TRACKING NEURODEGENERATION IN EARLY WOLFRAM SYNDROME (I-TRACK) Protocol
HRPO:
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OBJECTIVES:

Wolfram syndrome (WFS; OMIM #222300) is a rare autosomal recessive disease clinically defined in 1938 as the combination of childhood-onset insulin dependent diabetes, optic nerve atrophy, diabetes insipidus and deafness. Based on early descriptions, neurological features were thought to appear later in the disease with death occurring in middle adulthood. Importantly, the major causative gene (WFS1) was identified in 1998. This discovery allowed researchers to determine that the WFS1 gene encodes the protein wolframin, which helps protect cells from endoplasmic reticulum (ER) stress-mediated apoptosis, potentially via intracellular calcium homeostasis. Pathogenic mutations in WFS1 can result in death or dysfunction of cells that are under high ER stress, such as insulin-producing pancreatic β cells, causing insulin dependent diabetes. In addition, knowing the causative gene has allowed us to identify patients by their WFS1 mutation rather than the classic set of symptoms, leading to the increasing realization that the WFS1-related phenotype (including neurologic symptoms) is much more variable than previously understood. **The first iteration of this grant (HD070855 "Tracking Neurodegeneration in Early Wolfram Syndrome") contributed to this shift in understanding.** In this time, we have built a successful annual research clinic for WFS, met or exceeded our recruitment goals for patients and controls, validated a clinical severity rating scale for WFS, described an unexpectedly early neurophenotype of reduced balance, smell identification and ventral pons volume, identified alterations in traditional diffusion tensor imaging (DTI) metrics that suggest hypomyelination as a pervasive neuropathological feature of WFS and provided justification for the selection of two primary outcomes (visual acuity and ventral pons volume) in a newly funded clinical efficacy study in WFS (Barrett, PI).

In this new grant, we hypothesize that ER stress-related dysfunction could inhibit production of myelin during neurodevelopment in WFS, as active and developing oligodendrocytes (cells that produce myelin in the brain) are more vulnerable to ER stress than mature ones. However, standard DTI methods conflate inflammatory processes (which can also be associated with ER stress) in the extra-axonal space with metrics of axonal and myelin integrity, leading to potentially confounded measurements. We propose to collect novel, validated diffusion sequences on a new state of the art MRI scanner (Siemens Prisma) and apply cutting-edge analysis approaches to measure white matter integrity throughout the brain and in the optic nerve, improving our ability to draw conclusions about axonal and myelin integrity over time. We will assess WFS patients annually at our WU Wolfram Research Clinic using these methods.

Findings from this work may indicate future targets for brain-specific intervention, identify outcome measures or high-risk subgroups for clinical trials targeting neurological symptoms. These data will also greatly expand our understanding of the cross-sectional and longitudinal phenotype of WFS1-mutation related disorders, rather than classically defined Wolfram Syndrome. Such knowledge will have a significant impact on patients and families by allowing physicians to provide more accurate prognoses. Finally, forms of ER stress-mediated apoptosis have been implicated in more common neurodegenerative, endocrine and neurodevelopmental diseases, which may benefit from the insights gained here.

GOALS OF THIS PROTOCOL

Our primary goal is to determine the impact of WFS on myelination within white matter tracts. We will study Wolfram syndrome patients yearly for at least 3 years, each year adding new patients as they are identified. Patients will be scanned with MRI and tested to address our hypotheses.

We hypothesize that hypomyelination is a key pathological feature in WFS. To test this hypothesis, we will apply cutting-edge validated diffusion imaging sequences (whole brain and optic nerve) and analyses (Diffusion Basis Spectrum Imaging [DBSI], cortical myelin mapping) to dissociate myelin vs axonal integrity vs extra-fiber components (inflammation-associated cell infiltration and edema) in cross-sectional and longitudinal data. This unique dataset will allow for stronger neurobiological interpretations and will determine whether hypomyelination ultimately leads to axonal and neuronal loss in WFS.

METHODS

Overview: Traditionally, patients and families attend one group research clinic event per year. However, during the time of COVID 19, the 2022 clinic was not held as one research clinic for the year but a series of mini-clinics over a period of months. Mini-clinics throughout the year will continue until the end of the study. Mini-clinic activities will take place at Washington University in Saint Louis, mainly in the Pediatric Clinical Research Unit (PCRU) located on the 11th floor at St Louis Children's Hospital. MRI scans will be performed at the Center for Clinical Imaging Research (CCIR) located on the 10th floor of the Barnes-Jewish Hospital on the south campus of the Washington University Medical Center.

Patient population: We propose to collect new MRI sequences and apply novel analysis techniques to our existing WFS cohort, and we anticipate adding new participants. We will also add siblings of WFS patients who demonstrate no symptoms of Wolfram Syndrome and/or are determined to be non-WFS (carriers or Null-null for WFS1 mutations) to draw stronger neurobiological conclusions and identify better markers of neurodegeneration.

Description of activities and schedule:

1. **Phone Screen**: A phone screen will be conducted with participants to determine their interest in and eligibility for on-line, virtual and/or in-person mini-clinic sessions. The phone screen also serves as an opportunity to explain to participants who have not yet participated in a mini-clinic, the changes to the format of the clinics (i.e. having several smaller clinics instead of one large one, conducting assessments virtually) and to allow them to ask question in real time. The purpose of the phone screen will be explained, and participants will be asked to verbally consent to participating in the phone screen. Information from the phone screen will be documented directly in REDCap.

2. **Informed Consent** will be obtained prior to participants completing any REDCap surveys, virtual visits or in-person mini-clinic testing. To prepare for the consent form discussion with the participant/proxy, the consent form and communication waiver will be sent to participants via REDCap and reviewed with them via phone call or telehealth appointment. Participants will be encouraged to ask questions. Once all questions are answered and the consent forms are complete, the consent forms will be certified by a member of the study data management team, scanned, verified and stored electronically in the secure REDCap data base. Participants will be sent a completed and signed copy of the consent forms. Participants will be asked whether they would like to receive the consent forms via REDCap, e-mail, or USPS.

3. **Surveys:** Once all participants' questions are answered and the consent forms have been signed, families will be sent a questionnaire or "survey" via the secure REDCap website. Completing the

survey prior to the clinic saves time at the clinic and allows families to access their records at home in order to answer questions more accurately. Approved study staff are then able to access the REDCap database and retrieve the completed data. There are minimal hard copies to be transported or filed and there is much less chance that the participants' information is lost or incorrectly entered into the database. For those who do not complete the survey electronically, it will be available to them to complete electronically at a mini-clinic.

4. **Research Clinic:** Prior to COVID, we hosted large annual research clinics in July of each year where patients are evaluated by various members of the research team. Since 2022, we have hosted a series of mini-clinics involving only a few families at a time. At these mini-clinics, WFS patients and their non-WFS siblings, will be evaluated with multiple tests including blood and urine samples, endocrinology, brain imaging with MRI, ophthalmology, smell, cognition, balance and psychiatric symptoms. On any one of the testing days during the mini-clinics, the biological parent(s) of each patient will be asked to participate in the study. The parent will be asked to do a brief reading task.

5. **Post Clinic:** A summary of the clinically relevant information obtained (e.g. standard clinical measure such as visual acuity) will be sent to patients or parents post-mini-clinic.

Measures obtained:

Parents:

1) **Questionnaires:** A parent will fill out questionnaires about the participant with WFS via REDCap prior to the virtual data collection sessions and the mini-clinic sessions.
    a. Checkmate Plus DSM-based questionnaire(s): appropriate for the patient's age. The CASI-4R will be completed by a parent (parent-report on patient) if the patient is age 6 yrs or older. The Early Childhood Inventory-4 (ECI-4) will be completed by a parent (parent-report on patient) if the patient is age 3-5 yrs.
    b. Emotion Regulation Checklist (ERC): A parent report on how children exhibit various emotional states.
    c. Blood Glucose Questionnaire: A parent report to assess symptoms of hypo and hyperglycemia.
    d. Pediatric Sleep Questionnaire (PSQ): A parent report assessing the child's usual sleep habits.
    e. PROMIS Fatigue Scale: The PROMIS Fatigue Scales rate quality of the child's life based on symptoms of fatigue.
    f. Social Economic Scale (SES): administered to collect occupational and educational information.
    g. Neurogenic Bladder Symptom Score (NBSS): administered to assess neurogenic bladder dysfunction for participants age 14 and younger.
    h. NeuroQOL Cognitive Function Short Form: A parent report of daily cognitive function and potential cognitive difficulties.
    i. Observer Memory Questionnaire (OMQ): a parent report expected to directly assess the cognitive quality of life in patients and siblings and may capture subtle deficits with high sensitivity.
    j. Developmental and Motor History Form: a parent report expected to directly assess the participant's motor skills and motor coordination history.

WFS Patients and Siblings:

1) **Questionnaires:** WFS patients will fill out the following questionnaires with the help of a parent, if needed:
    a. Activities Questionnaire: A self-report completed by the child to assess how symptoms of Wolfram Syndrome may be impacting daily life.
    b. Bladder and Bowl Dysfunction Questionnaire (BBD): used to assess bladder/bowel symptoms.
    c. Checkmate Plus DSM-based questionnaire(s) appropriate for the patient's age: The Youths Inventory-4 (YI-4) will be completed by the patient (self-report) if the patient is age 12-18 yrs. The Adult Self-Report Inventory (ASRI-4) will be completed by the patient (self-report) if the patient is age 18 yrs or older.
    d. Composite Autonomic Symptom Score (COMPASS 31): a self-report questionnaire designed to assess autonomic function in patients in a comprehensive manner.
    e. Emotional Regulation Questionnaire (ERQ): a self-report questionnaire used to assess the respondents' tendencies to regulate their emotions.
    f. Epworth Sleepiness Scale: administered to the participant to measure the level of daytime sleepiness.
    g. Everyday Memory Questionnaire (EMQ): a self-report expected to directly assess the cognitive quality of life in patients and siblings and may capture subtle deficits with high sensitivity.
    h. Hearing Environments and Reflection on Quality of Life Measurement for Children (Hear-QL-26 and Hear-QL-28): administered to participants ages 7-12 yrs (Hear-QL-26) and participants ages 13-18 yrs (Hear-QL-28) to assess the impact of hearing on quality of life.
    i. Hypo-Hyper Unawareness questionnaire: will be completed to assess awareness of blood glucose extremes.
    j. Neurogenic Bladder Symptom Score (NBSS): administered to assess neurogenic bladder dysfunction for participants age 15 yrs and older.
    k. NeuroQOL Cognitive Function Short Form: A self-report of daily cognitive function and potential cognitive difficulties.
    l. Occupational Therapy Activities Questionnaire: a self-report questionnaire used to assess daily functioning.
    m. Pediatric Urinary Incontinence Questionnaire (PIN-Q): administered to assess the impact of urinary incontinence on quality of life.
    n. Pittsburgh Sleep Quality Index (PSQI) or Pediatric Sleep Questionnaire (PSQ): The PSQI and PSQ are questionnaires to measure sleep quality. Participants age 18 yrs or older will complete the PSQI and participants 17 yrs and younger will complete the PSQ.
    o. Profile of Fatigue and Discomfort Scale: administered to the participant to get a more detailed assessment of the nature of daytime sleepiness and fatigue and track them over time.
    p. PROMIS Fatigue Scale: The PROMIS Fatigue Scales rate quality of life based on symptoms of fatigue. Fatigue is a prominent symptom in our WFS patients, and we would like to explore further how it could impact daily function.
    q. Rand Visual Function Questionnaire: The VFQ-25 takes about 10 minutes to perform and will generate scales related to the visual disability and visual symptoms due to the neurodegenerative disease of wolfram syndrome
    r. Urine and Bowel diaries: will be completed approximately one to three weeks prior to the clinic.
    s. Voiding Questionnaire: administered to the patient to assess frequency and urgency of

daily urination.

Medical History: The research team could obtain written permission to examine the medical records of all WFS group and sibling subjects for data including previous blood sugar control, severe blood sugar events, complications from diabetes, dates of disease diagnosis, details relating to birth (term carried, birth weight, birth height), head circumference and other information related to Wolfram Syndrome. The medical history will be reviewed with the participant and/or their proxy either virtually or at the mini-clinic visit.

Genetics: A genetic counselor will meet with each family virtually or in-person to answer questions they have regarding Wolfram, family history and/or general genetics. A genogram and a pedigree will be created for new patients, and updated as necessary for returning patients.

Ophthalmology: The ophthalmologic evaluation will assess optic atrophy and central, peripheral and color vision and will take place at the mini-clinics. We will also conduct the OCT retina on some participants whose RNFL is near or above 60. Color vision will be tested with HRR color plates. In addition, persons with Wofram syndrome will be asked to complete a questionnaire about visual disability and visual symptoms.

Endocrinology evaluation: A pediatric endocrinologist will perform, oversee or interpret the following measures with Wolfram patients and their siblings without WFS at the mini-clinics only.

1) **A general comprehensive physical exam** including a personal medical history. This routine physical exam will examine the skin, eyes, ears, nose, mouth, neck, heart, lungs, abdomen, hands, and feet. Detailed medical history of occurrences of hypo- or hypernatremia will be gathered in addition to history of DDAVP or vasopressin treatment and sodium supplementation.
2) **Boost Test:** WFS participants diagnosed with diabetes for approximately $\leq$ 10 years will be asked to fast overnight and then come in for a blood draw at approx. 8 am and drink a Boost liquid mixed meal. They will then have a blood draw 30 min later for glucose and c- peptide. The purpose of the Boost test is to assess residual beta cell function. The total amount of blood to be drawn is less than two tsp. In addition, we will attempt to draw blood from all participants (Wolfram Syndrome patients, siblings, and biological parents) to determine if neurofilament light (NfL) measures in plasma are abnormal in Wolfram Syndrome when compared to controls, indicating on going neurodegeneration. The total amount of blood drawn for this is approximately 8 ml or 0.5 Tbsp.
3) **Blood and urine samples** will be taken. Diabetes insipidus will be assessed by measuring serum sodium and osmoles and urine specific gravity by drawing approximately 17 ml or 1 tablespoon of blood. Approximately 15 ml or 1 Tbsp of blood will be drawn to test for biomarkers of disease. A small vacutainer of blood (~5cc) will also be collected from WFS patients to obtain serum that will be stored for possible future tests. In total, including the Boost Test, approximately 3 Tbsp of blood will be drawn. A small (100cc) sample of urine will be collected and stored for possible future tests. It is possible that in the future a biochemical blood or urine test (marker) will be developed by other investigators in the Wolfram syndrome research field that will serve to better diagnose or track the course of the disease. If this happens, we will use the stored sample(s) available and conduct those test(s) to gain information that could better guide our research. These samples will be stored in a locked freezer in a locked room at

Washington University in Dr. Urano's (a co-investigator) laboratory. Only approved individuals will have access to the samples. It is possible that the samples will be shared with other qualified investigators in the field of Wolfram syndrome research. The samples are de-identified meaning the patient's name is not on the sample, only a code number. The code number list is kept in a secure file in a locked room. The samples will be stored for a minimum of 10 years and then destroyed at the completion of the study. Taking part in this part of the research study is voluntary. These exams/tests will be performed at the mini-clinic visit.

Balance: An assessment of balance for Wolfram patients and siblings will be completed using the Mini-BESTest, a rating scale for performance during balance challenging tests (e.g. standing on one foot). This exam will be performed at the mini-clinic visit.

Neurology: A pediatric neurologist will perform a standard neurological examination and the Wolfram Syndrome Unified Rating Scale (WURS) on Wolfram patients and siblings. The WURS merges information from parents about patient's daily activities and from the neurologic examination. The Scale for the Assessment and Rating of Ataxia (SARA) will also be performed to assess movement. The observations made from these tests will indicate the possibility of atypical neurodevelopment. This exam will be performed virtually or at the mini-clinic visit.

Neuropsychology: We will measure IQ at the first visit for WFS patients (Vocabulary and Similarities subtests from the Wechsler Abbreviated Scale of Intelligence; WASI) and siblings. The Wide Range Assessment of Memory and Learning (WRAML) will be administered to assess short- and long-term memory. In addition, the Woodcock Johnson III Test of Cognitive Abilities (WJ-III) will be administered to explore executive function with tests appropriate for those who are low vision/blind. We will aim for a targeted blood glucose level between 70 and 300 prior to testing. This testing will be performed via a virtual data collection method or at the mini-clinic visit.

Psychiatry: A psychiatrist will meet with patients (and/or their parents) and siblings (for approximately 30 minutes) either virtually or in-person to discuss their responses on the above questionnaires regarding the patient and clarify any questionnaire responses that may suggest a psychiatric disorder. Questionnaires will be scored according to standard scoring procedures, and, if appropriate, the psychiatrist will also provide an opinion regarding best-estimate possible psychiatric diagnoses, which will be recorded on a progress note sheet along with additional relevant information obtained during brief psychiatric interview by the psychiatrist. A comprehensive psychiatric evaluation cannot be performed in only 30 minutes, but if the patient has symptoms of a particular psychiatric disorder, the psychiatrist will provide information regarding that possible disorder (to parent and/or patient if patient is a minor, or to the patient if the patient is over 18 and does not have a guardian). This information will be provided in case the parent and/or patient may want to seek further evaluation/treatment in the future. The patient/family may be referred to "Facts for Families" handouts, published by the American Academy of Child & Adolescent Psychiatry and available at: http://www.aacap.org/cs/root/facts_for_families/facts_for families, if participants want more detailed information regarding psychiatric symptoms or diagnoses. This assessment may be split between a virtual data collection session and a visit to the mini-clinic.

Smell: University of Pennsylvania Smell Identification Test (UPSIT): The UPSIT is a scratch-and-sniff test of common odorants, with high reliability (test-retest r=0.94) and established sensitivity [19]. Given that WFS1 has high expression in the olfactory tubercle and several case studies of Wolfram syndrome have noted anosmia [9,15,18,20-22]a quantified measure of olfactory identification may be sensitive to early

neurological changes in Wolfram syndrome and siblings. The UPSIT test will be performed at the mini-clinic. If participants are unable to attend a mini-clinic, the UPSIT will be mailed to participants to complete and return. (10 min)

Neuroimaging: Scans take place in a research dedicated Siemens 3T Prisma MRI scanner with an iPAT compatible 64 channel head coil. Blood glucose will be targeted to be above 70 and below 300 mg/dl prior to scanning. Patients and siblings will be familiarized with the scanner's noises, requirements to lay still and the general scanner environment prior to scans to maximize the quality of the images. Images will be used to measure gray and white matter volumes and to measure myelination. These scans be performed at the mini-clinics.

These sequences will be acquired:

1) **3D T1-weighted MR:** Used for volumetric and cortical myelin analyses. Multi-echo, sagittal acquisition, TR=2500 ms, TE 1=1.81 ms, TE 2=3.6 ms, TE 3=5.39 ms, TE 4=7.18 ms, TI=1000 ms, voxel res=.8x.8x.8mm, frames=208, Flip Angle (FA)=8 deg, Time=7:07-8:22 min.
2) **3D T2-weighted MR:** Used for volumetric, cortical myelin, and resting BOLD analyses. Sagittal acquisition, TR=3200 ms, TE=564 ms, voxel res=.8x.8x.8mm, frames=208, Time=5:15-6:35 min. For T1 and T2 scans, we will use a novel method of prospectively compensating for subject motion. Short 3D echo-planar imaging volumetric navigators are embedded in a long 3D sequence, and the resulting image volumes are registered to provide an estimate of the subject's location in the scanner at a cost of less than 500 ms,∼ 1% change in contrast, and ∼3% change in intensity. This time fits into the existing gaps in sequences, thus giving a motion-corrected sequence with no extra time required.
3) **Diffusion Weighted Imaging (DWI), whole brain:** Used for DBSI analyses of the whole brain. Two echo planar sequences with a multiband factor of 3 and 102 volumes each, one with b-values ranging from 0 to 1500 and the other 0 to 3000 s/mm. Transverse acquisition, TR = 3500, TE = 83, voxel resolution = 2×2×2 mm, Time = 6:14 min.
4) **DWI, optic nerve FOV:** Used for DBSI analyses of the optic nerves (Fig 15). Optic nerve MRI data will be acquired with a 64-channel head coil using only the anterior elements for receiving. Structural: For optic nerve atrophy measurements, a CSF and fat suppressed SPACE vNav sequence will be used to acquire coronal T1w images at 0.53 mm isotropic resolution: FOV=170x170x102mm, matrix = 320 x 320 x 192, TR=900 ms, TE = 3.9 ms, FA=120 deg., slab selective binomial (1_2_1) water excitation, iPAT=2, bandwidth=446 Hz/Px, echo spacing = 5.24 ms, Time: 6:37 min. vNav will be set to "passive" mode, so that motion information will be acquired but not used prospectively in the sequence. dMRI: For DBSI analyses of the optic nerves, an inner-volume-imaging spin echo EPI diffusion sequence will be used to acquire reduced field-of-view (FOV) optic nerve images in both axial and coronal view in two protocols. (1) Axial view: 10 oblique slices (typically, the entire optic nerves for both eyes are covered in 3-5 center slices) along the nerve, TR=5000 ms, TE=44.6 ms, voxel res=1.3x1.3x1.3 mm, FOV=166x41.5x13 mm, matrix=128x32x10, phase-encoding (PE) direction=anterior->posterior, ETL=24, Time=4:41 min x 4 effective averages, (2) Coronal view: three 4 mm thick slices perpendicular to and at the center of the intraorbital portion of each optic nerve, TR=2500 ms, TE=47 ms, voxel res=1.1x1.1x4 mm, FOV=138x43.2x12mm, matrix=128x40x3, PE direction=foot->head, ETL=30, Time=2:24 min x 2 effective averages x two eyes. Common parameters: FA=90 deg, 6/8 partial Fourier, echo spacing=0.93 ms, bandwidth=1260 Hz/Px, monopolar diffusion encoding, optimized 25 multi-bval (linearly spaced) multi-bvec diffusion scheme with bmax=1000 s/mm and two b0, effective averages with alternating diffusion gradient polarity.

5) **Resting State BOLD:** Used for resting state functional connectivity analyses. Three 5:16 minute blood oxygenation level dependent (BOLD) scans with a multiband factor of 4 will be acquired for exploratory analyses of functional connectivity at rest. Sequence parameters (https://www.cmrr.umn.edu/multiband/): 250 frames, transverse orientation, TR=1230 ms, TE=33 ms, FA=63 degrees, FOV=216x216, 64 slices, voxel resolution=2.4x2.4x2.4 mm. Subjects stare at a fixation cross during the scans.
6) **Gradient Echo Plural Contrast Imaging (GEPCI)/SMART**: Used to quantify tissue alterations that reflect integrity of cellular brain structure. (1) GEPCI: TR=50 ms, 10 echo times equally spaced from 4-40ms, voxel res=1x1x2mm, FA=30 deg, Time=6:05 min. (2) SMART: TR=20 ms, TE 1,2,3=3,7,11 ms, voxel res=1.3x1.3x1.3mm, Time=2:01 min x 5 variations of flip angle (5, 10, 20, 40, 60 deg). This novel technique, which includes the more advanced GEPCI-based sequences qGRE and SMART, should provide quantitative in vivo high-resolution 3D measurements of several brain-tissue-specific relaxation properties (GEPCI metrics) of the gradient recalled echo (GRE) MRI signal. Since GEPCI metrics depend on the molecular constituents present in the brain, the GEPCI metrics can serve as surrogate markers of tissue alterations that reflect the integrity of brain cellular structure and disease-related tissue damage. These techniques are based on a standard Siemens GRE-based sequence and do not require contrast.
7) **3D ME-FLASH Quantitative Multi-Parameter Mapping (MPM) of R1, PD*, MT, and R2***: Used to assess volume loss, myelin reductions, and iron accumulations across the brainstem and nuclei, as well as other regions of interest. (1) MT-weighted: TR=37 ms, TE 1-6=2.46-14.76 ms, voxel res=1x1x1mm, FA=9 deg, Time=7:48 min. (2) T1-weighted: TR=25 ms, TE 1-8=2.46-19.68 ms, voxel res=1x1x1mm, FA=23 deg, Time=5:16 min. (3) PD-weighted: TR=25 ms, TE 1-8=2.46-19.68 ms, voxel res=1x1x1mm, FA=4 deg, Time=5:16 min.

Blood glucose monitoring: Prior to starting the neuroimaging procedures for patients and siblings, a finger stick blood glucose level will be obtained using a home glucose meter titrated to be within the targeted range of 70-300 mg/dL within 60 min of the start of the test. Insulin or food will be given at the discretion of a physician or nurse investigator and parent/guardian to achieve this goal. For both patients with WFS and non-WFS siblings, glucose levels will be rechecked as needed to ensure that subjects remain approximately in the range of 70-300 mg/dL. If a result is trending such that the subject may become low, checks may be done more frequently. If the glucose level is <70 mg/dL when checked, we will attempt to repeat any cognitive tests performed between the time of the check and the most recent check above approximately 70 mg/dL may need to be repeated. If the tester observes or the subject reports any symptoms of hypoglycemia during testing, glucose levels will be tested and titration performed again if necessary. Retesting will confirm that blood glucose levels are within the desired targeted range before testing begins (or resumes). At the end of the session, blood glucose levels will be rechecked. Ketones will be evaluated if blood glucose is >300 mg/dL. Testing can continue at investigator discretion as long as ketones are tested, and a urine result is no more than trace or a blood ketone result is <0.6 mmol/L. If more than trace urine ketones or >=0.6 mmol/L blood ketones are present, the testing may be rescheduled.

The same procedure outlined above to monitor blood glucose will also apply to neuropsychology testing for patients only (not siblings).

Data Storage: REDCap Database and Survey will be utilized to collect and maintain information on the Wolfram participants. In addition, we will add results of the testing to the REDCap database. These are standardized, widely used databases with enhanced security for sharing of information between researchers/clinicians involved in a project. Only approved users will be allowed access to the Wolfram

Clinic REDCap; users will be approved by Tamara Hershey, PI, Samantha Ranck, Study Coord or Tasha Doty, Clinic Data Management Coordinator. Current approved users are those listed in this protocol.

POTENTIAL RISKS

Likely

a. **Questionnaires**: Individuals may become fatigued or bored answering questions.
b. **Genetics**: Individuals may become upset or fatigued with the examination.
c. **Ophthalmology**: None
d. **Endocrinology**: Possible fatigue from sitting in one place (lack of activity) for an extended period of time. Possible bruising from the blood draw. Possible hyperglycemia from the Boost test. To deal with that, we have pediatric endocrinologists present who can assist with appropriate insulin dosing and follow up blood glucose checks if needed.
e. **Balance**: None
f. **Neurology**: Individuals may become fatigued with the examination.
g. **Neuropsychology**: Taking cognitive tests could cause fatigue or boredom.
h. **Psychiatry**: Individuals may become fatigued or bored answering questions.
i. **Smell**: Some individuals may find the smells unpleasant.
j. **Neuroimaging**: None
k. **Blood Glucose Monitoring**: The finger prick done as part of the blood glucose check is associated with the same minor discomfort as the routine blood sugar checks that are part of daily diabetes management. These include bruising and soreness on the pricked finger. To minimize the discomfort, participants will be allowed to test their own blood glucose levels or have their parent perform the test, whichever method is standard for them. Blood glucose checks are standard medical care for all children and adults with diabetes.

Less Likely

a. **Questionnaires**: None
b. **Genetics**: None
c. **Ophthalmology**: Individuals may experience blurry vision, eye discomfort or headaches after their eyes are dilated for the exam. This would last for a few hours. Mild anxiety is also a possibility.
d. **Endocrinology**: None
e. **Balance**: None
f. **Neurology:** None
g. **Neuropsychology:** None
h. **Psychiatry**: Subjects may feel discomfort in answering questions about themselves. If this occurs, the importance of a question can be discussed, and a child/parent may choose not to answer any question that makes them uncomfortable.
i. **Smell:** None
j. **Neuroimaging**: Lying in the MRI scanner for approximately 60 minutes may cause physical discomfort from trying to lie still. Some people get muscle aches and pains from lying on their backs. This will be minimized by providing cushions at pressure points and beneath the knees as desired. Participants will be encouraged to bring their own music or story CD or DVD with them to the scan. These CDs or DVDs will be played through headphones and shown on a video screen to help participants relax and to dampen the noise of the MRI scanner. Some people feel claustrophobic in the scanner. If a participant feels claustrophobic, we will end the experiment. We will also provide each participant a chance to get used to being in an MRI scanner before the scan is performed in our mock MRI scanner. There are no known risk factors associated with

MRI scans for healthy individuals or individuals with diabetes. The MRI uses strong magnetic fields and weak radio waves. The magnitude of these and the duration of exposure will be similar to that used by a radiologist in standard clinical use of MRI. These exposures are thought to be extremely safe, and presently no limit exists for the number of MRI studies one may have in a lifetime. Of course, there is the possibility that some unknown risk does exist. If a participant has any surgically placed metallic objects, he/she will not be allowed to participate since this is a known risk. These issues will be addressed before the first visit. Parents will be informed that the purpose of the brain MRI is not for clinical diagnosis but for research on the brain and that this research MRI is not a complete clinical MRI examination. We will fully discuss any questions parents or participants might have about the severity, frequency, and duration of these risks and discomforts.

k. **Blood Glucose Monitoring:** None

Unlikely

a. **Questionnaires:** None
b. **Genetics:** None
c. **Ophthalmology:** None
d. **Endocrinology:** There is a rare risk of infection at the site of a blood draw. Nausea from the taste or texture of the Boost is rare. If treatment is needed, patients will rest in recliners and will be provided with water and have their blood sugar closely monitored and insulin will be provided as needed.
e. **Balance:** None
f. **Neurology:** None
g. **Neuropsychology:** None
h. **Psychiatry:** None
i. **Smell:** None
j. **Neuroimaging:** Discovery of a brain problem on MRI. All scans will be reviewed by a pediatric neuroradiologist affiliated with the project (Dr. Shimony). Parents or adult participants are notified if the neuroradiologist believes the finding warrants medical follow-up.
k. **Blood Glucose Monitoring:** None

In addition, there is always the possibility of a breach of confidentiality and of unknown risks occurring.

PROTECTION AGAINST RISKS

a. **Questionnaires:** Subjects may discontinue answering questions or skip questions at any time. The REDCAP questionnaires are entirely self-paced and do not require completion.
b. **Genetics:** At any point during the assessments/procedures, if the individual looks fatigued or uncomfortable or expresses that s/he is tired or uncomfortable, we will allow them to take a break and resume when s/he is ready.
c. **Ophthalmology:** At any point during the assessments/procedures, if the individual looks fatigued or uncomfortable or expresses that s/he is tired or uncomfortable, we will allow them to take a break and resume when s/he is ready.
d. **Endocrinology:** Pediatric endocrinologists will be available to monitor blood glucose and provide insulin as needed before, during, and after the Boost test.
e. **Gait and Balance**: At any point during the assessments/procedures, if the individual looks fatigued or uncomfortable or expresses that s/he is tired or uncomfortable, we will allow them to take a break and resume when s/he is ready.

f. **Neurology:** At any point during the assessments/procedures, if the individual looks fatigued or uncomfortable or expresses that s/he is tired or uncomfortable, we will allow them to take a break and resume when s/he is ready.
g. **Neuropsychology:** To minimize the possibility of fatigue or boredom during the cognitive tests, participants will be allowed to take as many rest breaks as they need.
h. **Psychiatry:** At any point during the assessments/procedures, if the individual looks fatigued or uncomfortable or expresses that s/he is tired or uncomfortable, we will allow them to take a break and resume when s/he is ready.
i. **Smell:** At any point during the assessments/procedures, if the individual looks fatigued or uncomfortable or expresses that s/he is tired or uncomfortable, we will allow them to take a break and resume when s/he is ready.
j. **Neuroimaging:** To minimize physical discomfort during the MRI scan, we will provide cushions at pressure points and beneath the knees as desired. Participants will be encouraged to bring their own music or movie DVD with them to the scan. These DVDs will be played through headphones to help participant relax and to dampen the noise of the MR scanner. If a participant feels claustrophobic, we will end the experiment. We will also provide each participant a chance to get used to being in an MRI scanner before the scan is performed in our mock MRI scanner. We will screen the participant for claustrophobia, metal implants or other contraindications for MR scanning before the first visit. Trained technicians will run the scanner and certified Radiology Technicians and medical staff are available by pager or in person at all times. A parent may accompany the participant into the scanner room, if necessary and if the parent passes MRI safety screening, and wears ear-plugs.
k. Blood Glucose Monitoring: To minimize the discomfort during the blood glucose check, participants will be allowed to test their own blood glucose levels or have their parents perform the test, whichever method is standard for them. Blood glucose checks are standard medical care for all children and adults with insulin dependent diabetes.

DATA AND SAFETY MONITORING PLAN

For **all WU participants**, the data and safety monitoring plan includes, but is not limited to, the following features:

- Review of adverse events: All personnel who will be in contact with participants and/or data are prepared to identify adverse events and have been instructed to report their occurrence immediately to the principal investigator(s).
- Reporting of serious adverse events: In the unlikely event of a serious adverse event, the principal investigators, in collaboration with any relevant personnel, will take the appropriate actions to report the event to the local IRB offices.
- Criteria for stopping the study: Circumstances that would warrant stopping the study are not anticipated. However, should any circumstances arise that compromise the safety of the participants, they will be reported to the principal investigators who will suspend research until appropriate safeguards allow continuation of the study.
- Qualified individual(s) monitoring the study: The principal investigator and collaborators have experience in this area of research and are qualified to monitor the study.

ELIGIBILITY

Wolfram Syndrome Group (WFS):

Inclusion Criteria:

• Has had genotyping
• Willingness to share result of genotyping

• Participant has confirmation of WFS mutation

Exclusion Criteria:

• Participant is unaware of their diagnosis.
• Inability of patient or guardian to understand informed consent.
• Advanced disease that makes traveling too problematic and/or uncomfortable for the patient and/or guardian, such as use of ventilator or inability to walk.

Non-Wolfram Syndrome Sibling Group (WFS):

Inclusion Criteria:

• Willingness to share result of genotyping

Exclusion Criteria:

• Participant has confirmation of WFS mutation and a diagnosis of Wolfram syndrome
• Inability of patient or guardian to understand informed consent.
• Advanced disease that makes traveling too problematic and/or uncomfortable for the patient and/or guardian, such as use of ventilator or inability to walk.

Proxy Group: Adult biological parent(s), biological caregiver, or non-biological caregiver of adult and minor participants in any of the groups.

Inclusion criteria:

• Biological or non-biological parent/caregiver (proxy) of a participant.

Exclusion criteria:

• Proxy is unaware of the participant's diagnosis (as it applies).

HANDLING OF BIOLOGICAL SAMPLES

- Blood samples drawn in the Pediatric Clinical Research Unit for research purposes will go to our Core Lab for processing.
- A serum sample (1cc) will be stored at -80 for future studies in Dr. Urano's lab.

PROTECTION OF PATIENT INFORMATION AND INFORMATION STORAGE

A secured database will be created to store and retrieve patient information. Data will be de-identified and stored in REDCap and/or Box. There is always the risk of loss of confidentiality. MRI data will be number coded only and master files will be kept on a removable disk in a locked drawer and will only be available to members of the research team. Data from the eye exam, medical interviews, neuropsychological evaluations, and blood tests will be stored in a locked drawer and will only be available to members of the research team.

BENEFITS

Benefits to patients: Patients have the immediate benefit of access to experts in a specialized clinic. Additionally, the data collected from these participants will help us better understand Wolfram syndrome and its effects. This information is critical for selecting outcome measures for clinical trials. For control participants, there will be no direct benefit to undergoing the procedures in the study. However, the data collected from these subjects will help us better understand Wolfram syndrome.

Benefits to others: This initiative will raise awareness of Wolfram syndrome at the regional level, and diagnosis and prognosis would be improved in future patients. Clinicians will also gain expertise in a rare disease. Furthermore, investigators may gain insight from this monogenic disease that will relate to more common ER stress-mediated disorders.

ETHICS

Informed consent will be obtained from the patient and/or their appropriate guardian. Participation of children is critical in this initiative. Associated risks with participation in this project include confidentially breach, pain or anxiety during blood draw, anxiety with MRI or testing.

COLLABORATORS/RESEARCHERS

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Medicine: Fumi Urano, Stacy Hurst
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Radiology: Josh Shimony
Neurology: Amy Viehoever
Audiology: Judith Lieu
Siteman (Genetic counseling): Linda Manwaring
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Reference List

1. Takeda,K. *et al.* WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. *Human Molecular Genetics* **10**, 477-484 (2001).
2. Hilson,J.B., Merchant,S.N., Adams,J.C. & Joseph,J.T. Wolfram syndrome: a clinicopathologic correlation. *Acta Neuropathol. (Berl.)* **118**, 415-428 (2009).
3. Barrett,T.G., Bundey,S.E., Fielder,A.R. & Good,P.A. Optic atrophy in Wolfram (DIDMOAD) syndrome. *Eye* **11**, 882-888 (1997).
4. Hardy,C. *et al.* Clinical and molecular genetic analysis of 19 Wolfram syndrome kindreds demonstrating a wide spectrum of mutations in WFS1. *Am. J. Hum. Genet.* **65**, 1279-1290 (1999).
5. D'Annunzio,G. *et al.* Wolfram syndrome (diabetes insipidus, diabetes, optic atrophy, and deafness) - Clinical and genetic study. *Diabetes Care* **31**, 1743-1745 (2008).
6. Pakdemirli,E., Karabulut,N., Bir,L.S. & Sermez,Y. Cranial magnetic resonance imaging of Wolfram (DIDMOAD) syndrome. *Australas. Radiol.* **49**, 189-191 (2005).
7. Scolding,N.J., KellarWood,H.F., Shaw,C., Shneerson,J.M. & Antoun,N. Wolfram syndrome: Hereditary diabetes mellitus with brainstem and optic atrophy. *Ann. Neurol.* **39**, 352-360 (1996).
8. Khardori,R., Stephens,J.W., Page,O.C. & Dow,R.S. Diabetes-Mellitus and Optic Atrophy in 2 Siblings - A Report on A New Association and A Review of the Literature. *Diabetes Care* **6**, 67-70 (1983).
9. Jackson,M.J. *et al.* Biochemical and Molecular Studies of Mitochondrial-Function in Diabetes-Insipidus, Diabetes-Mellitus, Optic Atrophy, and Deafness. *Diabetes Care* **17**, 728-733 (1994).
10. Gregorios,J.B. Wolframs Syndrome with Schizophrenia and Central Hypoventilation - A Neuropathological Study. *Journal of Neuropathology and Experimental Neurology* **48**, 308 (1989).
11. Carson,M.J., Slager,U.T. & Steinberg,R.M. Simultaneous occurrence of diabetes mellitus, diabetes insipidus, and optic atrophy in a brother and sister. *Am. J. Dis. Child* **131**, 1382-1385 (1977).
12. Kehl,O. & Keller,U. [DIDMOAD syndrome (diabetes insipidus, diabetes mellitus, optic atrophy, deafness) with cerebello-pontine atrophy]. *Schweiz. Med. Wochenschr.* **112**, 348-352 (1982).
13. Rando,T.A., Horton,J.C. & Layzer,R.B. Wolfram Syndrome - Evidence of A Diffuse Neurodegenerative Disease by Magnetic-Resonance-Imaging. *Neurology* **42**, 1220-1224 (1992).
14. Galluzzi,P., Filosomi,G., Vallone,I.M., Bardelli,A.M. & Venturi,C. MRI of Wolfram syndrome

(DIDMOAD). *Neuroradiology* **41**, 729-731 (1999).
15. Lessell,S. & Rosman,N.P. Juvenile diabetes mellitus and optic atrophy. *Arch. Neurol.* **34**, 759-765 (1977).
16. Okten,A. *et al.* Various clinical aspects of DIDMOAD (Wolfram) syndrome. *Turk. J. Pediatr.* **37**, 235-240 (1995).
17. Nickl-Jockschat,T., Kunert,H.J., Herpertz-Dahlmann,B. & Grozinger,M. Psychiatric symptoms in a patient with Wolfram syndrome caused by a combination of thalamic deficit and endocrinological pathologies. *Neurocase.* **15**, 47-52 (2008).
18. Shannon,P., Becker,L. & Deck,J. Evidence of widespread axonal pathology in Wolfram syndrome. *Acta Neuropathol. (Berl.)* **98**, 304-308 (1999).
19. Doty,R.L., Bromley,S.M. & Stern,M.B. Olfactory testing as an aid in the diagnosis of Parkinson's disease: development of optimal discrimination criteria. *Neurodegeneration.* **4**, 93-97 (1995).
20. Genis,D., Davalos,A., Molins,A. & Ferrer,I. Wolfram syndrome: A neuropathological study. *Acta Neuropathol. (Berl.)* **93**, 426-429 (1997).
21. Leiva-Santana,C., Carro-Martinez,A., Monge-Argiles,A. & Palao-Sanchez,A. [Neurologic manifestations in Wolfram's syndrome]. *Rev. Neurol. (Paris)* **149**, 26-29 (1993).
22. Marquardt,J.L. & Loriaux,D.L. Diabetes mellitus and optic atrophy with associated findings of diabetes insipidus and neurosensory hearing loss in two siblings. *Arch. Intern. Med.* **134**, 32-37 (1974).
23. Wechsler,D. Wechsler Abbreviated Scale of Intelligence. Psychological Corporation, San Antonio, TX (1999).
24. Carver,C.S. & White,T.L. Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS scales. *Journal of Personality and Social Psychology* **67**, 319-333 (1994).
25. Swift,R.G., Sadler,D.B. & Swift,M. Psychiatric findings in Wolfram syndrome homozygotes. *Lancet* **336**, 667-669 (1990).
26. Beck,A.T. & Beamsderfer,A. Psychological measurements in psychopharmacology. Pichot,P. (ed.), pp. 151-169 (Karger, Basel,1974).
27. Gioia,G.A., Isquith,P.K., Guy,S.C. & Kenworth,L. Behavior Rating Inventory of Executive Function. Psychological Assessment Resources, Odessa, FL (2000).
28. Khan,A.R., Wang,L. & Beg,M.F. FreeSurfer-initiated fully-automated subcortical brain segmentation in MRI using Large Deformation Diffeomorphic Metric Mapping. *Neuroimage.* **41**, 735-746 (2008).
29. Adams HR, Beck CA, Levy E, Jordan R, Kwon JM, Marshall FJ, Vierhile A, Augustine EF, de Blieck EA, Pearce DA, Mink JW. Genotype does not predict severity of behavioural phenotype in juvenile neuronal ceroid lipofuscinosis (Batten disease). Dev Med Child Neurol. 2010 Jul;52(7):637-43. Epub 2010 Feb 19.
30. Paul A. Harris, Robert Taylor, Robert Thielke, Jonathon Payne, Nathaniel Gonzalez, Jose G. Conde, Research electronic data capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support, J Biomed Inform. 2009 Apr;42(2):377-81.