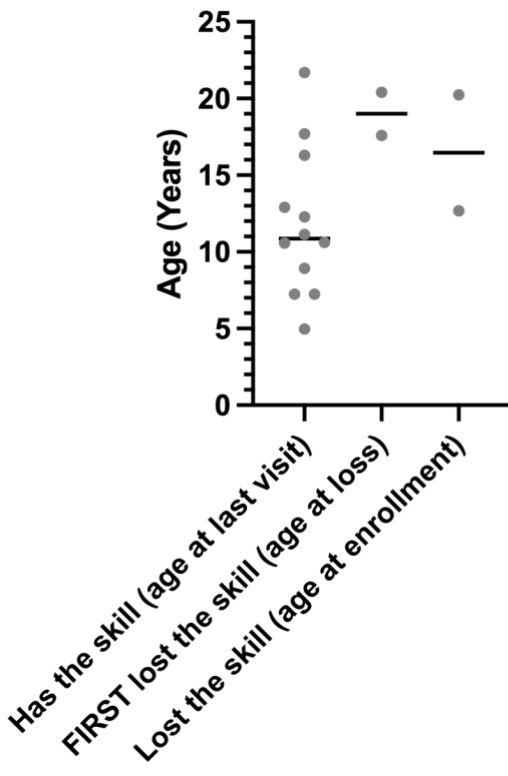


• Subject without fluctuation in regression

*Note.* Participants were categorized into one of three groups: 'Has the skill', 'First lost the skill', and 'Lost the skill'. A black horizontal line represents a median. Descriptive statistics can be found in Table 2.

**Appendix Figure 20B**

*Longitudinal Plot—Regression of Milestone 20*
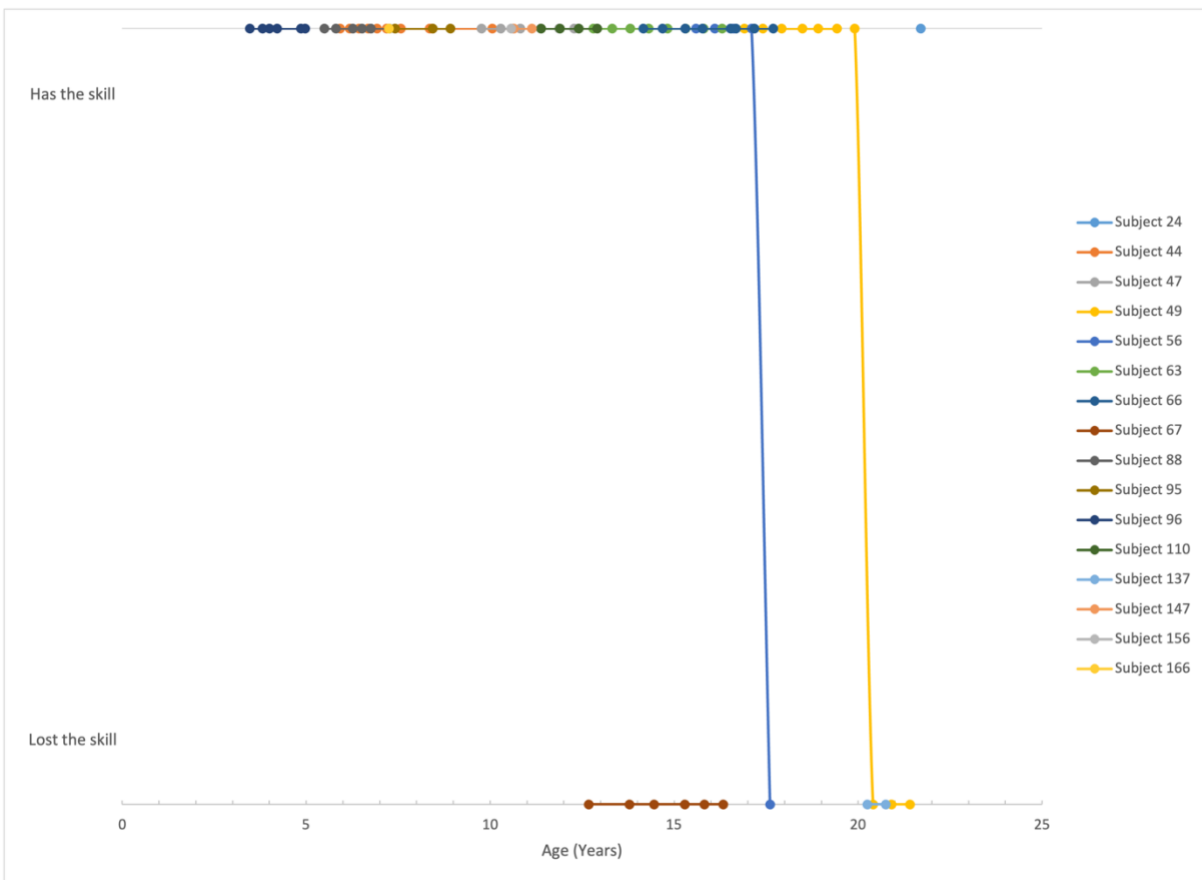


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate the subject has the skill, while dots at the bottom indicate the skill was lost. If present, a negative slope represents regression of milestone 20, and a positive slope indicates fluctuations in regression.

**Appendix Table 20**

*Kaplan-Meier Curve Analysis—Regression of Milestone 20*

| Age Range for Regression (Years) | | | No Regression (%) |
|---|---|---|---|
| 0.0 | to | 17.6 | 100 |
| 17.6 | to | 20.4 | 75 |
| 20.4 | to | 21.7 | 38 |

**Appendix Figure 20C**

*Kaplan-Meier Curve—Regression of Milestone 20*
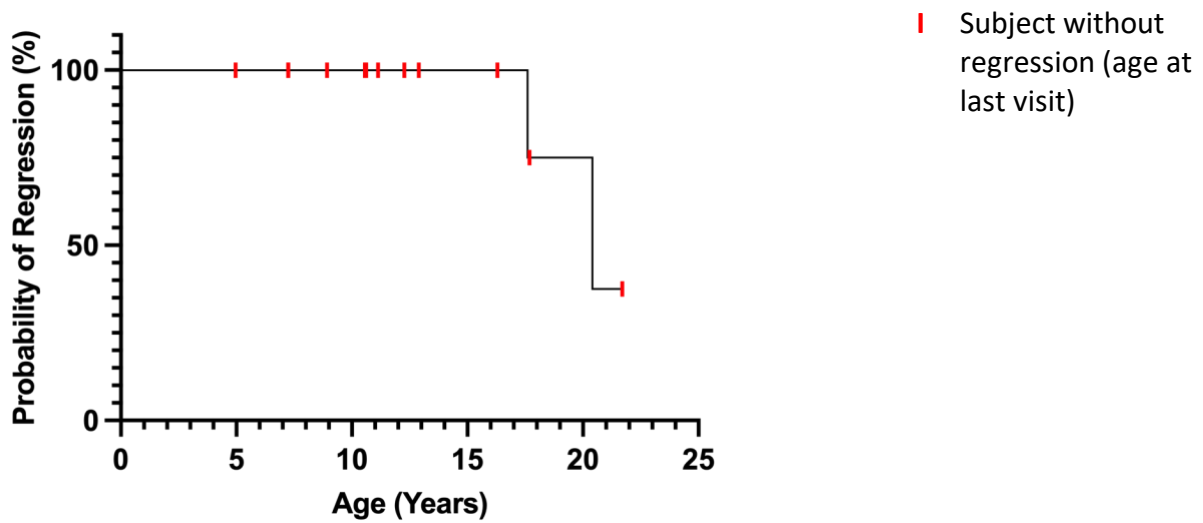


❙ Subject without regression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time regression of milestone 20 for one individual subject. Subjects who experienced regression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 20 and Table 6.

**Appendix Figure 20D**

*Grouped Columns—Regression of Milestone 20*
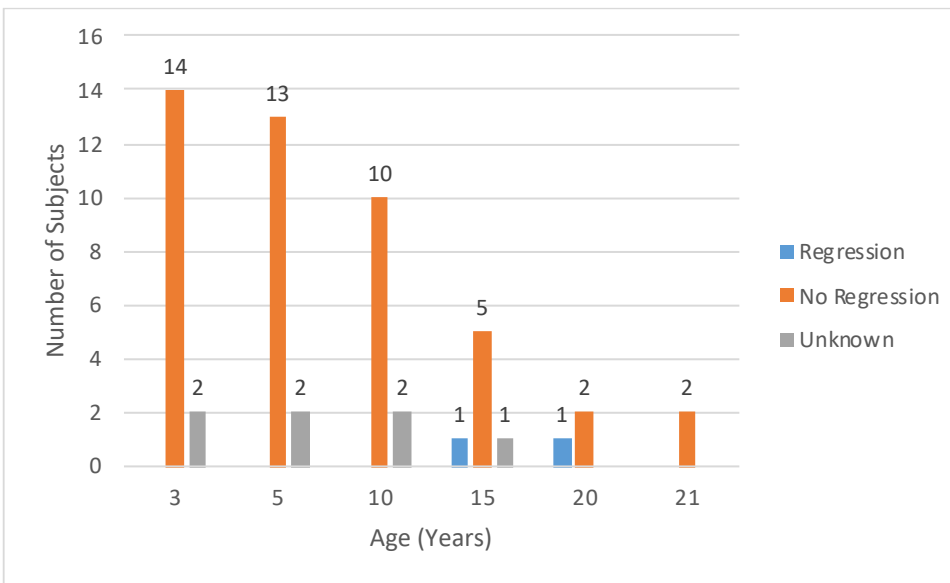


*Note.* At visit 1, if a subject reported no regression to milestone 20, they were assumed to have had no prior regression and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no regression'). If a subject reported regression, their prior status was marked as 'unknown' due to the uncertainty of when regression occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when regression first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Disease-Related Milestone 21**

*Uses wheelchair/stroller*

**Appendix Figure 21A**

*Scatter Plot—Progression of Milestone 21*
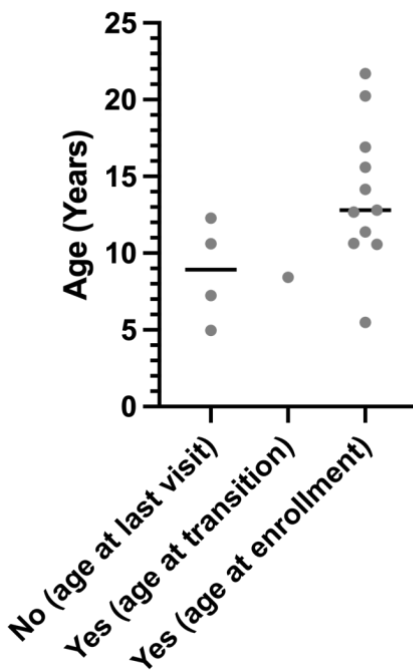


● Subject without fluctuation in progression

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 21B**

*Longitudinal Plot—Progression of Milestone 21*
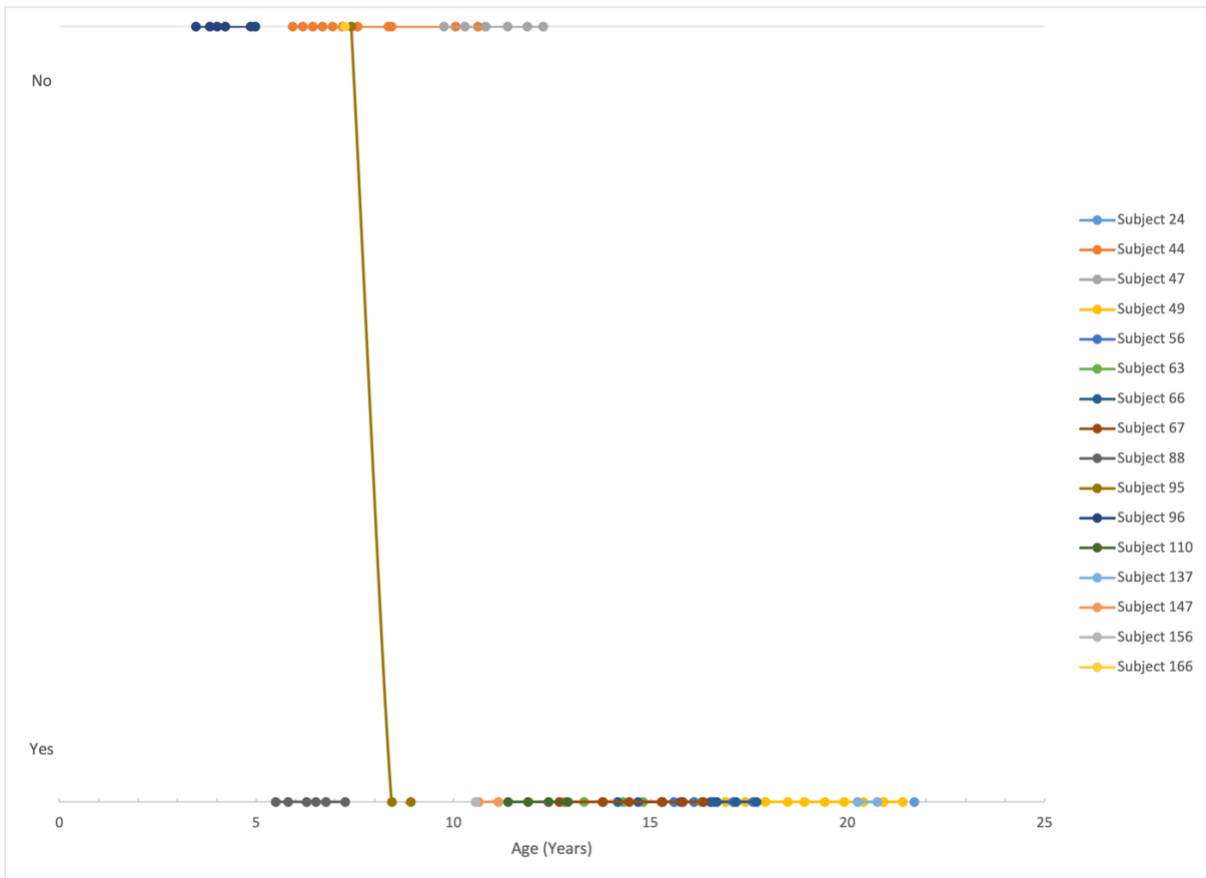


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 21, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature.

**Appendix Table 21**

*Kaplan-Meier Curve Analysis—Progression of Milestone 21*

| Age Range for Progression (Years) | | | No Progression (%) |
|---|---|---|---|
| 0.0 | to | 8.4 | 100 |
| 8.4 | to | 12.3 | 67 |

**Appendix Figure 21C**

*Kaplan-Meier Curve—Progression of Milestone 21*
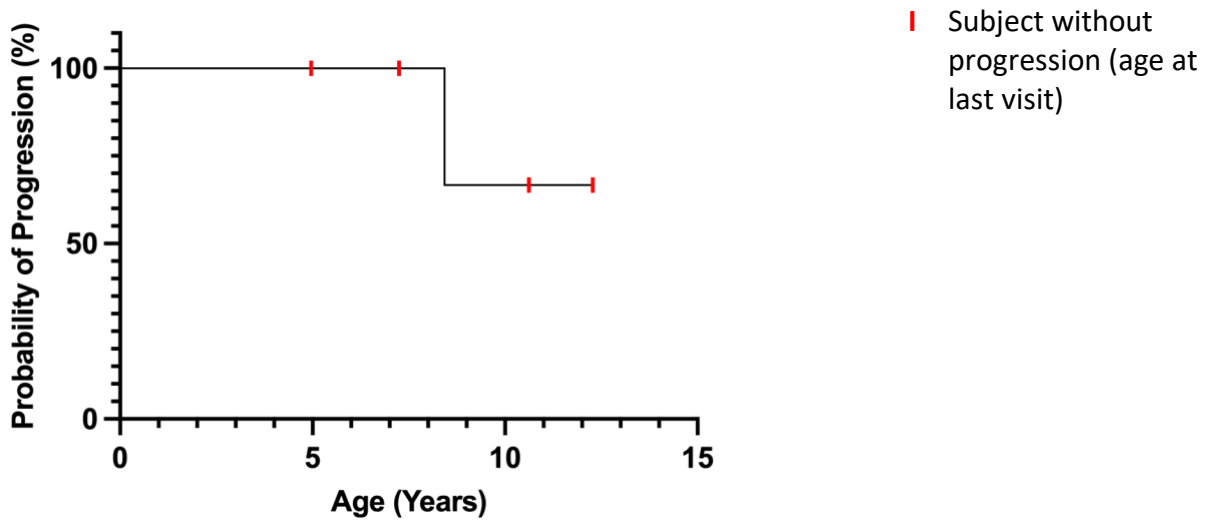


| Subject without progression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 21 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 21 and Table 7.

**Appendix Figure 21D**

*Grouped Columns—Progression of Milestone 21*
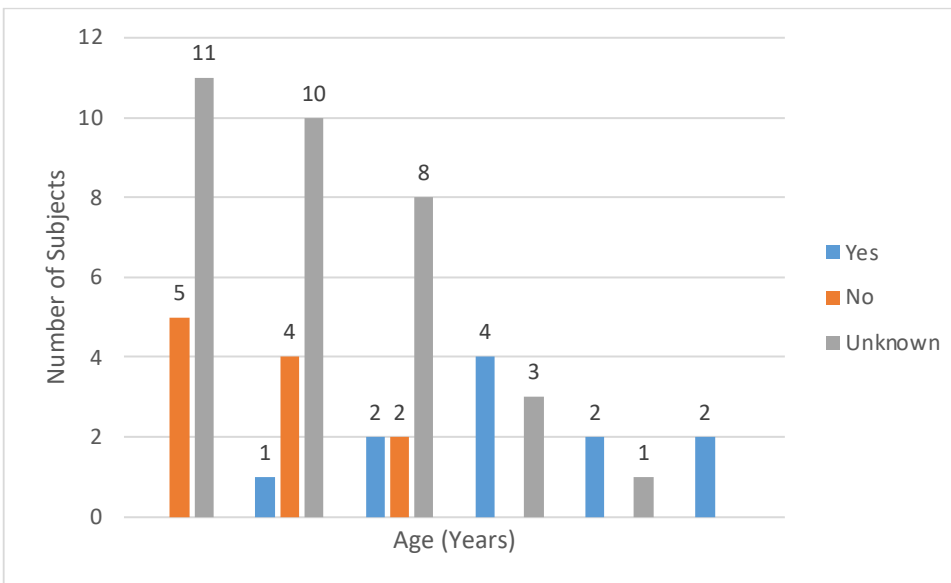


*Note.* At visit 1, if a subject reported 'no' to milestone 21, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 21 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Disease-Related Milestone 22**

*Falls frequently while walking (at least once/day)*

**Appendix Figure 22A**

*Scatter Plot—Progression of Milestone 22*
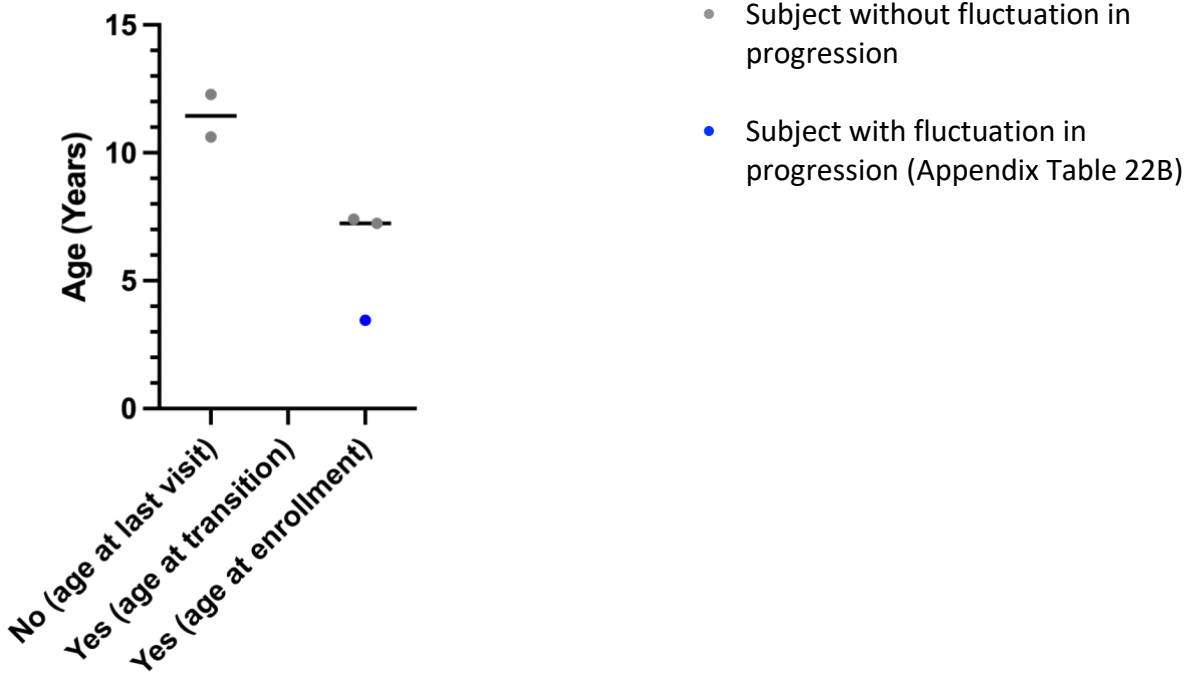


- Subject without fluctuation in progression

- Subject with fluctuation in progression (Appendix Table 22B)

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 22B**

*Longitudinal Plot—Progression of Milestone 22*



*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 22, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature (Appendix Table 22B).
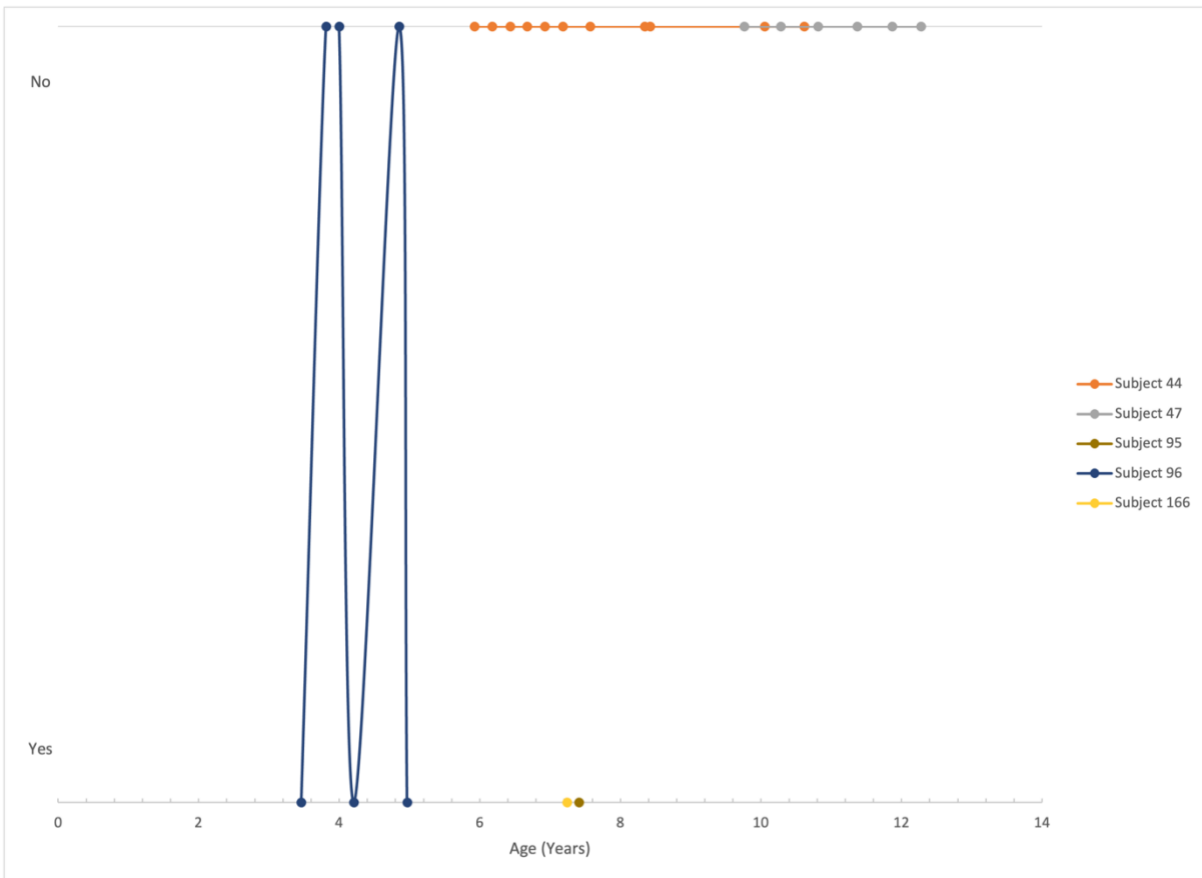
**Appendix Table 22A**

*Kaplan-Meier Curve Analysis—Progression of Milestone 22*

| Age Range for Progression (Years) | | | No Progression (%) |
|---|---|---|---|
| 0.0 | to | 12.3 | 100 |

**Appendix Figure 22C**

*Kaplan-Meier Curve—Progression of Milestone 22*
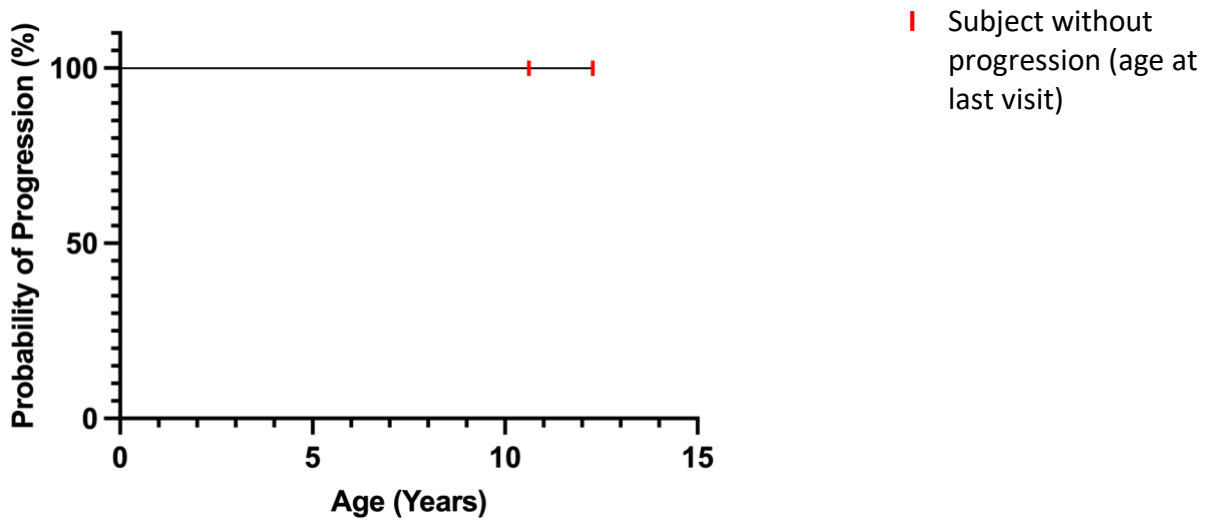


| Subject without progression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 22 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 22A and Table 7.

**Appendix Figure 22D**

*Grouped Columns—Progression of Milestone 22*
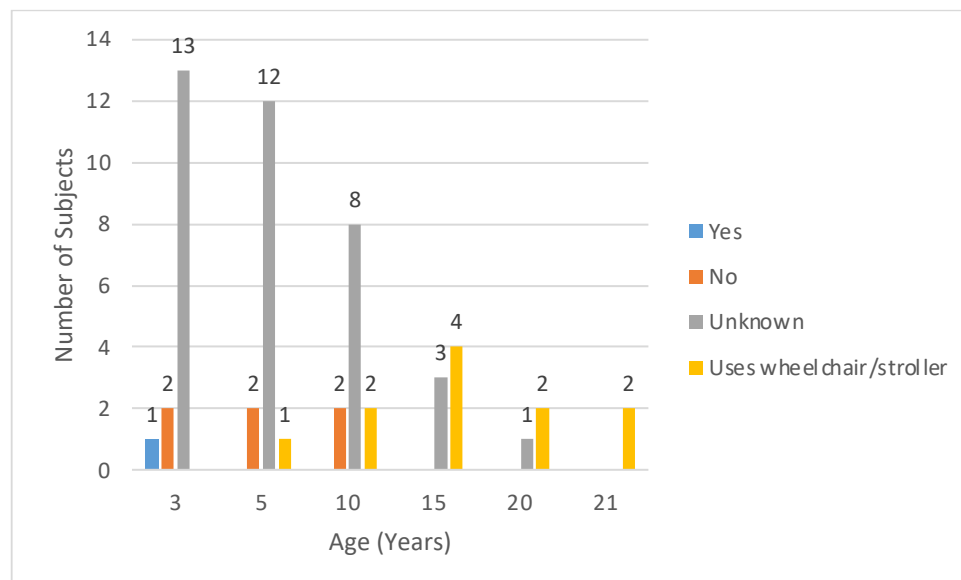


*Note.* At visit 1, if a subject reported 'no' to milestone 22, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 22 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 22B**

*Fluctuations—Regression of Milestone 22*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
| 96      | 1     | 3.5         | Yes         |
|         | 2     | 3.8         | No          |
|         | 3     | 4.0         | No          |
|         | 4     | 4.2         | Yes         |
|         | 5     | 4.9         | No          |
|         | 6     | 5.0         | Yes         |

**Disease-Related Milestone 23**

*Has seizures (or treated for them)*

**Appendix Figure 23A**

*Scatter Plot—Progression of Milestone 23*
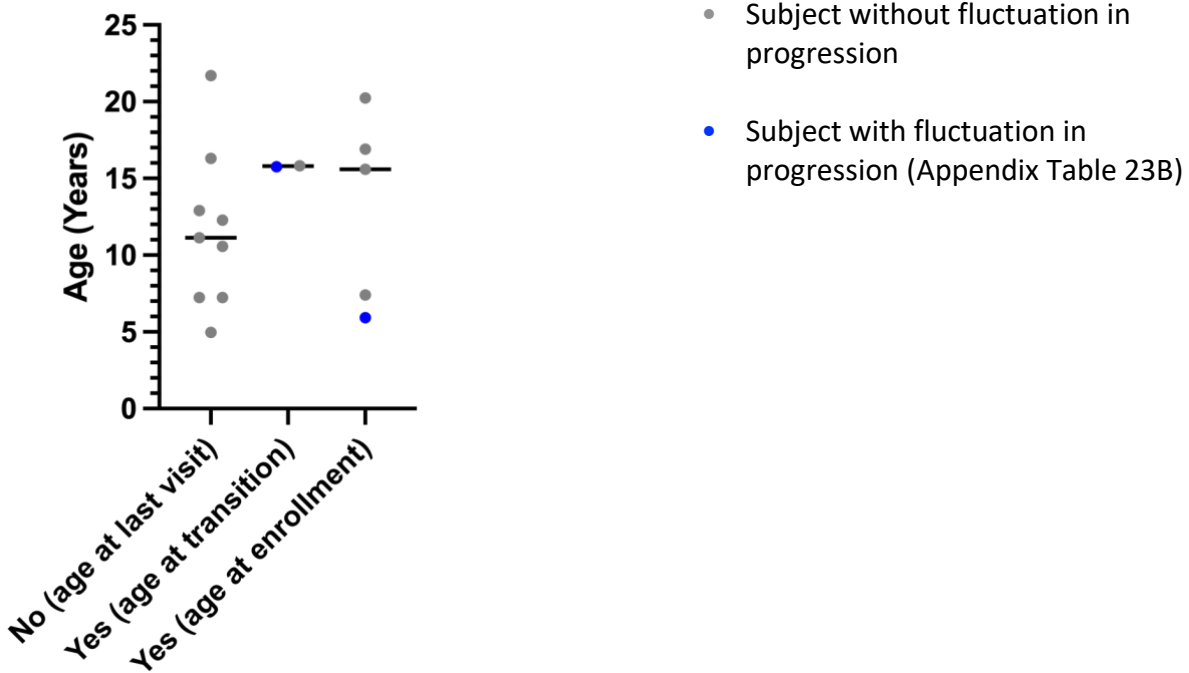


- Subject without fluctuation in progression

- Subject with fluctuation in progression (Appendix Table 23B)

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 23B**

*Longitudinal Plot—Progression of Milestone 23*
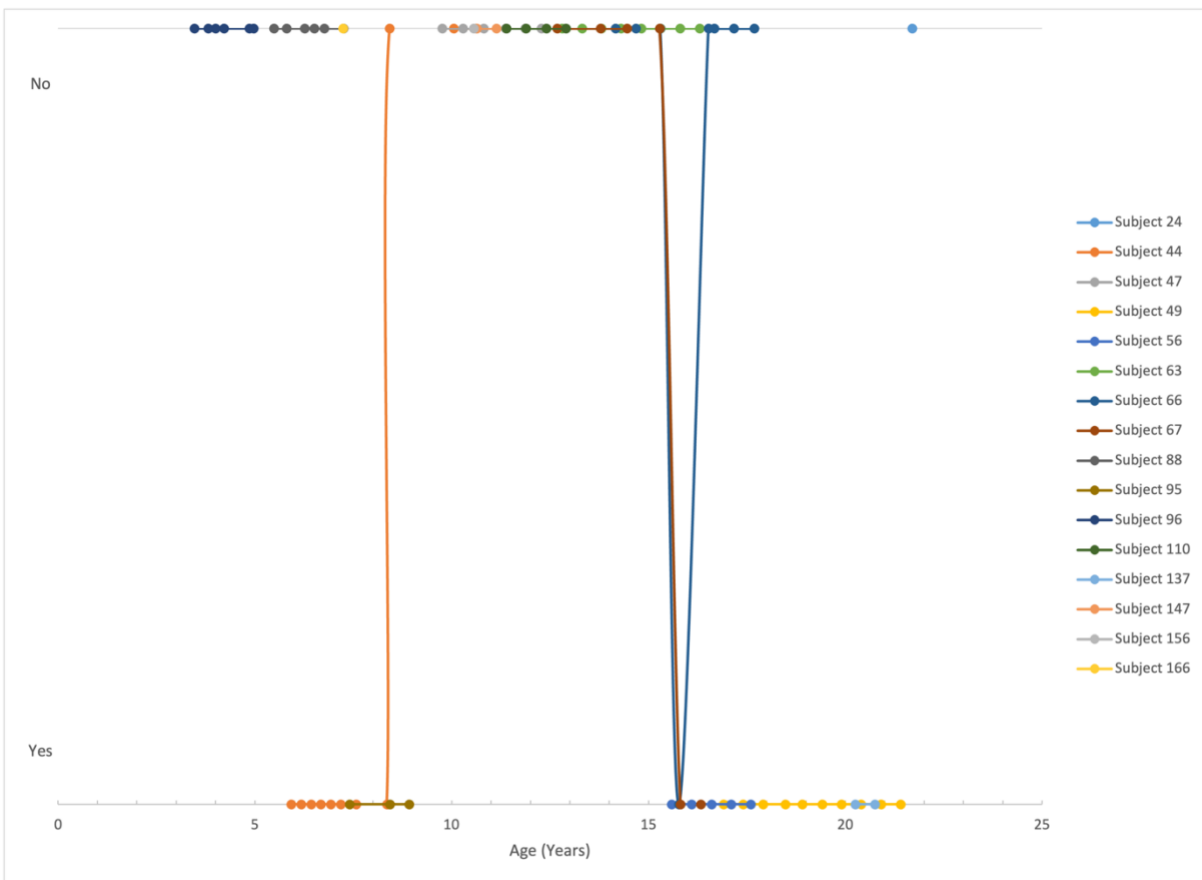


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 23, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature (Appendix Table 23B).

**Appendix Table 23A**

*Kaplan-Meier Curve Analysis—Progression of Milestone 23*

| Age Range for Progression (Years) | | | No Progression (%) |
|---|---|---|---|
| 0.0 | to | 15.8 | 100 |
| 15.8 | to | 15.8 | 75 |
| 15.8 | to | 21.7 | 50 |

**Appendix Figure 23C**

*Kaplan-Meier Curve—Progression of Milestone 23*
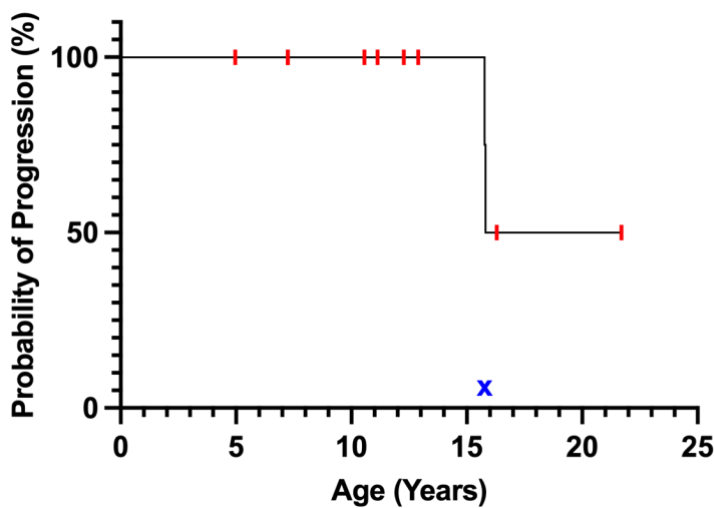


**I**   Subject without progression (age at last visit)

**X**   Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 23B).

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 23 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 23A and Table 7.

**Appendix Figure 23D**

*Grouped Columns—Progression of Milestone 23*
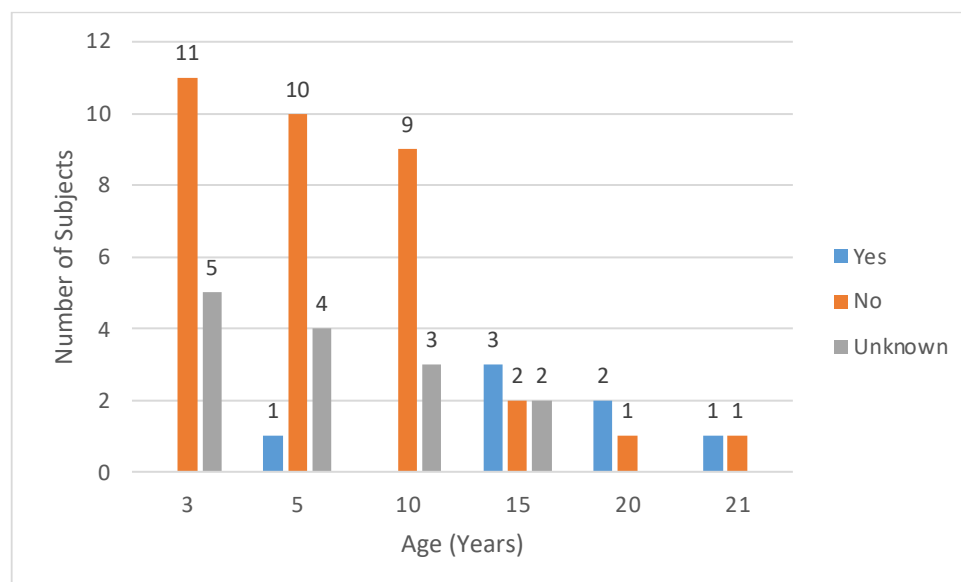


*Note.* At visit 1, if a subject reported 'no' to milestone 23, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 23 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 23B**

*Fluctuations—Progression of Milestone 23*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
| 44      | 1     | 5.9         | Yes         |
|         | 2     | 6.2         | Yes         |
|         | 3     | 6.4         | Yes         |
|         | 4     | 6.7         | Yes         |
|         | 5     | 6.9         | Yes         |
|         | 6     | 7.2         | Yes         |
|         | 7     | 7.6         | Yes         |
|         | 8     | 8.3         | Yes         |
|         | 9     | 8.4         | No          |
|         | 10    | 10.1        | No          |

**Appendix Table 23B Continued**

*Fluctuations—Progression of Milestone 23*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
|         | 11    | 10.6        | No          |
|         |       |             |             |
| 66      | 1     | 14.2        | No          |
|         | 2     | 14.7        | No          |
|         | 3     | 15.3        | No          |
|         | 4     | 15.8        | Yes         |
|         | 5     | 16.5        | No          |
|         | 6     | 16.7        | No          |
|         | 7     | 17.2        | No          |
|         | 8     | 17.7        | No          |

**Disease-Related Milestone 24**

*Requires diet modifications (soft diet, blending, dicing, thickening liquids, etc.)*

**Appendix Figure 24A**

*Scatter Plot—Progression of Milestone 24*
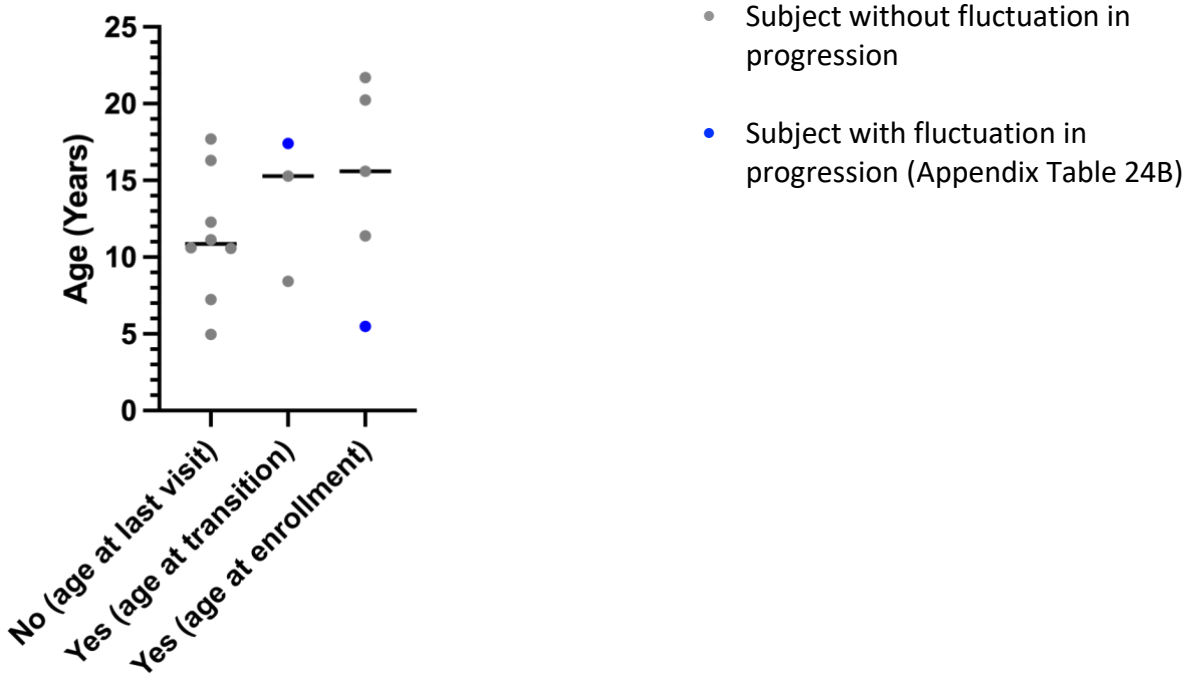


- • Subject without fluctuation in progression

- • Subject with fluctuation in progression (Appendix Table 24B)

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 24B**

*Longitudinal Plot—Progression of Milestone 24*
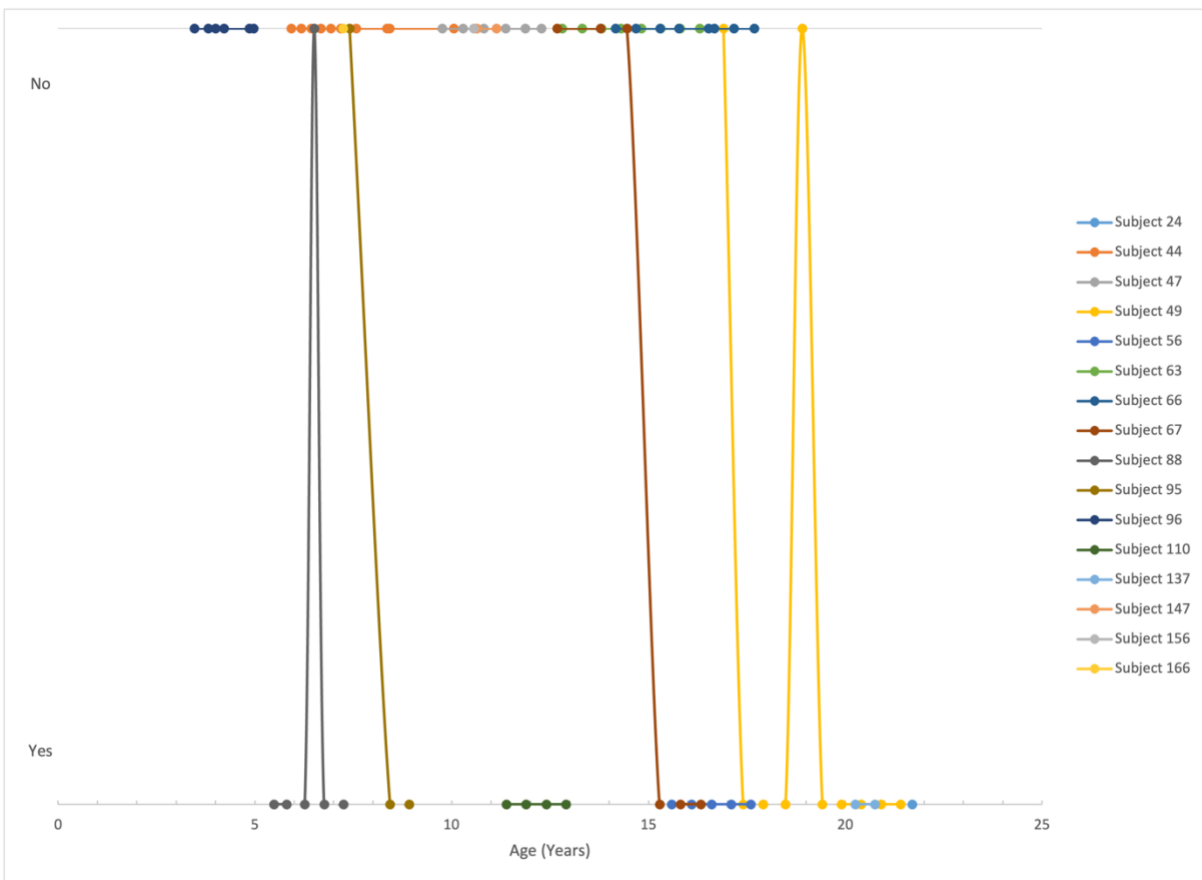


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 24, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature (Appendix Table 24B).
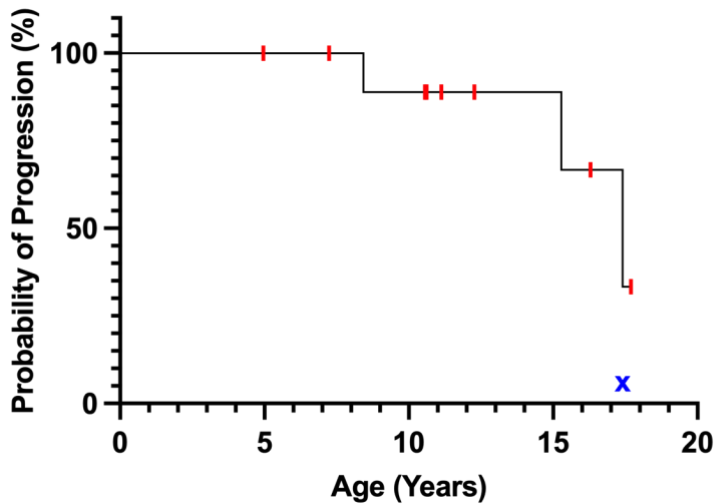
**Appendix Table 24A**

*Kaplan-Meier Curve Analysis—Progression of Milestone 24*

| Age Range for Progression (Years) | | | No Progression (%) |
|---|---|---|---|
| 0.0 | to | 8.4 | 100 |
| 8.4 | to | 15.3 | 89 |
| 15.3 | to | 17.4 | 67 |
| 17.4 | to | 17.7 | 33 |

**Appendix Figure 24C**

*Kaplan-Meier Curve—Progression of Milestone 24*



**I** Subject without progression (age at last visit)

**X** Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 24B).

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 24 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 24A and Table 7.

**Appendix Figure 24D**

*Grouped Columns—Progression of Milestone 24*
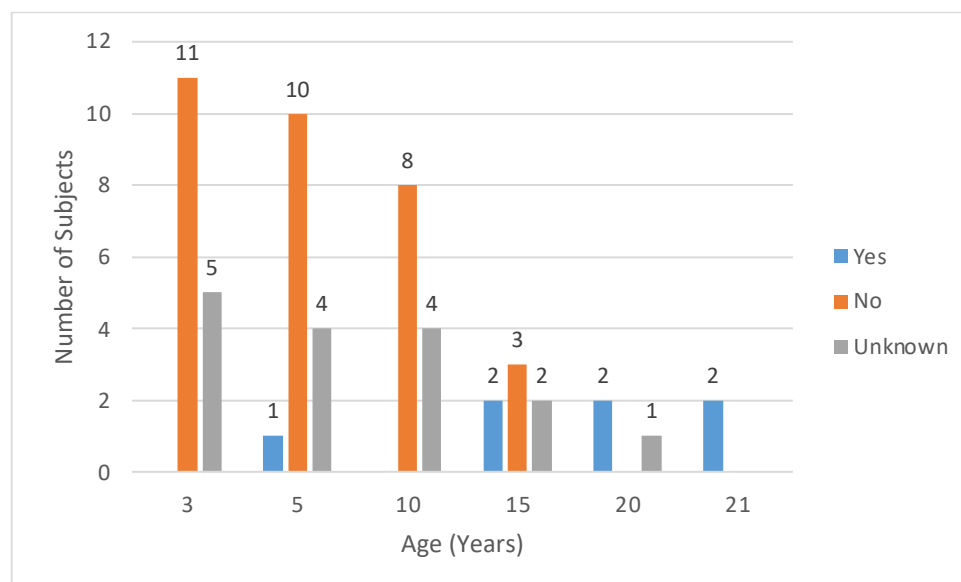


*Note.* At visit 1, if a subject reported 'no' to milestone 24, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 24 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 24B**

*Fluctuations—Progression of Milestone 24*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
| 49      | 1     | 16.9        | No          |
|         | 2     | 17.4        | Yes         |
|         | 3     | 17.9        | Yes         |
|         | 4     | 18.5        | Yes         |
|         | 5     | 18.9        | No          |
|         | 6     | 19.4        | Yes         |
|         | 7     | 19.9        | Yes         |
|         | 8     | 20.4        | Yes         |
|         | 9     | 20.9        | Yes         |
|         | 10    | 21.4        | Yes         |

**Appendix Table 24B Continued**

*Fluctuations—Progression of Milestone 24*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
| 88      | 1     | 5.5         | Yes         |
|         | 2     | 5.8         | Yes         |
|         | 3     | 6.3         | Yes         |
|         | 4     | 6.5         | No          |
|         | 5     | 6.8         | Yes         |
|         | 6     | 7.2         | Yes         |

**Disease-Related Milestone 25**

*Has swallowing difficulty / coughing with foods or liquids*

**Appendix Figure 25A**

*Scatter Plot—Progression of Milestone 25*
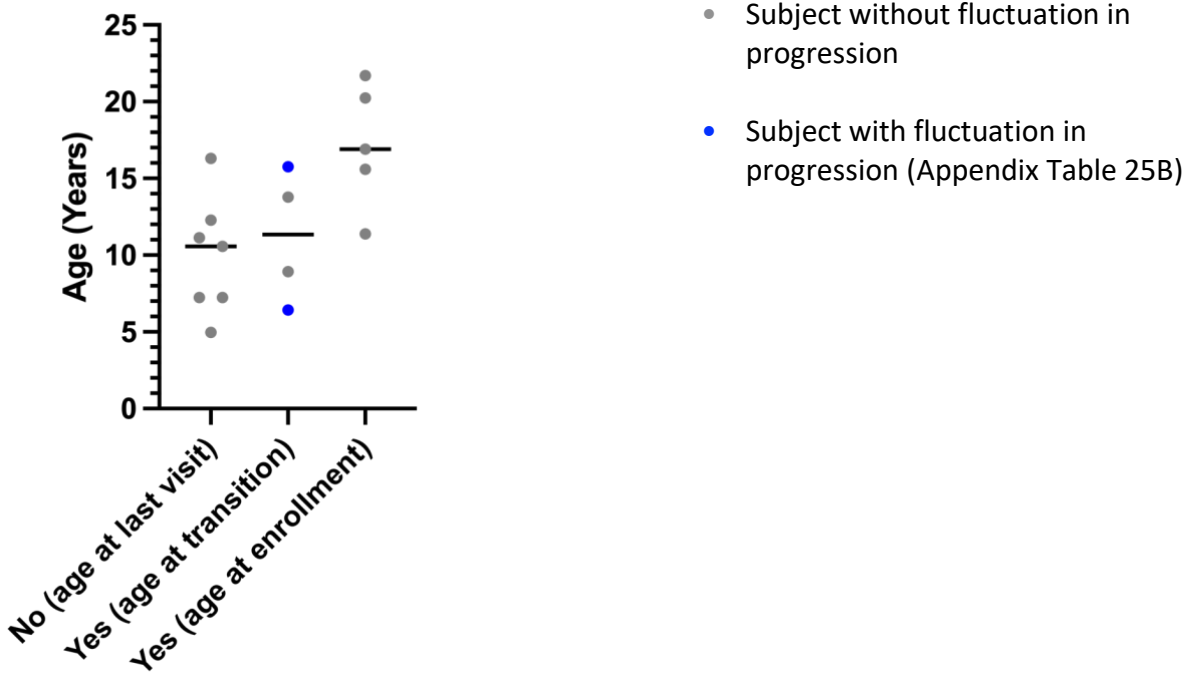


- Subject without fluctuation in progression

- Subject with fluctuation in progression (Appendix Table 25B)

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 25B**

*Longitudinal Plot—Progression of Milestone 25*
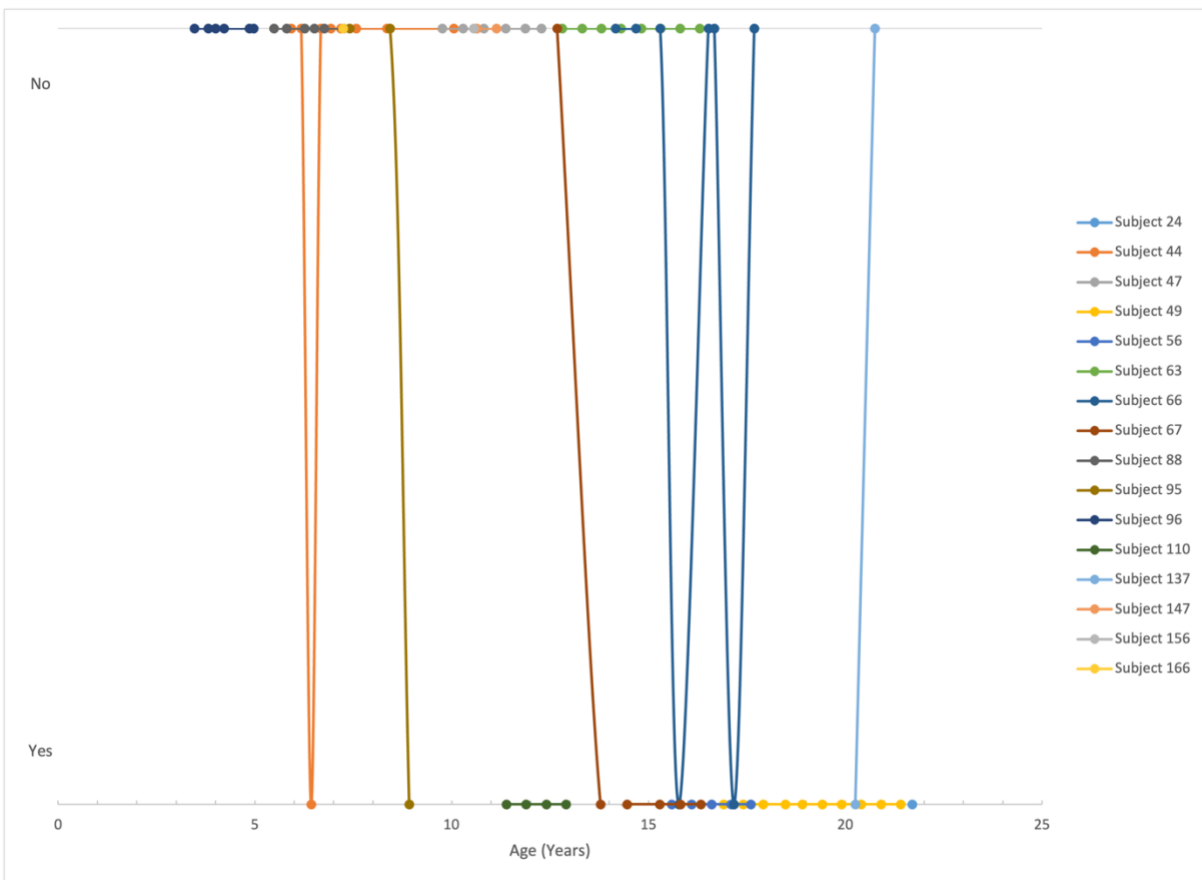


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 25, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature (Appendix Table 25B).
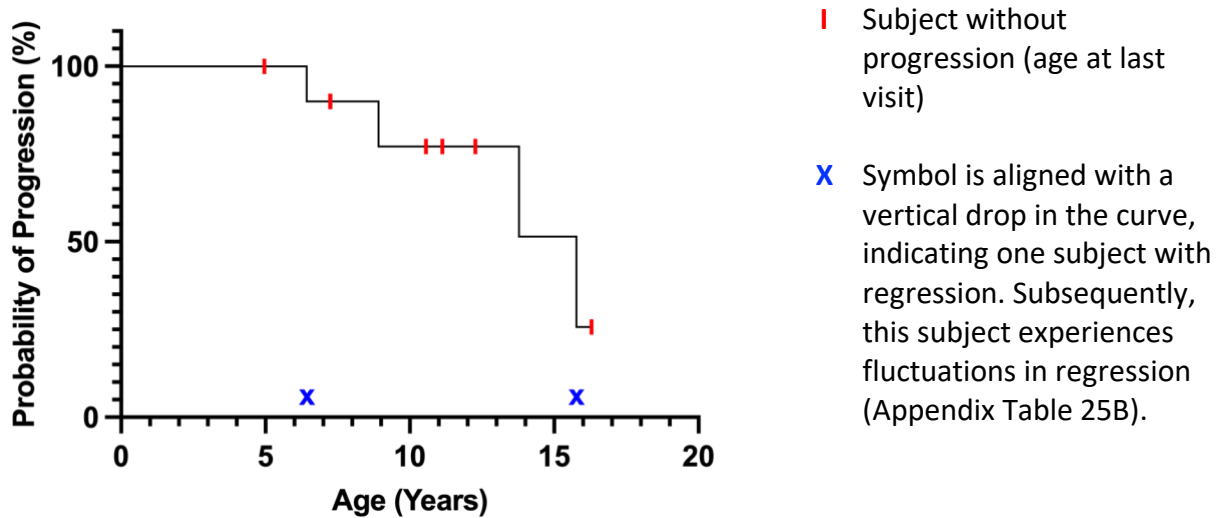
**Appendix Table 25A**

*Kaplan-Meier Curve Analysis—Progression of Milestone 25*

| Age Range for Progression (Years) | | | No Progression (%) |
|---|---|---|---|
| 0.0 | to | 6.4 | 100 |
| 6.4 | to | 8.9 | 90 |
| 8.9 | to | 13.8 | 77 |
| 13.8 | to | 15.8 | 51 |
| 15.8 | to | 16.3 | 26 |

**Appendix Figure 25C**

*Kaplan-Meier Curve—Progression of Milestone 25*



**I** Subject without progression (age at last visit)

**X** Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 25B).

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 25 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 25A and Table 7.

**Appendix Figure 25D**

*Grouped Columns—Progression of Milestone 25*
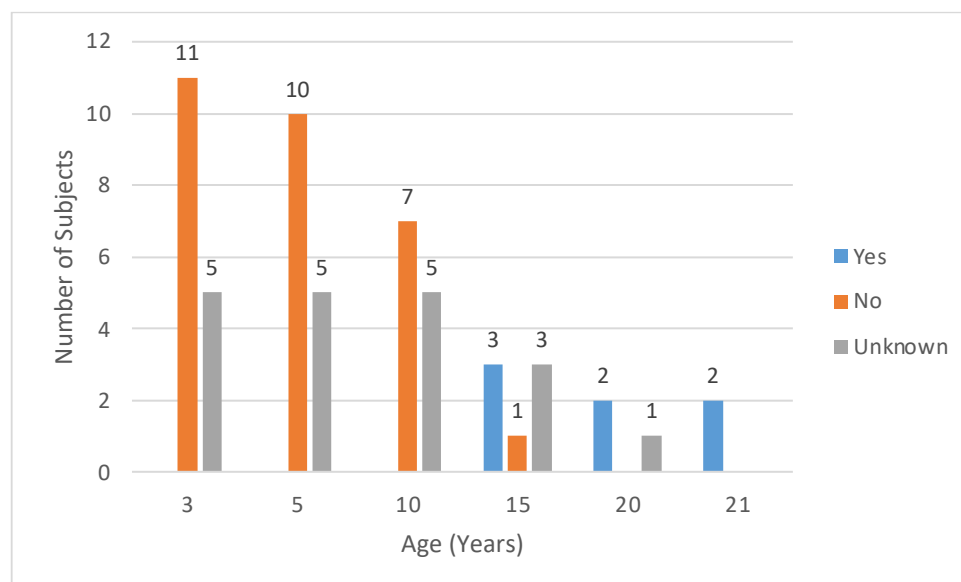


*Note.* At visit 1, if a subject reported 'no' to milestone 25, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 25 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 25B**

*Fluctuations—Progression of Milestone 25*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
| 44      | 1     | 5.9         | No          |
|         | 2     | 6.2         | No          |
|         | 3     | 6.4         | Yes         |
|         | 4     | 6.7         | No          |
|         | 5     | 6.9         | No          |
|         | 6     | 7.2         | No          |
|         | 7     | 7.6         | No          |
|         | 8     | 8.3         | No          |
|         | 9     | 8.4         | No          |
|         | 10    | 10.1        | No          |

**Appendix Table 25B Continued**
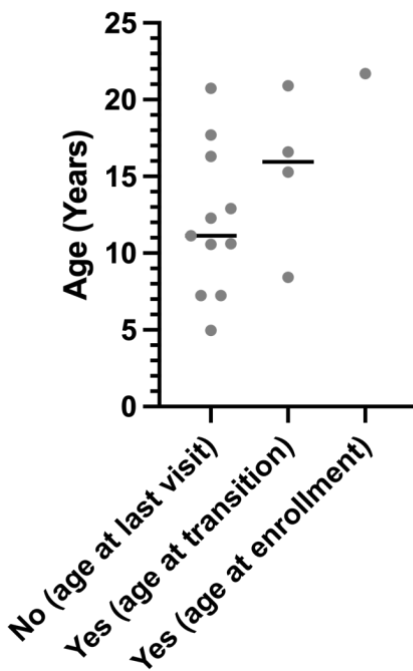
*Fluctuations—Progression of Milestone 25*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
|         | 11    | 10.6        | No          |
|         |       |             |             |
| 66      | 1     | 14.2        | No          |
|         | 2     | 14.7        | No          |
|         | 3     | 15.3        | No          |
|         | 4     | 15.8        | Yes         |
|         | 5     | 16.5        | No          |
|         | 6     | 16.7        | No          |
|         | 7     | 17.2        | Yes         |
|         | 8     | 17.7        | No          |

**Disease-Related Milestone 26**

*Has a gastrostomy tube (feeding tube)*

**Appendix Figure 26A**

*Scatter Plot—Progression of Milestone 26*



• Subject without fluctuation in progression

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 26B**

*Longitudinal Plot—Progression of Milestone 26*
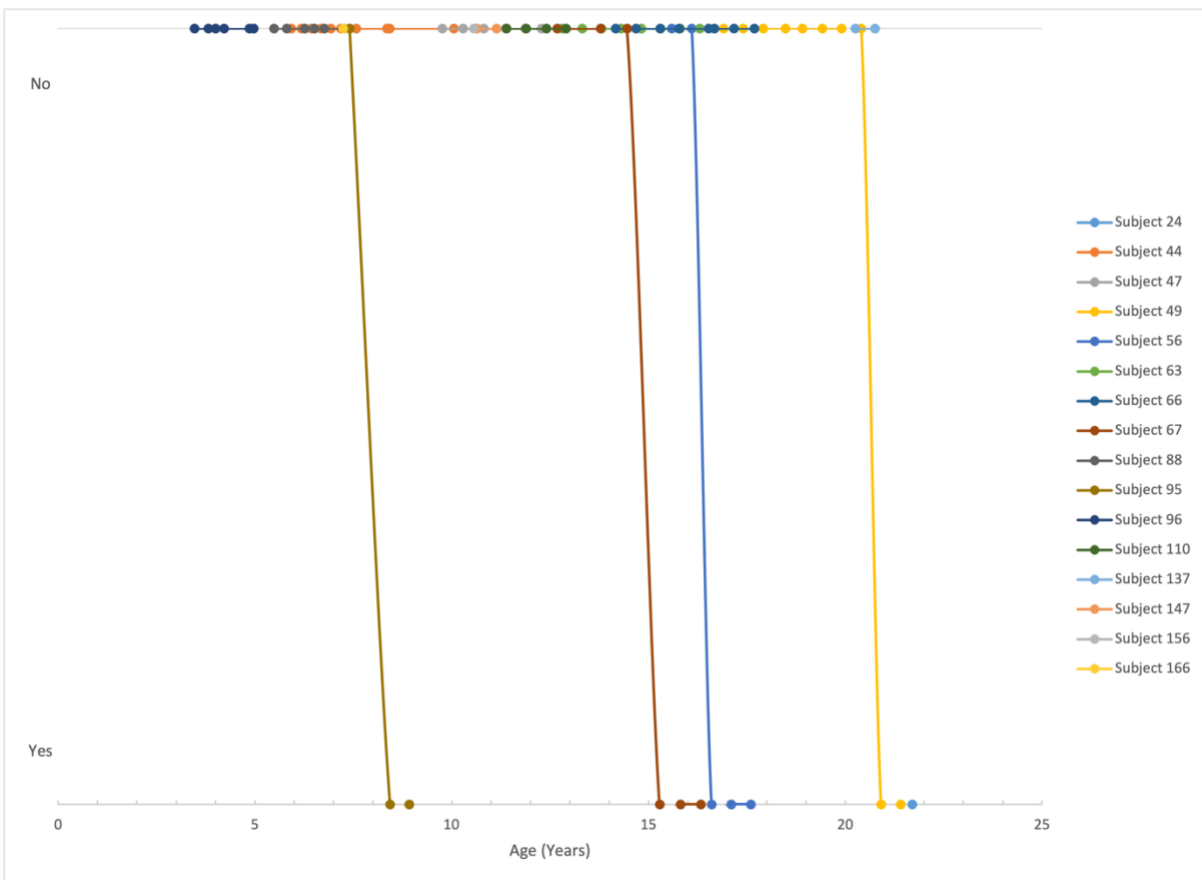


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 26, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature.
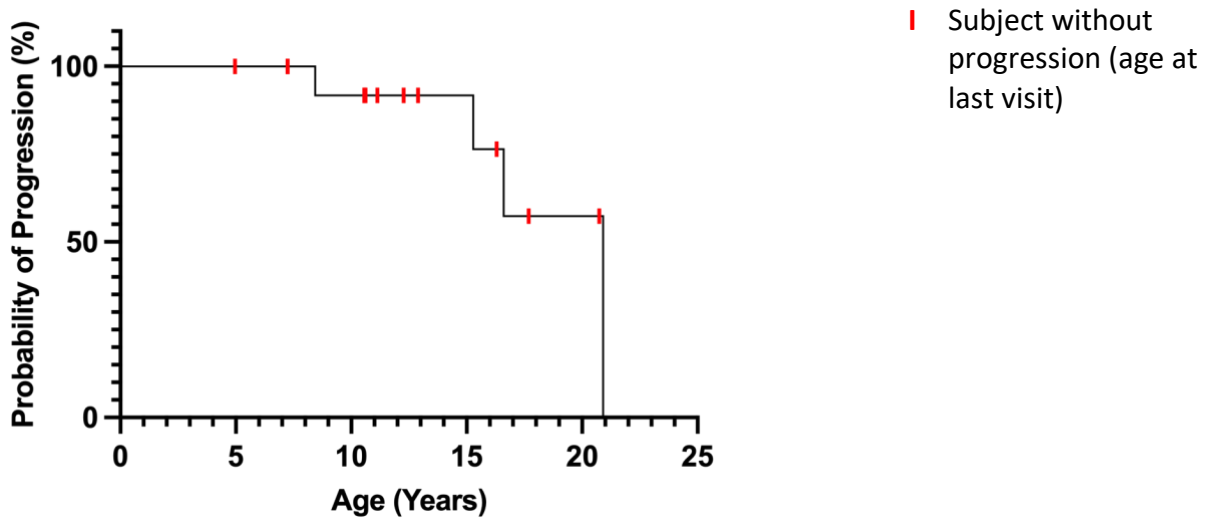
**Appendix Table 26**

*Kaplan-Meier Curve Analysis—Progression of Milestone 26*

| Age Range for Progression (Years) | | | No Progression (%) |
|---|---|---|---|
| 0.0 | to | 8.4 | 100 |
| 8.4 | to | 15.3 | 92 |
| 15.3 | to | 16.6 | 76 |
| 16.6 | to | 20.9 | 57 |
| 20.9 | to | - | 0 |

**Appendix Figure 26C**

*Kaplan-Meier Curve—Progression of Milestone 26*



I  Subject without progression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 26 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 26 and Table 7.

**Appendix Figure 26D**
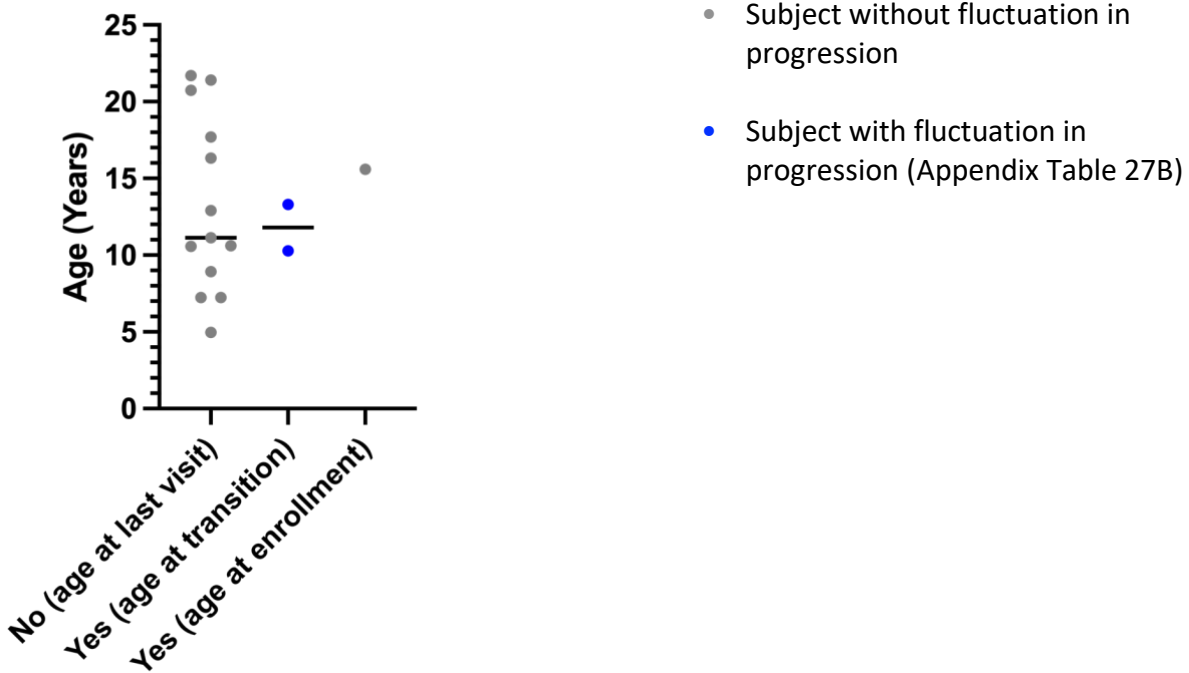
*Grouped Columns—Progression of Milestone 26*
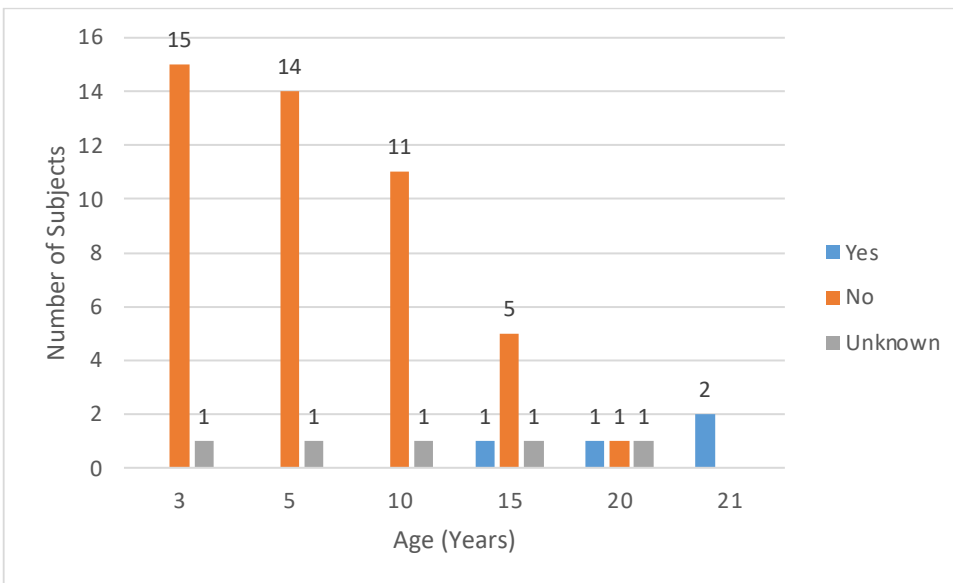


*Note.* At visit 1, if a subject reported 'no' to milestone 26, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 26 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Disease-Related Milestone 27**

*Has optic nerve atrophy or 'pale' optic nerve*

**Appendix Figure 27A**

*Scatter Plot—Progression of Milestone 27*



- Subject without fluctuation in progression

- Subject with fluctuation in progression (Appendix Table 27B)

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 27B**

*Longitudinal Plot—Progression of Milestone 27*
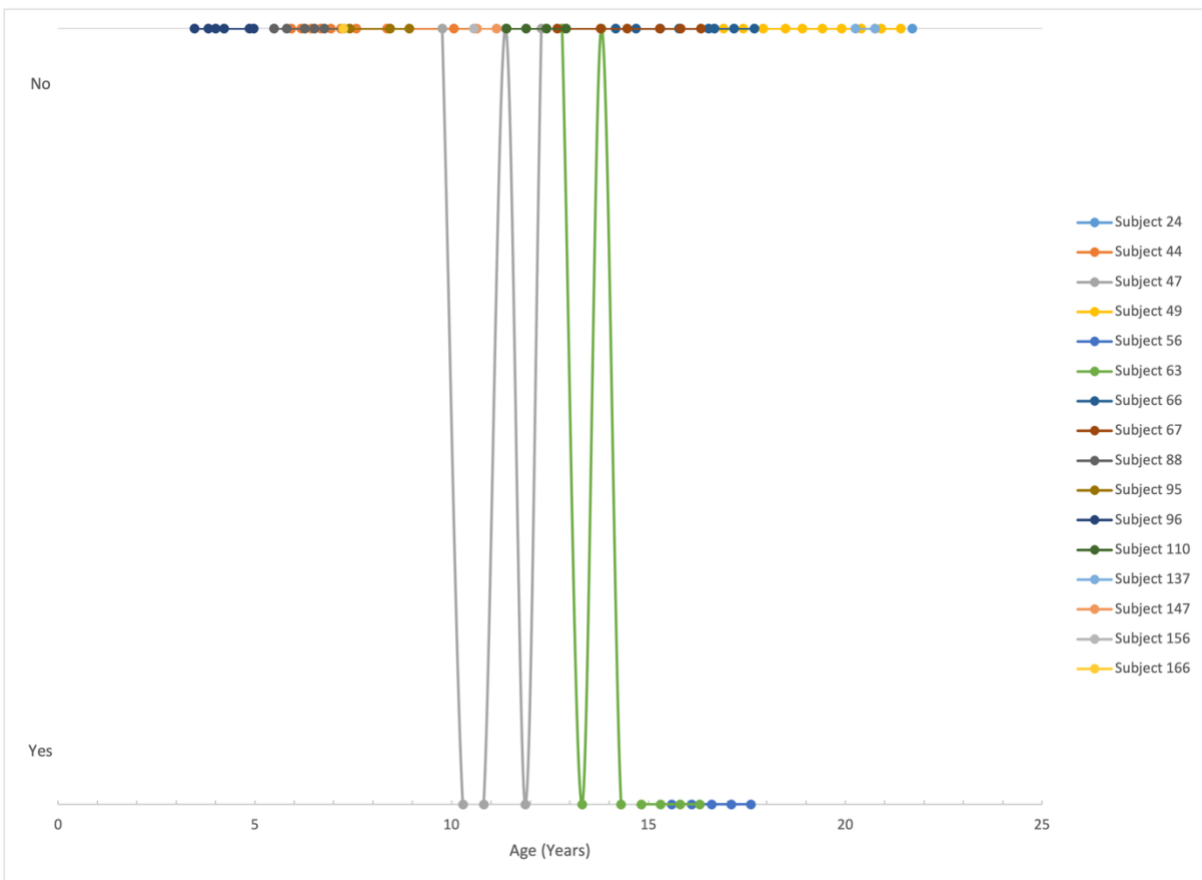


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 27, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature (Appendix Table 27B).
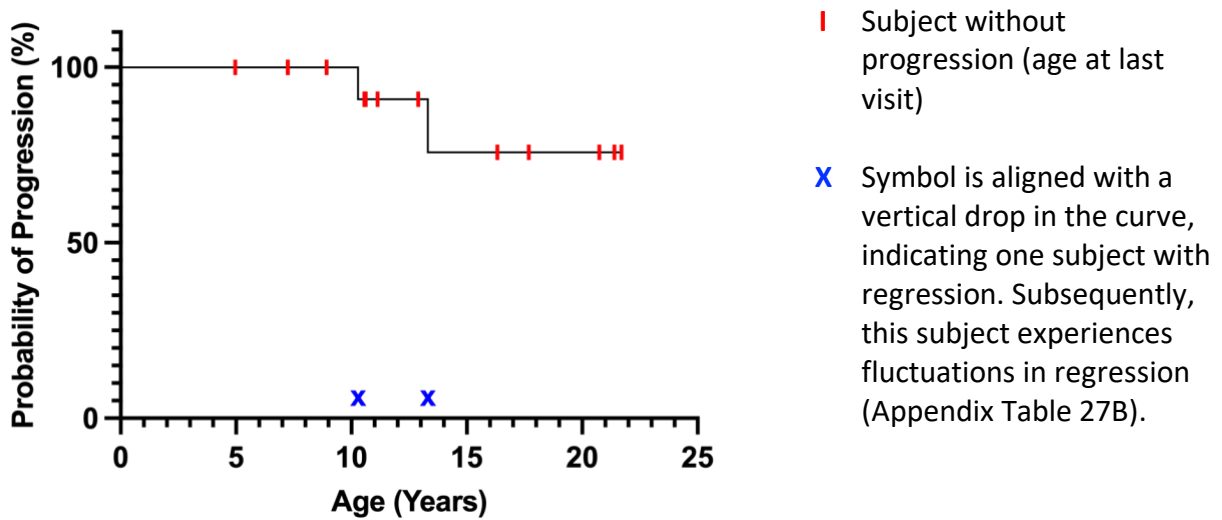
**Appendix Table 27A**

*Kaplan-Meier Curve Analysis—Progression of Milestone 27*

| Age Range for Progression (Years) | | | No Progression (%) |
|---|---|---|---|
| 0.0 | to | 10.3 | 100 |
| 10.3 | to | 13.3 | 91 |
| 13.3 | to | 21.7 | 76 |

**Appendix Figure 27C**

*Kaplan-Meier Curve—Progression of Milestone 27*



**I** Subject without progression (age at last visit)

**X** Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 27B).

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 27 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 27A and Table 7.

**Appendix Figure 27D**

*Grouped Columns—Progression of Milestone 27*
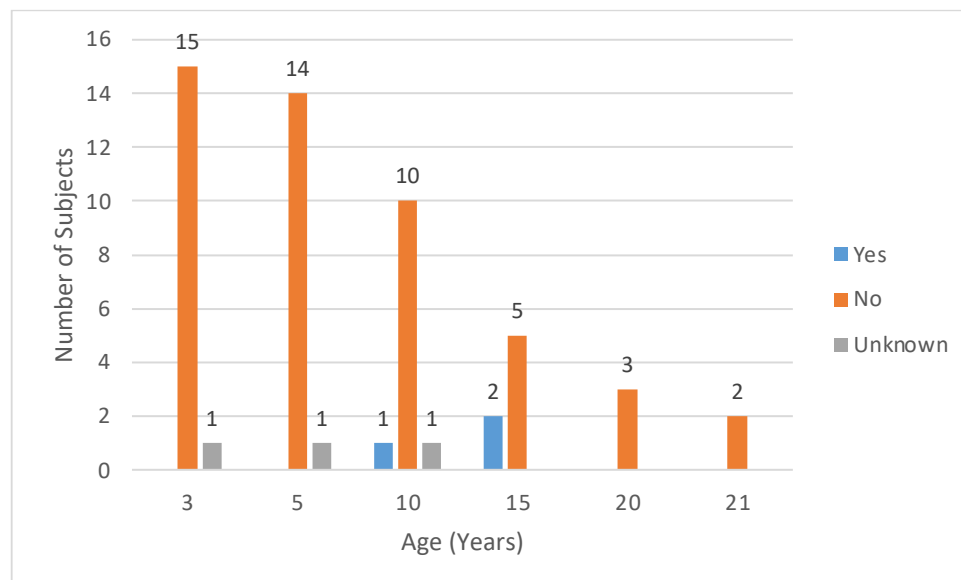


*Note.* At visit 1, if a subject reported 'no' to milestone 27, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 27 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 27B**

*Fluctuations—Progression of Milestone 27*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
| 47 | 1 | 9.8 | No |
| | 2 | 10.3 | Yes |
| | 3 | 10.8 | Yes |
| | 4 | 11.4 | No |
| | 5 | 11.9 | Yes |
| | 6 | 12.3 | No |
| 63 | 1 | 12.8 | No |
| | 2 | 13.3 | Yes |
| | 3 | 13.8 | No |

**Appendix Table 27B Continued**

*Fluctuations—Progression of Milestone 27*

| Subject | Visit | Age (Years) | Progression |
|---|---|---|---|
| | 4 | 14.3 | Yes |
| | 5 | 14.8 | Yes |
| | 6 | 15.3 | Yes |
| | 7 | 15.8 | Yes |
| | 8 | 16.3 | Yes |

**Disease-Related Milestone 28**

*Has constipation (or uses treatments for it)*

**Appendix Figure 28A**

*Scatter Plot—Progression of Milestone 28*
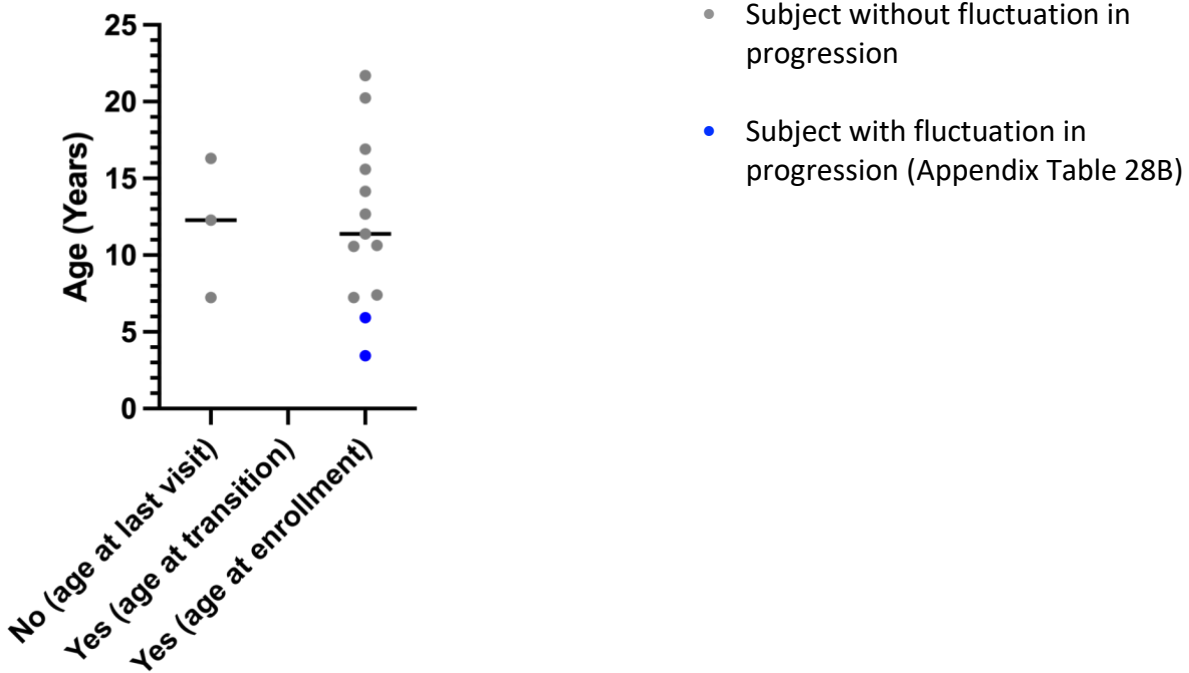


- Subject without fluctuation in progression

- Subject with fluctuation in progression (Appendix Table 28B)

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 28B**

*Longitudinal Plot—Progression of Milestone 28*
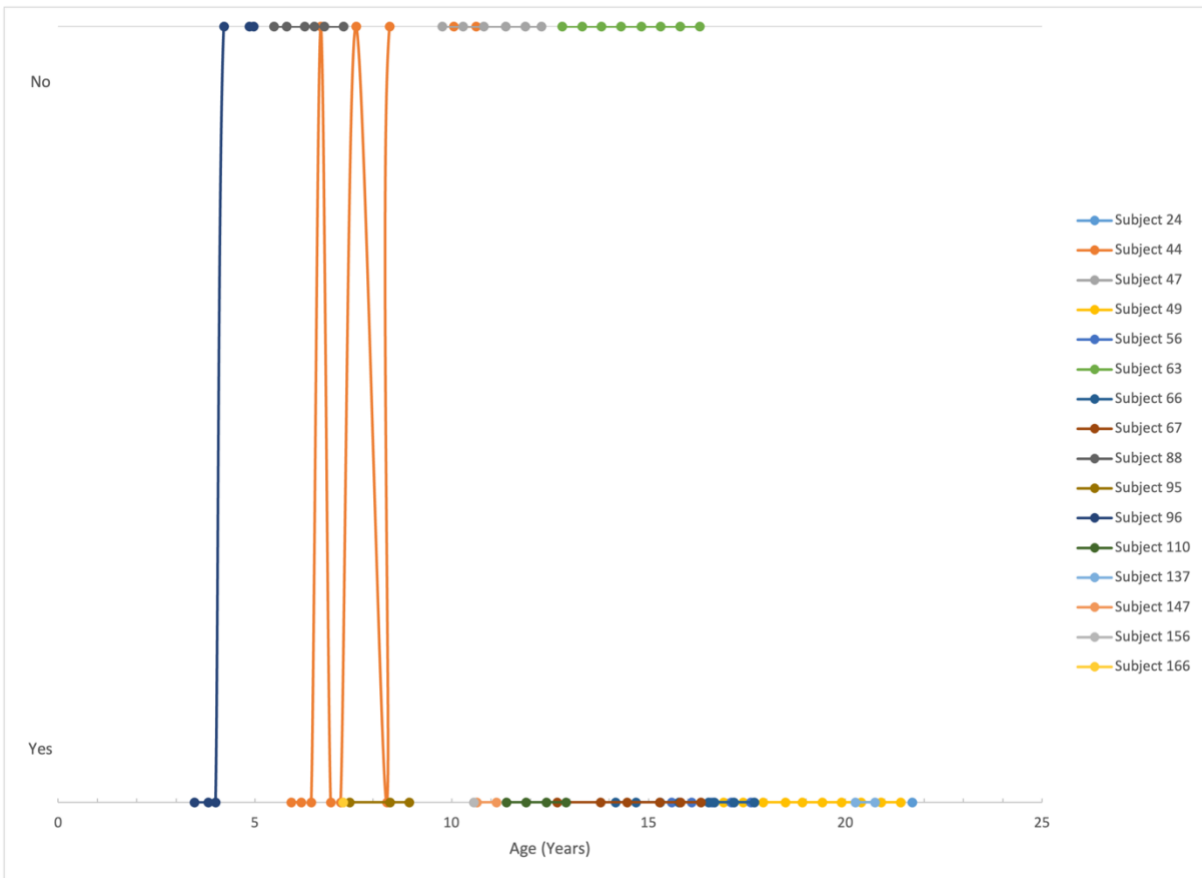


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 28, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature (Appendix Table 28B).
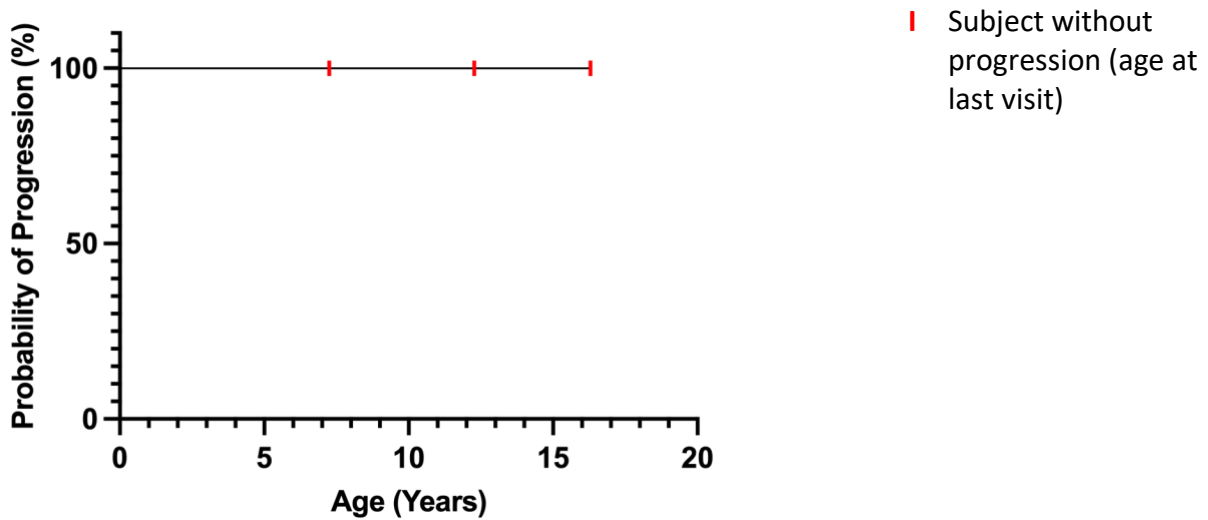
**Appendix Table 28A**

*Kaplan-Meier Curve Analysis—Progression of Milestone 28*

| Age Range for Progression (Years) | No Progression (%) |
|---|---|
| 0.0    to    16.3 | 100 |

**Appendix Figure 28C**

*Kaplan-Meier Curve—Progression of Milestone 28*



| Subject without progression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 28 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 28A and Table 7.

**Appendix Figure 28D**

*Grouped Columns—Progression of Milestone 28*
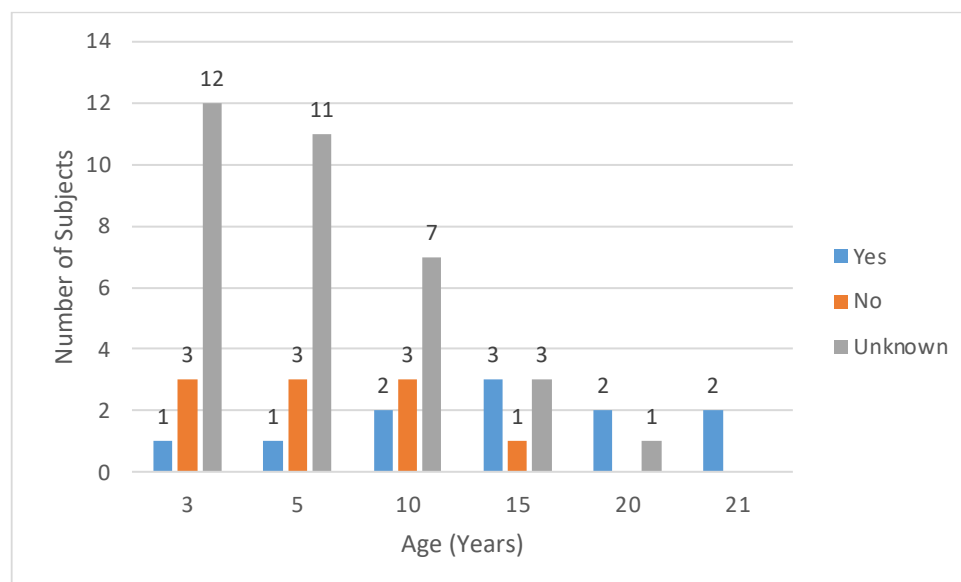


*Note.* At visit 1, if a subject reported 'no' to milestone 28, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 28 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 28B**

*Fluctuations—Progression of Milestone 28*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
| 44      | 1     | 5.9         | Yes         |
|         | 2     | 6.2         | Yes         |
|         | 3     | 6.4         | Yes         |
|         | 4     | 6.7         | No          |
|         | 5     | 6.9         | Yes         |
|         | 6     | 7.2         | Yes         |
|         | 7     | 7.6         | No          |
|         | 8     | 8.3         | Yes         |
|         | 9     | 8.4         | No          |
|         | 10    | 10.1        | No          |

**Appendix Table 28B Continued**
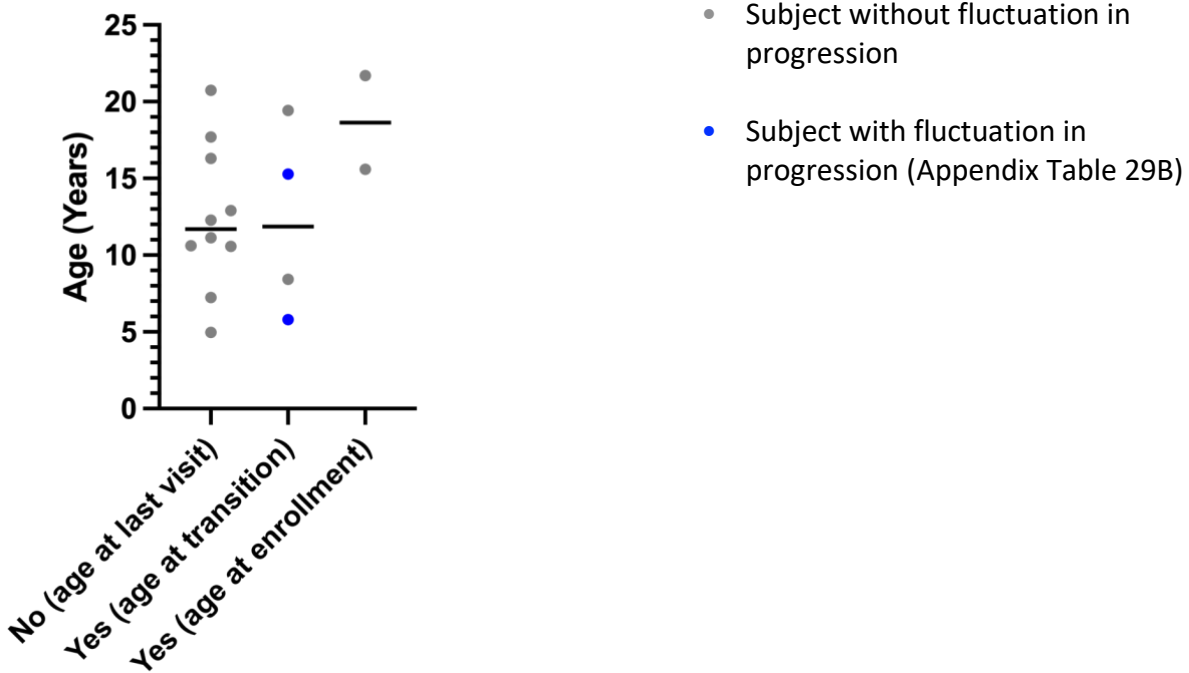
*Fluctuations—Progression of Milestone 28*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
|         | 11    | 10.6        | No          |
|         |       |             |             |
| 96      | 1     | 3.5         | Yes         |
|         | 2     | 3.8         | Yes         |
|         | 3     | 4.0         | Yes         |
|         | 4     | 4.2         | No          |
|         | 5     | 4.9         | No          |
|         | 6     | 5.0         | No          |

**Disease-Related Milestone 29**

*Has gastroesophageal reflux (or uses treatments for it)*

**Appendix Figure 29A**

*Scatter Plot—Progression of Milestone 29*



● Subject without fluctuation in progression

● Subject with fluctuation in progression (Appendix Table 29B)

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 29B**

*Longitudinal Plot—Progression of Milestone 29*
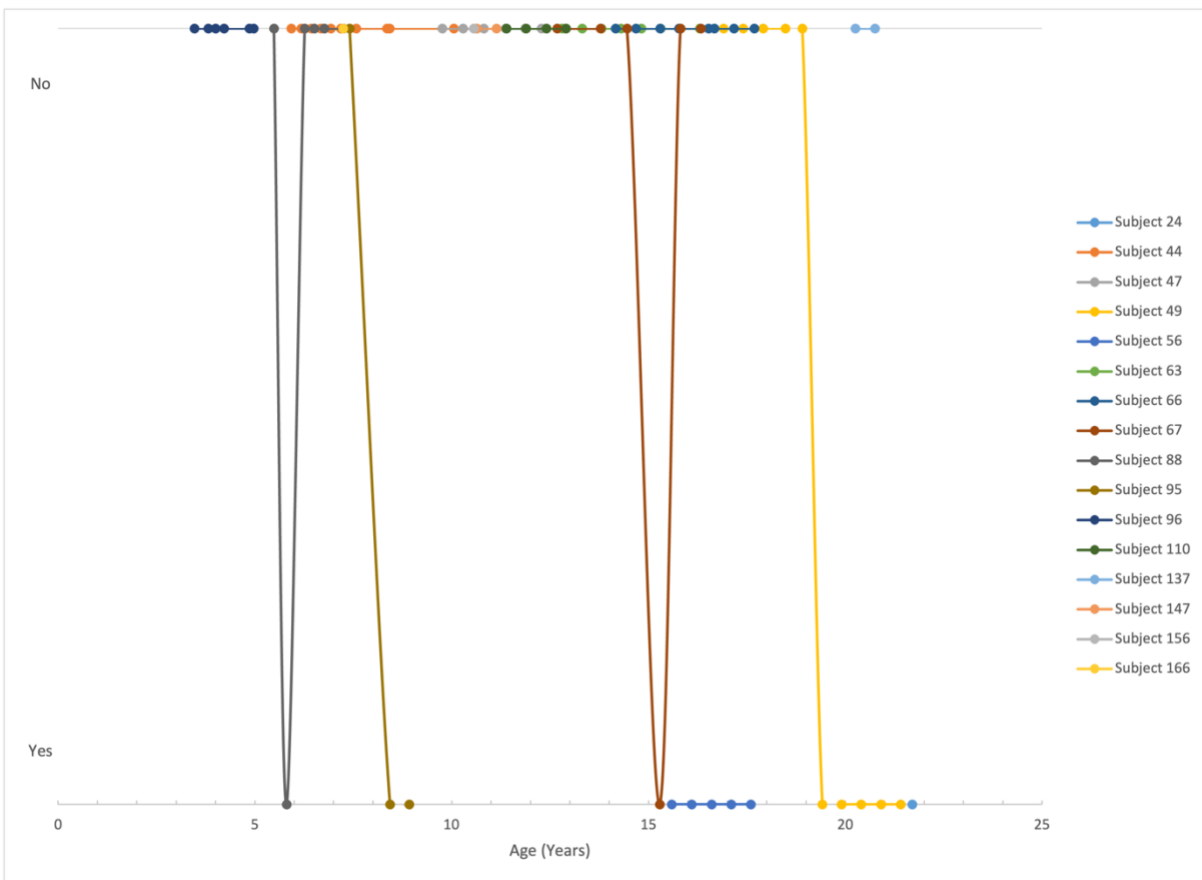


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 29, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature (Appendix Table 29B).
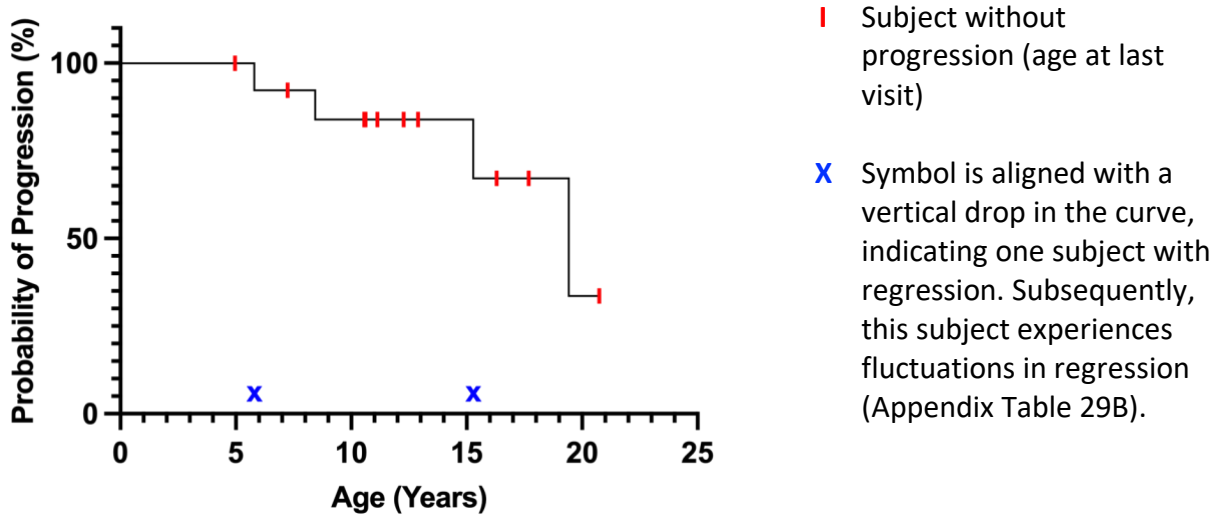
**Appendix Table 29A**

*Kaplan-Meier Curve Analysis—Progression of Milestone 29*

| Age Range for Progression (Years) | | | No Progression (%) |
|---|---|---|---|
| 0.0 | to | 5.8 | 100 |
| 5.8 | to | 8.4 | 92 |
| 8.4 | to | 15.3 | 84 |
| 15.3 | to | 19.4 | 67 |
| 19.4 | to | 20.7 | 34 |

**Appendix Figure 29C**

*Kaplan-Meier Curve—Progression of Milestone 29*



**I** Subject without progression (age at last visit)

**X** Symbol is aligned with a vertical drop in the curve, indicating one subject with regression. Subsequently, this subject experiences fluctuations in regression (Appendix Table 29B).

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 29 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 29A and Table 7.

**Appendix Figure 29D**

*Grouped Columns—Progression of Milestone 29*
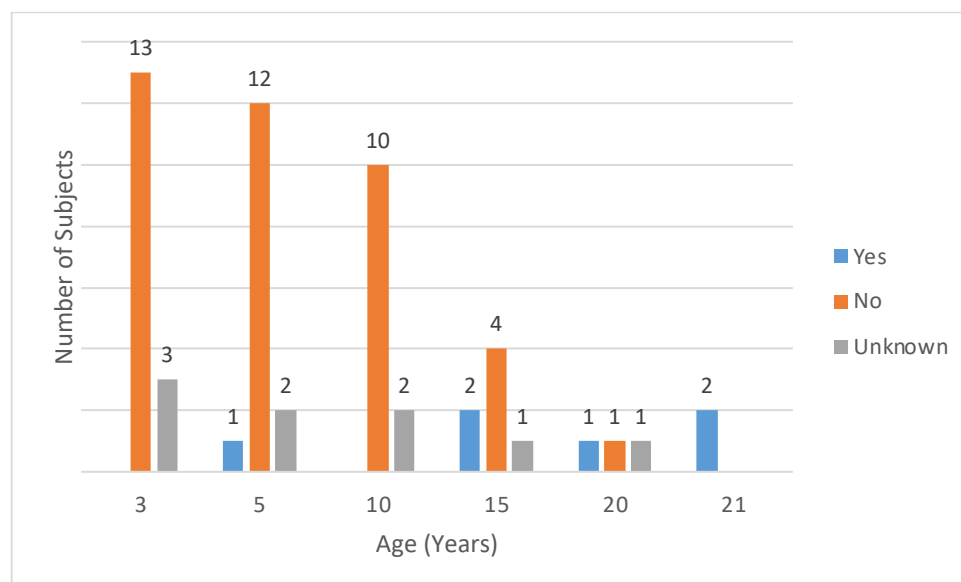


*Note.* At visit 1, if a subject reported 'no' to milestone 29, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 29 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Appendix Table 29B**

*Fluctuations—Progression of Milestone 29*

| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
| 67      | 1     | 12.7        | No          |
|         | 2     | 13.8        | No          |
|         | 3     | 14.5        | No          |
|         | 4     | 15.3        | Yes         |
|         | 5     | 15.8        | No          |
|         | 6     | 16.3        | No          |
| 88      | 1     | 5.5         | No          |
|         | 2     | 5.8         | Yes         |
|         | 3     | 6.3         | No          |

**Appendix Table 29B Continued**

*Fluctuations—Progression of Milestone 29*

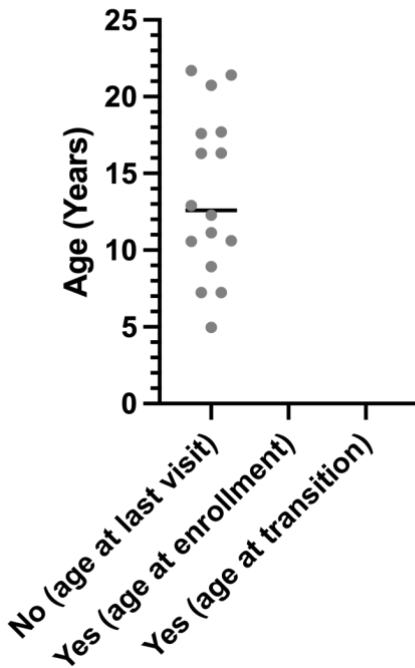| Subject | Visit | Age (Years) | Progression |
|---------|-------|-------------|-------------|
|         | 4     | 6.5         | No          |
|         | 5     | 6.8         | No          |
|         | 6     | 7.2         | No          |

**Disease-Related Milestone 30**

*Has a tracheostomy*

**Appendix Figure 30A**

*Scatter Plot—Progression of Milestone 30*



• Subject without fluctuation in progression

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 30B**

*Longitudinal Plot—Progression of Milestone 30*
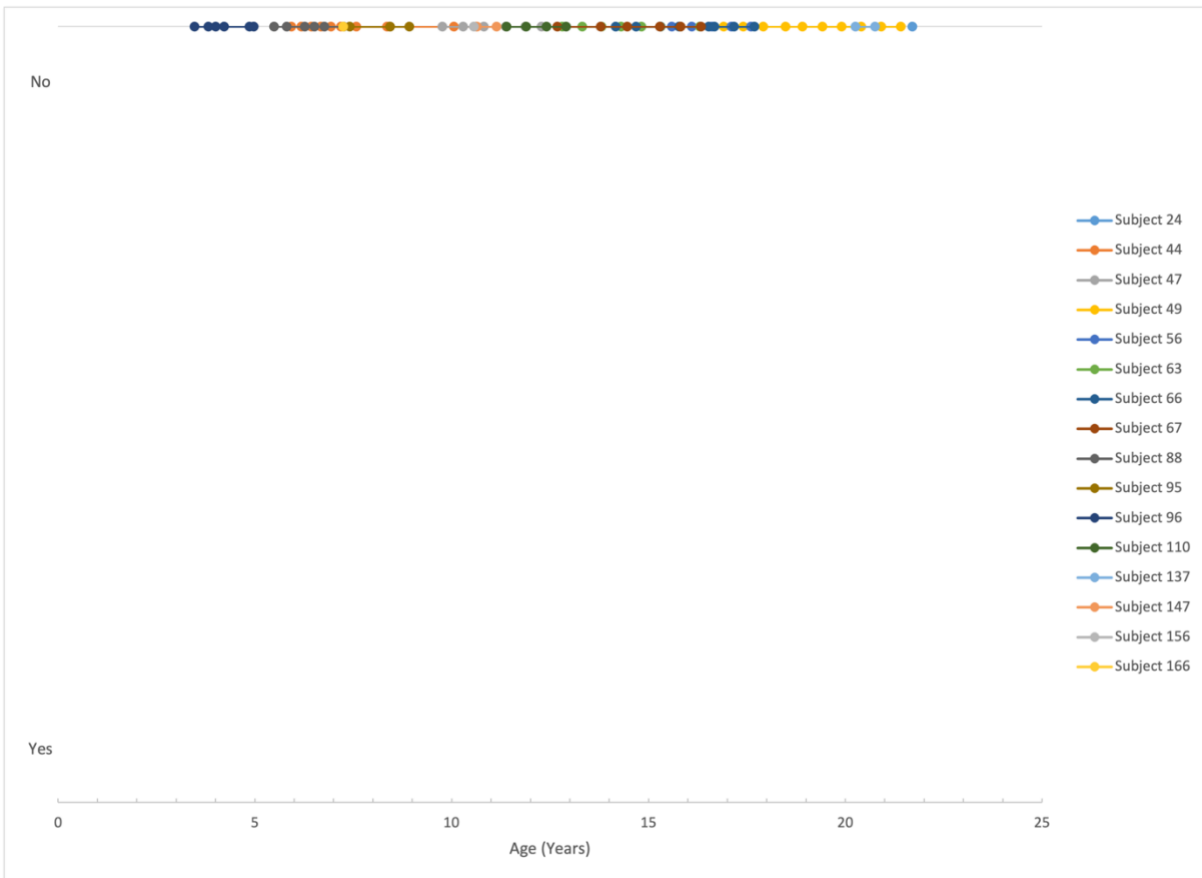


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 30, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature.
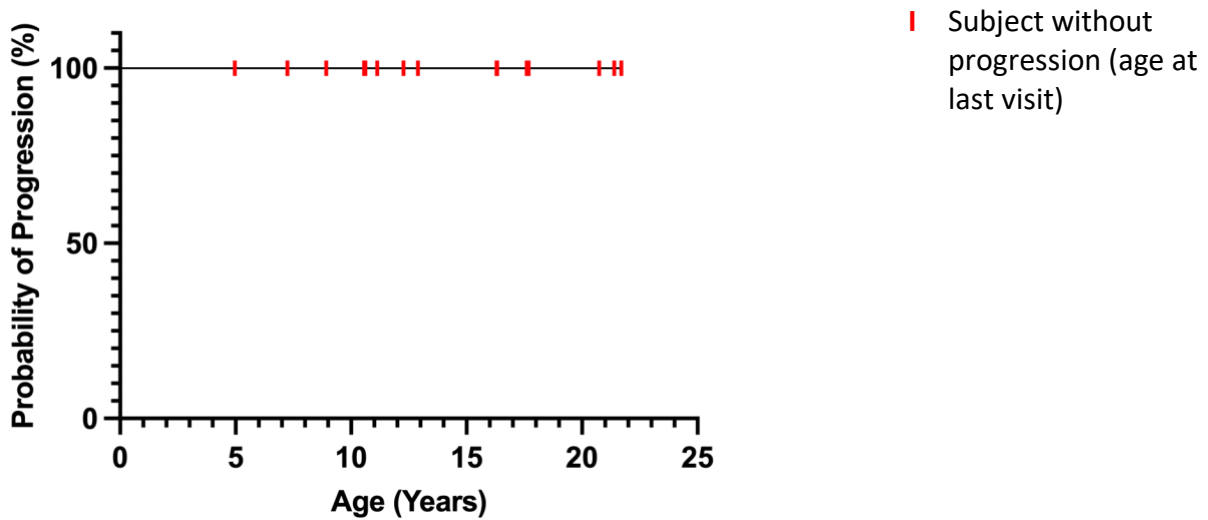
**Appendix Table 30**

*Kaplan-Meier Curve Analysis—Progression of Milestone 30*

| Age Range for Progression (Years) | No Progression (%) |
|---|---|
| 0.0    to    21.7 | 100 |

**Appendix Figure 30C**

*Kaplan-Meier Curve—Progression of Milestone 30*



| Subject without progression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 30 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 30 and Table 7.

**Appendix Figure 30D**

*Grouped Columns—Progression of Milestone 30*
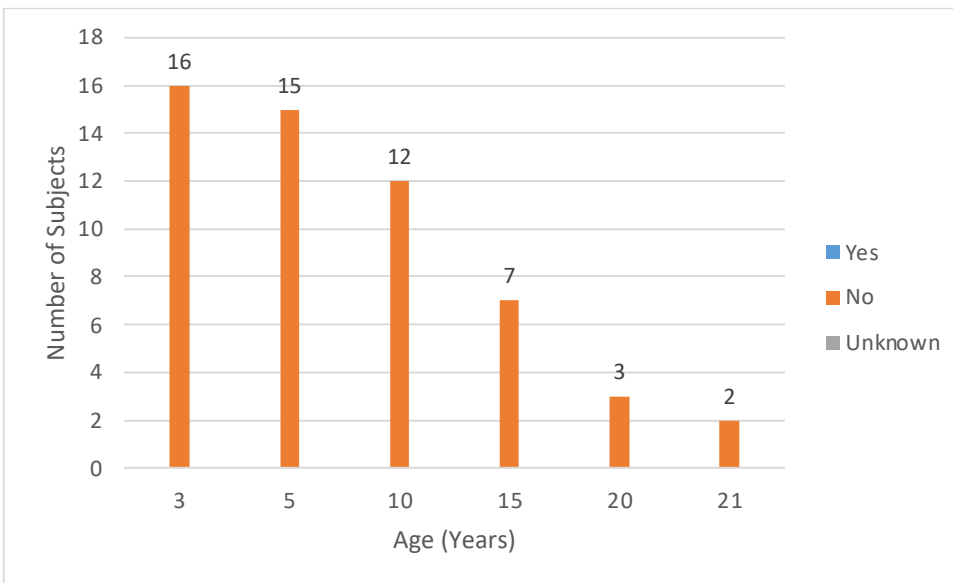


*Note.* At visit 1, if a subject reported 'no' to milestone 30, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 30 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).

**Disease-Related Milestone 31**

*Has abnormal autonomic function (such as cold hands/feet or sudden changes in core body temperature)*

**Appendix Figure 31A**

*Scatter Plot—Progression of Milestone 31*
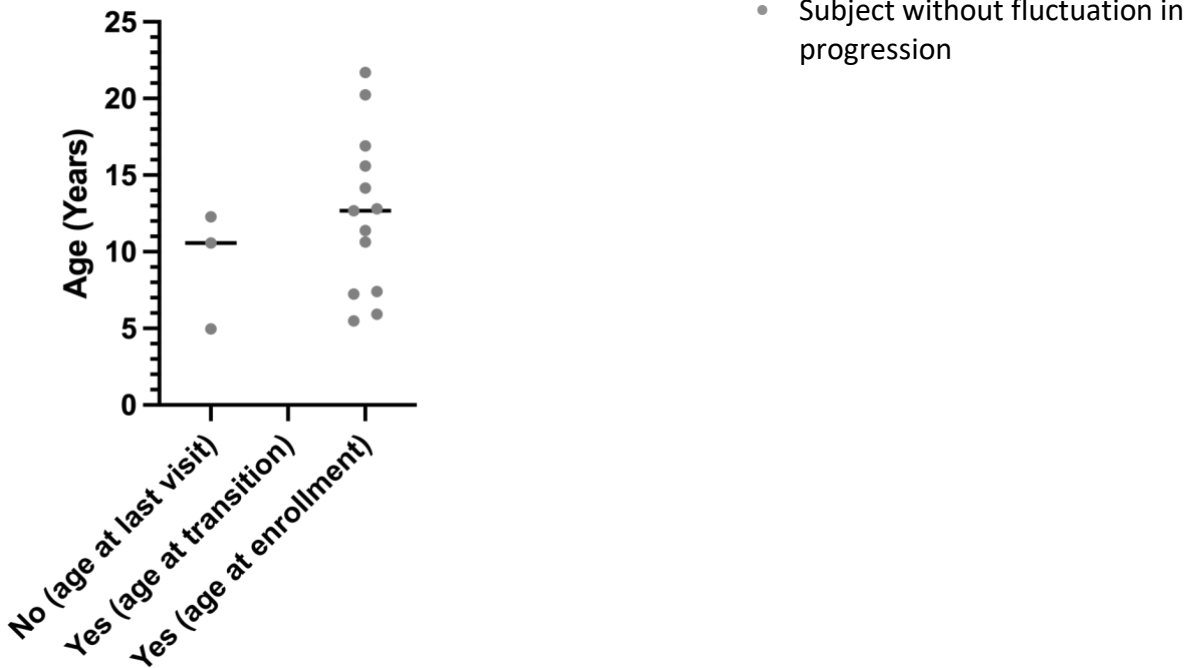


• Subject without fluctuation in progression

*Note.* Participants were categorized into one of three groups: 'No (age at last visit)' for those who have not experienced progression, 'Yes (age at transition)' for progression captured in real-time, and 'Yes (age at enrollment)' for progression that occurred before enrollment in PLANready. A black horizontal line represents a median. Descriptive statistics can be found in Table 4.

**Appendix Figure 31B**

*Longitudinal Plot—Progression of Milestone 31*
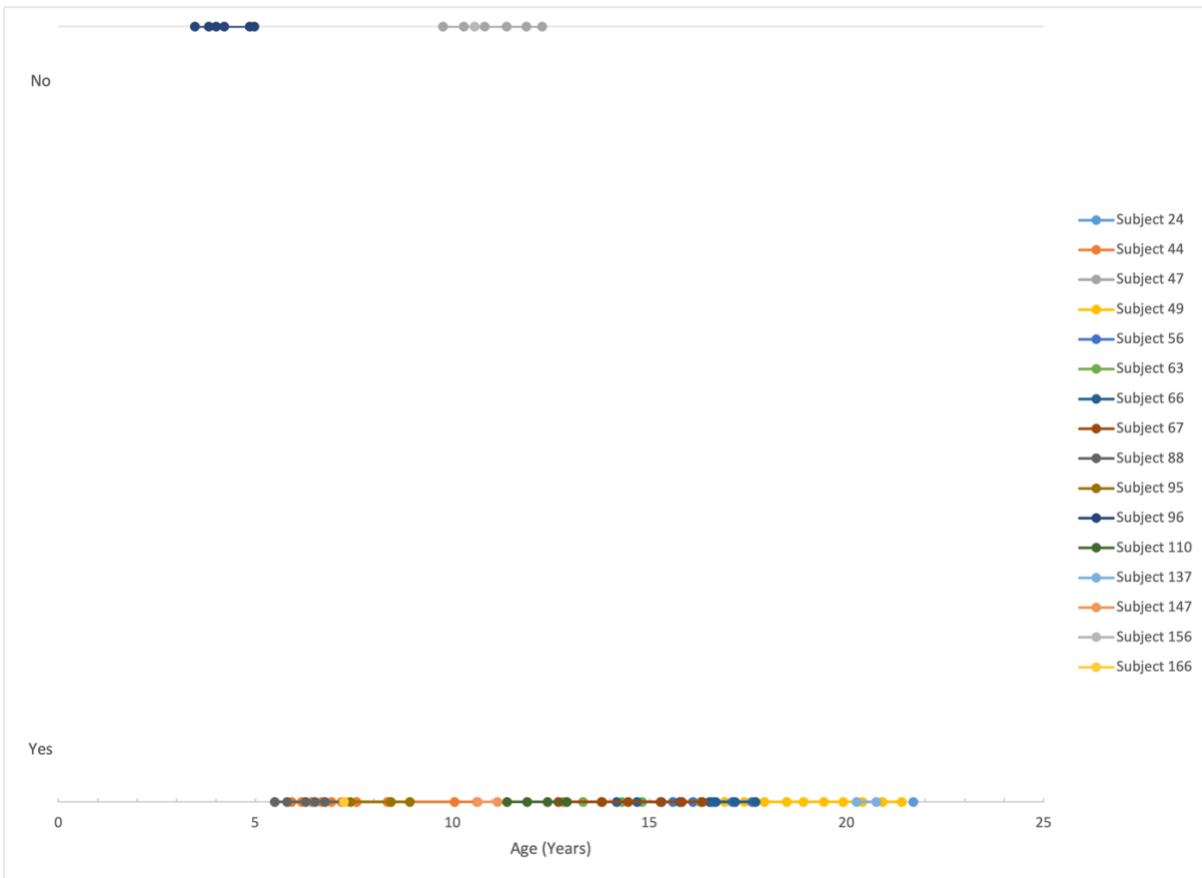


*Note.* Each subject was represented by a unique color, and dots indicate study visits relative to the subject's age. Dots at the top indicate that the subject reported 'no' to milestone 31, while dots at the bottom indicate 'yes'. If present, a negative slope represents a real-time transition from 'no' to 'yes', while a positive slope indicates fluctuations in this feature.
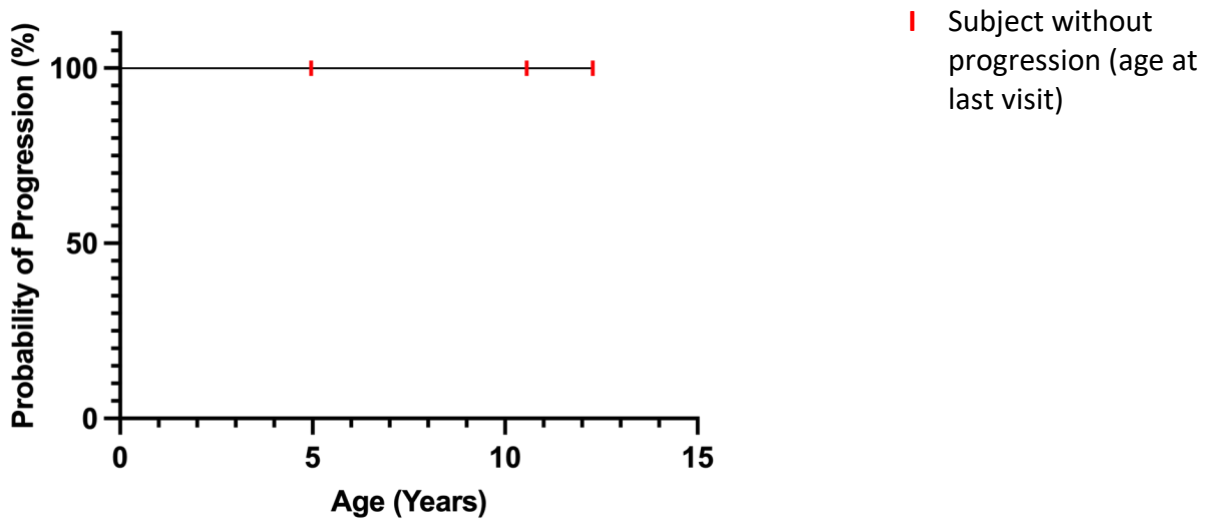
**Appendix Table 31**

*Kaplan-Meier Curve Analysis—Progression of Milestone 31*

| Age Range for Progression (Years) | No Progression (%) |
|---|---|
| 0.0 to 12.3 | 100 |

**Appendix Figure 31C**

*Kaplan-Meier Curve—Progression of Milestone 31*



| Subject without progression (age at last visit)

*Note.* If present, a vertical drop in the solid black line represents real-time progression of milestone 31 for one individual subject. Subjects who experienced progression before enrolling in PLANready (age unknown) were not included to prevent left-censored data. For descriptive statistics, see Appendix Table 31 and Table 7.

**Appendix Figure 31D**

*Grouped Columns—Progression of Milestone 31*
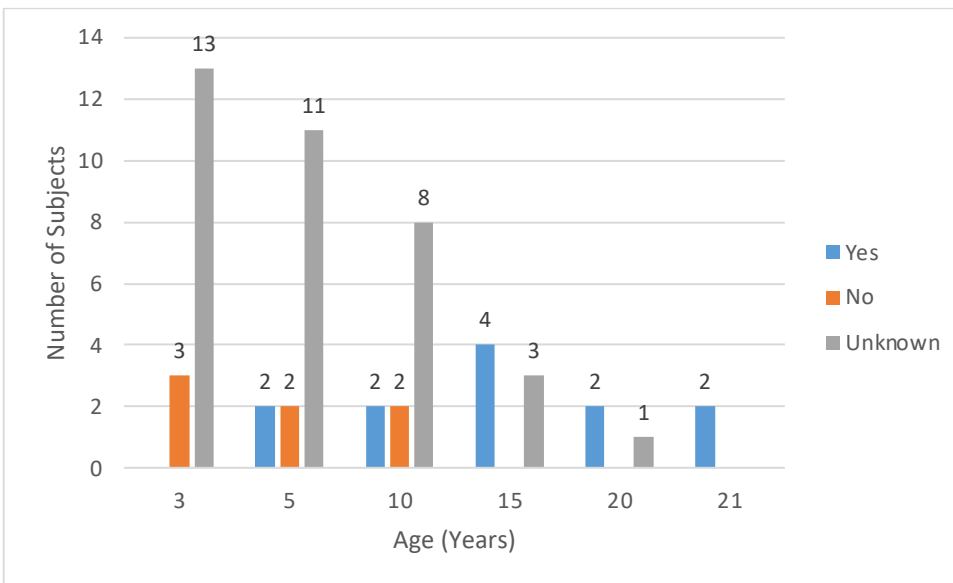


*Note.* At visit 1, if a subject reported 'no' to milestone 31, they were assumed not to have had this feature prior to enrollment and were marked accordingly (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'no'). If a subject reported 'yes', their prior status was marked as 'unknown' due to the uncertainty of when milestone 31 first occurred (e.g., visit 1 occurred at age 10, ages 1-9 are marked as 'unknown'). Note that the focus was on the number of individuals affected at specific ages, not the identification of when this feature first occurred. Bar graphs for each age group may not always equal 16, as not all individuals have reached certain ages (Table 8).