Radhika Dhamija, MBBS

Mayo Clinic Protocol for Minimal Risk Research

General Study Information

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Study Title: PIONEER: **P**OLR2 **I**nvestigation **O**f **N**atural History, Outcom**E**s, and **E**valuation for **R**esearch

Current Protocol Version Number: Version 1.4

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Research Question and Aims

Hypothesis: We hypothesize that a longitudinal characterization of individuals with pathogenic variants in *POLR2A* or *POLR2B* will define the natural history of *POLR2*-related neurodevelopmental disorder, establish robust genotype-phenotype correlations stratified by gene and variant mechanism, and identify clinically meaningful outcome measures suitable for use as endpoints in future therapeutic trials.

Aims, purpose, and/or objectives (primary, secondary, and exploratory):

PURPOSE: To establish a multi-source natural history registry for individuals with molecularly confirmed *POLR2*-related neurodevelopmental disorder (including *POLR2A*-related disorder (NEDHIB) and the *POLR2B*-related neurodevelopmental phenotype), in order to comprehensively characterize the clinical, developmental, neuroimaging, and biochemical features of this rare condition over time and to support future translational and therapeutic research.

PRIMARY OBJECTIVES:
To define the natural history of *POLR2*-related neurodevelopmental disorder and advance clinical trials readiness by defining the spectrum, frequency, age of onset, severity, and longitudinal evolution of clinical features, management, and outcomes in individuals with POLR2A or POLR2B related disorders.

This objective will be achieved through collection and analysis of both **non-concurrent** data, including historical medical records, diagnostic reports, genetic testing reports, imaging, laboratory results, prior clinical assessments, and other available standard-of-care information.

SECONDARY OBJECTIVES:

1. To define longitudinal trajectories of disease progression using both non-concurrent data, including historical medical records, diagnostic reports, imaging, laboratory results, genetic testing reports, and

other standard-of-care data, and concurrent data collected prospectively through real-world follow-up, clinical assessments, and biospecimen collection.

2. To establish genotype-phenotype correlations based on the affected gene, variant type, variant location, inheritance pattern, predicted molecular consequence, and clinical severity.
3. To characterize the prevalence, severity, age of onset, and clinical impact of medical comorbidities and organ-system involvement.
4. To describe clinical management patterns, healthcare utilization, surveillance practices, supportive interventions, quality-of-care indicators, and variability or gaps in care.
5. To evaluate associations between management strategies and clinical outcomes in an observational framework.
6. To identify candidate clinical outcome assessments, patient- and caregiver-reported outcomes, functional measures, disease milestones, biomarkers, and endpoints that may support future interventional clinical trials.
7. To develop and maintain a coded registry of individuals with POLR2-related neurodevelopmental disorder to support longitudinal follow-up, future recontact, recruitment for research studies, and clinical trial readiness.
8. To evaluate the feasibility, tolerability, and longitudinal utility of standardized developmental, rehabilitation, gait, neuropsychological, adaptive behavior, and quality-of-life measures as candidate clinical outcome assessments for POLR2-related neurodevelopmental disorder.
9. To characterize motor function, manual dexterity, gait, mobility, adaptive behavior, cognition, participation, and caregiver/family impact over a 2–3 year follow-up period.

EXPLORATORY OBJECTIVES:

1. To investigate genetic, molecular, cellular, and biological mechanisms underlying POLR2-related disorders.
2. To support biomarker discovery and development using linked clinical data, biospecimens, imaging, laboratory data, omics data, cellular models, and other research-derived measures.
3. To create and maintain research resources, including coded datasets, biospecimen repositories, DNA/RNA resources, fibroblast lines, iPSC-derived models, engineered cellular models, and other derivative materials.
4. To enable controlled sharing of coded data, biospecimens, cell lines, derivative materials, and research findings with Mayo Clinic investigators and approved external academic, nonprofit, government, commercial, laboratory, biotechnology, pharmaceutical, and patient-advocacy collaborators under appropriate agreements.
5. To collect real-world treatment exposure, clinical outcome, and safety data from participants receiving approved, investigational, off-label, compassionate-use, or other disease-directed therapies outside this protocol.
6. To support post-approval or post-marketing evidence generation, when applicable, by collecting longitudinal data on treatment exposure, safety, tolerability, clinical outcomes, durability of response, healthcare utilization, and disease progression.

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Background:

Pathogenic variants in the genes encoding the catalytic core subunits of RNA polymerase II (pol II) cause recently described, ultra-rare neurodevelopmental disorders. The disorder associated with *POLR2* - Neurodevelopmental Disorder with Hypotonia and Variable Intellectual and Behavioral Abnormalities (NEDHIB), was first delineated as a distinct Mendelian condition in 2019. *POLR2A* (chromosome 17p13.1) encodes RPB1, the largest and catalytic subunit of pol II, and *POLR2B* (chromosome 4q12) encodes RPB2, the second-largest subunit; together, RPB1 and RPB2 form the catalytic core of the enzyme responsible for transcription of all messenger RNA and several non-coding RNAs in eukaryotic cells. Because both genes are among the most evolutionarily constrained in the human genome, germline pathogenic variation was not recognized as a cause of Mendelian disease until the widespread adoption of trio exome and genome sequencing. The true prevalence of each disorder is unknown but is presumed to be on the order of <1:1,000,000.

The *POLR2A*-related disorder presents with a clinical spectrum that ranges from a severe, infantile-onset encephalopathy with profound hypotonia, absent speech, refractory epilepsy, and progressive cerebellar atrophy, to a milder phenotype characterized by delayed motor milestones, learning difficulties, and mild dysmorphism. Given the obligate functional partnership of RPB1 and RPB2 within the catalytic core of pol II, pathogenic variants in *POLR2B* are anticipated to disrupt pol II–mediated transcription through analogous mechanisms, and emerging evidence suggests that they give rise to a clinically overlapping neurodevelopmental phenotype. Because of this shared underlying biology, the overlapping clinical presentation, and the fact that each disorder is individually too rare to study in isolation, this protocol encompasses pathogenic variants in both *POLR2A* and *POLR2B*. There are currently no disease-specific or disease-modifying therapies for either disorder, and clinical management is entirely symptomatic and supportive.

Initial work has shown that disease severity correlates with the mechanism by which a variant disrupts RNA polymerase II function:

- Missense variants exert a dominant-negative effect on pol II, producing a malfunctioning enzyme that interferes with transcription. These variants are associated with the most severe phenotype, dominated by profound infantile-onset hypotonia, severe global developmental delay, feeding difficulties, and structural brain abnormalities including delayed myelination, thin corpus callosum, and ventriculomegaly.
- Loss-of-function variants result in haploinsufficiency, with the unaffected allele providing reduced but functional pol II. These variants are generally associated with milder phenotypes, including delayed walking, mild to moderate intellectual impairment, and subtler dysmorphic features.
- A growing number of variants of intermediate effect have been associated with additional phenotypic features including refractory epilepsy, ataxia, progressive cerebellar atrophy, autism spectrum disorder, joint hypermobility, congenital cardiac anomalies, immune dysfunction, hip dysplasia, and isolated reports of central nervous system tumors.

Despite this rapidly expanding phenotypic literature, no natural history study of *POLR2*-related neurodevelopmental disorder has been undertaken. The existing evidence base consists almost exclusively of

retrospective case reports and small cross-sectional series, with substantial heterogeneity in phenotyping depth, follow-up duration, and outcome measures used. As a result, the longitudinal trajectory of motor, cognitive, and language development is unknown; it is unclear whether neuroimaging findings are static or progressive; the true prevalence and clinical impact of medical comorbidities have not been systematically characterized; and there are no validated, disease-specific outcome measures suitable for use as endpoints in future interventional trials. The rarity of the condition and the lack of clinician familiarity with it contribute to diagnostic delay, prolonged diagnostic odysseys for affected families, and inconsistent multidisciplinary care.

This study is a longitudinal natural history study for individuals with molecularly confirmed POLR2-related neurodevelopmental disorder, conducted at the Mayo Clinic Department of Clinical Genomics. The general objective of the study is: a) to establish the natural history of POLR2-related neurodevelopmental disorder, including its clinical, developmental, neuroimaging, and biochemical characteristics and their evolution over time; b) to refine genotype-phenotype correlations stratified by affected gene, variant mechanism, and variant location; c) to characterize the prevalence and longitudinal course of medical comorbidities, including neurological, cardiac, immune, musculoskeletal, and gastrointestinal manifestations; d) to assess quality of life and caregiver burden using validated caregiver-reported outcome measures; and e) to provide an inventory of well-characterized participants who may be eligible for recruitment into future interventional studies.

The vision of this study is to build a patient-centered, collaborative natural history and clinical trial readiness framework for **POLR2-related disorders** that defines disease progression, captures patient and caregiver priorities, enables biomarker and therapeutic development, and accelerates future studies aimed at improving outcomes for affected individuals and families.

Study Design and Methods

Methods: This study is an non-interventional, prospective, natural history study designed to enable the collection of comprehensive clinical data and biospecimens (including blood, cerebrospinal fluid, and fresh or archival tissue samples) to better understand developmental progress, serious medical events and behavioral changes in individuals with confirmed POLR2-related neurodevelopmental disorder. This study will include prospective standardized developmental, motor, functional, gait, cognitive, adaptive behavior, and quality-of-life assessments to characterize longitudinal disease trajectory and identify candidate outcome measures for future interventional trials. These assessments are observational in nature and are not intended to diagnose, treat, or alter clinical management, although clinically relevant findings may be documented in the medical record and communicated to the participant's care team when appropriate. This registry will remain open for registration indefinitely, with no planned termination or closure date. All individuals with a confirmed, suspected, or pending diagnosis are eligible to join. Participants will be excluded if they do not meet the inclusion criteria, and information may be excluded if the person submitting it is not the participant or their legally authorized representative. Genetic testing to confirm a genetic diagnosis will not be performed under this protocol and should be ordered exclusively for clinical purposes.

A maximum of 50 subjects will be accrued in this clinical study. 10 screen failures will be allowed, for a maximum of 60 subjects to be screened in order to reach the maximum accrual.

Information about the study will be shared with interested members of the *POLR2* community, including patients, parents, physicians, and researchers for recruitment purposes. Data will be collected through future clinical assessments and past medical records review. Primary outcome measures will include the frequency and severity of serious medical events such as respiratory infections, cardiovascular complications, or other disorders.

Participant data will be recorded in an electronic database using code-generated aliases and identifiable private health information (name, date of birth, health record number, date of medical events). Only IRB approved study staff will have electronic access to the database.

In addition, we may also collect whole blood biospecimens from participants, which will be stored in a secure location at Mayo clinic. Samples collected will be at minimal risk to the participants. These samples will be collected, processed, and dispersed to researchers by the Mayo Clinic Biorepository's Biospecimen Accessioning and Processing (BAP) Lab according to established standard operating procedures. Researchers on this protocol will utilize samples for disease modeling, researching variant mechanisms, molecular pathogenesis, develop strategies for targeted therapeutics, and improve clinical understanding of the disease.

Subjects may be asked to provide a punch biopsy of the skin (3mm) if they are capable and willing. These samples will be utilized to create fibroblasts for various disease modeling studies. The study will only collect up to 10 skin biopsies total, all from different subjects. Subjects will choose to take part in the optional skin biopsy within the informed consent form after being informed of the risks.

Decentralized clinical trial practices will be enabled for this study, given the rare nature of the disease and considering proximity to the study site(s). Subjects who are unable or unwilling to travel to the study site will work with study staff to arrange remote phlebotomy. Instructions for local facilities to collect, process, and ship blood samples will be provided to ensure consistent samples across all subjects. The samples will all be sent to Mayo Clinic for central usage and for ease of retrieval.

Participants may be invited to complete standardized assessments at enrollment and again during longitudinal follow-up, approximately annually for up to 3-years post-enrollment, depending on participant availability, clinical feasibility, and burden. Those unable to complete physical and occupational assessments, gait assessments, cognitive assessments, or other in-person assessments will undergo a standard virtual visit with a Physical Medicine and Rehabilitation provider to assess basic developmental markers based on basic and noninvasive telerehabilitation protocols.

Participants may be recruited by Mayo Clinic physicians or other study staff. Recruitment may also be supported through Dr. Craig Kaplan's University of Pittsburgh registry, "POLR2A and related POLR2 Patient Registry." Dr. Kaplan may serve as an external referring provider because of his involvement in POLR2

research. Any sharing of participant-identifying information between Mayo Clinic and the University of Pittsburgh will occur only as permitted by participant consent/authorization and under an appropriate data use or similar agreement.

Professor William Murphy, an external collaborator in Biomedical Engineering at the University of Wisconsin, may receive coded human biospecimens to support organoid development and related gene-delivery or therapeutic research. Any transfer of specimens or associated information will occur only under appropriate institutional agreements and in accordance with participant consent/authorization. Participants who do not consent to sharing information or specimens with external collaborators will not have their data or samples sent to external institutions, including the University of Wisconsin or the University of Pittsburgh.

Subject Information

Target enrollment: 150 subjects

Target accrual: 150 subjects (50 affected subjects for primary subject; up to 100 caregiver subjects providing answers to questionnaire in the cases where the affected subjects are unable).

Subject population(s): Children, adults lacking capacity to consent, and developmentally delayed subjects will be recruited for this study. Researchers delegated to perform informed consent will be trained in specific regulations as outlined in 45 CFR 46 and guidelines in ICH Good Clinical Practice.

Inclusion Criteria:

1. Clinically confirmed *POLR2 (A or B)* genetic mutation **OR**
2. Suspected *POLR2 (A or B)* genetic mutation **OR**
3. Pending results for *POLR2 (A or B)* genetic mutation
4. May be included if deemed primary caregiver of affected and consented subject (caregiver subject population only, above three criteria do not apply)

Exclusion Criteria:

1. No clinically confirmed *POLR2* genetic mutation
2. Patient or family members (Legally authorized representative, if applicable) unable/unwilling to provide informed consent or assent.
3. Unable or unwilling to complete blood draw for the research study
4. Not deemed a primary caregiver by the study team (caregiver subject population only, above three criteria do not apply)

Study Procedures

Schedule of Activities:

Procedure	Baseline/Enrollment Visit	If performed clinically	Annual follow-up (Y1, Y2, and Y3) +/-60 days	Follow-up (indefinitely**)
Informed Consent/Assent	X			
Retrospective Assessments				
Medical Record Review	X			X
Research Assessments				
Physical/Occupational	X		X	
Gait	X		X	
Cognitive	X		X	
QoL Questionnaires	X		X	
Biospecimen Collections				
Blood Draw (on-site or remote)	X			
*Skin Biopsy (Max N=10)**	X			
*Fresh or Archival Tissue**		X		
*Cerebrospinal Fluid**		X		

*** = optional**
**** = or until study closure**

Informed Consent/Assent and Screening:

Prior to initiating any study-related procedures, legally effective informed consent (and assent, if applicable) will be obtained from the participant or their legally authorized representative (LAR) / primary caregiver. Screening will consist of verifying a documented, pathogenic, or likely pathogenic mutation in the *POLR2A* gene via clinical genetic report, if available. See Inclusion/Exclusion criteria for more details.

Given the developmental nature of the disorder, it is estimated that all subjects will require written permission from their parents or caregivers. Given that this study provides no direct benefit to the subjects and that it is considered Minimal Risk, permission from only one parent or guardian will be required.

Medical Record Review

The study team will conduct a comprehensive retrospective and prospective review of the participants' available medical records. Both baseline and longitudinal reviews will be completed. Baseline reviews will extract medical history, diagnostic reports (including genetic testing), neuroimaging, clinical laboratory results, and documentation of physical, neurological, and developmental examinations.

Longitudinal review may allow researchers to update medical history following the date of initial consent to track disease progression, clinical milestones, and other health-related outcomes.

Research Assessments (Physical Medicine and Rehabilitation)

Assessments for physical/occupational, gait, cognition, and quality of life will be performed by physicians and delegated staff within the Department of Physical Medicine and Rehabilitation for the purpose of understanding various measures of development. Assessments may be paused, shortened, split across visits, or discontinued at participant/caregiver request or at investigator discretion due to fatigue, behavioral distress, medical instability, developmental limitations, or scheduling constraints. Descriptions of individual assessments and estimated times are noted below:

Physical and Occupational Assessments

- **Bruininks-Oseretsky Test of Motor Proficiency, Third Edition (BOT-3; estimated time: approximately 25–90 minutes, depending on form administered).** The participant will complete standardized, performance-based motor tasks administered by trained study staff. Tasks may include fine motor precision and integration activities, manual dexterity tasks using small objects, upper-limb coordination activities such as catching or throwing, balance activities, bilateral coordination activities, and strength or dynamic movement tasks. The assessment is used to characterize gross and fine motor function; testing may be stopped, modified, or omitted if the participant is unable to safely or meaningfully complete an activity.BOT-3 – using this as has both gross and fine motor assessments, performance based for ages 4-25 years. While BOT-4 just released, expense, change in documentation/reporting, and anticipated delay in uptake by external providers makes this less optimal.
- **Bayley Scales of Infant and Toddler Development, Fourth Edition (Bayley-4; estimated time: approximately 30–70 minutes).** For participants who are younger or whose developmental level makes other age-based measures inappropriate, trained study staff will administer structured developmental activities and observe the participant's responses. The participant may be asked to engage in age-appropriate play-based tasks involving objects, pictures, movement, communication, problem solving, and motor activities. Caregiver input may also be collected for social-emotional and adaptive behavior domains, as applicable.Bayley-4 – developmental assessment of foundational cognitive, language (expressive/receptive), and motor abilities through observation and direct play. Validated for infants up to 42 months. This will be used for children under 2 years of age or with developmental delays that make other age-validated measures not applicable.
- **Six-Minute Walk Test (6MWT; estimated time: approximately 10–15 minutes, including instructions and rest as needed).** The participant will be asked to walk back and forth along a marked, level course for up to six minutes while study staff record the total distance walked and observe tolerance, need for assistance, and any safety concerns. Participants may use their usual assistive devices or orthotics, as clinically appropriate, and may slow down, pause, or stop at any time.
- **Box and Block Test (BBT; estimated time: approximately 5–10 minutes).** The participant will be seated at a table and asked to move small blocks, one at a time, from one side of a divided box to the other using one hand at a time during a timed trial. Study staff will record the

number of blocks transferred to provide a standardized measure of gross manual dexterity for each upper extremity, when feasible.Box and Blocks Test – validated for ages 3 years and up. Utilized for gross measure of upper extremity manual dexterity.

- **Nine-Hole Peg Test (9HPT; estimated time: approximately 1–3 minutes per hand, with additional time for instructions or practice as needed).** The participant will be asked to pick up small pegs one at a time, place them into holes on a pegboard, and then remove them and return them to the container as quickly as possible using one hand at a time. Study staff will time the task in seconds to characterize fine finger dexterity for each hand, when feasible.Nine hole Peg test - Validated for ages 3 years and up. Utilized for measure of fine motor manual dexterity.
- **Pinch-Grip Strength Testing (estimated time: approximately 5–10 minutes).** The participant will be asked to squeeze or pinch a calibrated hand-strength device using standardized hand positions, when able. Study staff will record grip and/or pinch force for the right and left hands, with repeated attempts as tolerated, to characterize upper-extremity strength. Testing will be discontinued if the participant experiences pain, fatigue, or inability to safely complete the task.Pinch-grip test – Measure of hand strength
- **Upper Extremity Range of Motion and Manual Muscle Testing (ROM/MMT; estimated time: approximately 15–30 minutes).** Study staff will assess shoulder, elbow, wrist, and hand movement using standard clinical positioning and goniometric measurement, as feasible. Strength may be assessed by asking the participant to move or hold the limb against gravity or gentle examiner resistance using a standard manual muscle testing scale. The assessment is observational and functional in nature and will be limited or omitted if the participant cannot safely tolerate positioning or resistance.Upper extremity ROM (goniometric) and Strength testing (Medical Manual Muscle Testing Rating Scale)

Gait Lab Assessments

- **Instrumented Gait Laboratory Assessment with Physical Examination (estimated time: approximately 2–3 hours).** The participant may undergo a gait laboratory evaluation in which trained staff observe and record walking patterns, including movement and force data, with and without braces or orthotics when applicable. Reflective markers or sensors may be placed on the participant's body and the participant may be asked to walk across a walkway multiple times at a comfortable pace. A related physical examination may include assessment of strength, range of motion, selective motor control, balance, and mobility. Rest breaks will be provided as needed, and activities will be limited to those the participant can safely tolerate.Kinetics (brace and no brace) and Physical Exam (strength, ROM, selective control) (2-3 hours)

Cognitive Assessments

- **Neuropsychology Intake Interview (estimated time: approximately 30 minutes).** A neuropsychology provider or delegated study staff member will meet with the participant and/or caregiver to review developmental, educational, behavioral, medical, and functional history relevant to cognitive and adaptive functioning. Information may be obtained through interview

and review of available records to guide selection and interpretation of age- and ability-appropriate cognitive measures.Neuropsychometric intake with provider 30 minutes

- **Differential Ability Scales, Second Edition (DAS-II; estimated time: approximately 30–60 minutes for core testing, depending on age, ability, and subtests administered).** The participant will complete standardized cognitive tasks administered individually by trained personnel. Tasks may include age-appropriate activities involving verbal reasoning, nonverbal problem solving, pattern construction, memory, processing speed, and visual-spatial reasoning. The assessment will be used to characterize cognitive abilities and developmental level; subtests may be selected, discontinued, or omitted based on participant age, attention, communication, fatigue, and safety.Differential Ability Scale – lower floor for testing, age –2.5 to 18 yrs 30 minutes
- **Bayley Scales of Infant and Toddler Development, Fourth Edition (Bayley-4; estimated time: approximately 30–70 minutes).** For younger participants or participants functioning developmentally below the age range of other cognitive measures, trained study staff will administer structured developmental tasks and observe the participant's responses. Activities may involve play-based assessment of cognitive, language, and motor skills, with caregiver questionnaires or caregiver input collected for social-emotional and adaptive behavior domains when applicable.Bayley-4 for younger kids 60 minutes
- **Vineland Adaptive Behavior Scales, Third Edition (Vineland-3; estimated time: approximately 20–60 minutes, depending on form and domains administered).** A trained examiner will complete a structured caregiver interview or obtain caregiver/teacher rating forms to assess the participant's everyday adaptive functioning. Domains may include communication, daily living skills, socialization, motor skills, and maladaptive behavior, as applicable. The participant is not required to perform physical tasks for this measure; information is based on observed real-world functioning reported by the caregiver or other knowledgeable respondent.Vineland 3 – validated for infants through adults. Adaptive Behavior assessment of real-world life skills across Communication, Daily Living Skills, Socialization, and Motor Skills. Structured clinician interview or caregiver/teacher rating scales. 60 minutes
- **Behavior Assessment System for Children, Third Edition (BASC-3; estimated time: approximately 30 minutes).** A parent/caregiver, teacher, and/or age-appropriate participant may complete standardized rating forms about the participant's behavior, emotions, adaptive skills, and functioning in daily settings. The assessment does not involve physical procedures; respondents answer structured questions based on observed behavior over the specified rating period.Behavioral Assessment for Children (BASC-3) – Validated for Ages 2-21. Behavioral & Emotional assessment via measures of internalizing and externalizing behaviors, emotional control, and behavioral symptoms. Option for multi-rater system using Parent Scales, Teacher Scales, and child/adolescent Self-Reports. 30 minutes

Quality of Life

- **Pediatric Quality of Life Inventory (PedsQL; estimated time: approximately 5–10 minutes per form).** The participant, when developmentally able, and/or the parent/caregiver will complete standardized questionnaires about the participant's health-related quality of life. Items



may address physical, emotional, social, and school functioning. Responses are recorded on paper or electronically and do not involve physical procedures.PedsQL – validated for ages 2-18 years with extensions to age 25 years. A validated instrument for measuring health-related quality of life (HRQOL) in children and adolescents.

- **Pediatric Quality of Life Inventory Family Impact Module (PedsQL-FIM; estimated time: approximately 5–10 minutes).** The parent/caregiver will complete a standardized questionnaire about the effect of the participant's health condition on parent/caregiver functioning and family activities. Items may address physical, emotional, social, cognitive, communication, worry, daily activity, and family relationship domains. This measure is completed by questionnaire and does not involve physical procedures for the participant.PedsQL Family Impact Module - parent self-report instrument that measures how a child's chronic health condition affects the health-related quality of life and functioning of the parents and family.

Return of Research Results

The results of tests performed for study purposes in a research lab or a clinical laboratory on samples that do not meet CLIA quality standards shall not be returned to individual research subjects nor made available in the subject's medical record. The information will be provided to the investigator and care team providers. If the test is available in the clinical setting and, in the opinion of the investigator, returning the test result would be in the best interest of the research subject, the investigator may suggest the subject have the test performed in a CLIA certified lab. The IRB may require genetic counseling for the research subject for the return of a genetic test result. The research subject has the option to either choose or decline to learn the result. When possible and in agreement with the research subject, the investigator will inform the primary care provider of the possible research finding and the primary care provider will follow-up with recommendations regarding clinical confirmatory testing and additional genetic counseling. Confirmatory testing will be billed directly to the subjects and not covered under this protocol.

Biospecimens

Blood will be collected once at enrollment when feasible, with a target amount of approximately 29 mL or less, consistent with minimal-risk blood collection limits. Blood specimens may be processed into plasma and serum aliquots for research use. If remaining samples are unused, they may be stored for future research related to POLR2-related neurodevelopmental disorders under the present protocol.

If **cerebrospinal fluid (CSF)** is obtained as part of clinically indicated care or surgery, residual CSF may be collected for research when available and only after clinical needs have been met. Up to approximately 10 mL of CSF may be retained, if available, and aliquoted/stored for future studies, including cell-free DNA testing and other molecular analyses relevant to POLR2-related disease biology.



If a participant develops a brain **tumor** or other tumor and undergoes clinically indicated biopsy or surgery, residual tumor tissue, adjacent normal tissue when available, matched blood, and/or CSF may be collected for research use after all diagnostic requirements have been satisfied. These specimens may be used to evaluate whether tumor development is associated with the participant's germline POLR2 variant or whether the tumor has acquired a new somatic driver mutation. No tissue will be collected solely for research if it is needed for clinical diagnosis with the exception of the singular skin biopsy.

Participants may optionally provide a single **3 mm skin punch biopsy** if they are capable and willing, and only after this optional procedure is specifically addressed during the informed consent process. Up to 10 total skin punch biopsy specimens will be collected across the study, with no more than one biopsy collected from any individual participant. Skin biopsy specimens may be used to generate primary fibroblast cultures for POLR2-related disease modeling and related translational studies. These samples may be processed, stored, and used for future research related to POLR2-related disease biology; however, permanent or immortalized cell lines will not be established under this protocol.

Summary collection of biospecimen samples:

For blood:

- Volume per blood draw: 29 mL maximum (may be less due to age/low weight)
  Frequency of collection: **Once**

For Tissue:

- 3 mm skin punch biopsy (optional for subject)
  Frequency of collection: **Once**
- Tissue samples: up to 2 unstained slides or 0.5 cm fresh tissue, if available
  Only collected if performed clinically and residual is available for protocol.

Cerebrospinal fluid:

- Cerebrospinal fluid: 10 mL maximum
  Only collected if performed clinically and residual is available for protocol.

Will permanent cell lines be established? No. Permanent or immortalized cell lines will not be established under this protocol. Skin punch biopsy specimens may be used to generate primary fibroblast cultures for disease modeling studies, but samples will not be immortalized in the laboratory.

Will DNA be extracted from the specimens? Yes. DNA may be extracted from blood, tissue, CSF, or other collected biospecimens for future research analyses related to POLR2-related disease mechanisms. Potential analyses may include germline and somatic variant assessment, whole genome sequencing, methylation

profiling, and/or cell-free DNA testing. Any future testing not covered by this protocol will be conducted under an appropriate IRB-approved protocol and consent, as applicable.

Review of medical records, images, specimens

Check all sections that apply (data includes medical records, images, specimens).

☐ ____ Only retrospective data that exists before the IRB submission date will be collected.

Date Range for Specimens and/or Review of Medical Records:
Examples: *01/01/1999 through 12/31/2015*, or all records through *mm/dd/yyyy*.

Note: The Date Range must include the period for collection of baseline data, as well as follow-up data, if applicable.

__X__The study involves data that exist at the time of IRB submission **and** data that will be generated after IRB submission. Include this activity in the Methods section.
Examples

- The study plans to conduct a retrospective chart review for all noted medical events, diagnoses, medications, imagings, and assessments.
- The study plans to include subjects previously diagnosed with a specific disease and prospectively add newly diagnosed subjects in the future.

☐ _____The study will use data that have been collected under another IRB protocol. Include this activity in the Methods section and enter the IRB number from which the research material will be obtained.

Data Analysis

Statistical analysis will be done using R statistical software. These analyses include assessment of mean values and variability of responses to binary response questions, assessment of free text responses, and data visualizations. All analyses will be performed on Mayo Clinic clinical workstations, and data will be stored in secured folders as required. The bulk of the collected data will be descriptive in nature. In characterizing the clinical features of the disease, we will compare various characteristics, diagnoses, treatments, and outcomes. We will pay particular attention in subsequent analyses to those feastures that are distinctive among the group.

Protocol Versions

Protocol version	Date	Summary of Changes
V1.1	**28JUL2026**	Original Protocol
V1.2	**07AUG2026**	Adjusted slides from 5 to 2 per biospecimen subcomittee
V1.3	**25AUG2026**	Updated language requiring consent from both parents to only one parent, since the study is minimal risk.

****The study team is responsible to keep the Protocol Versions section up to date. The IRB will <u>not</u> review this table for accuracy.**