Research Protocol

Natural History in ATP1A3-related disease: a deep phenotyping – genotyping project

STUDY TITLE

Natural history in ATP1A3-related disease: a deep phenotyping-genotyping Project

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INTRODUCTION

In recent years medical science has focused on the results of randomized clinical trials (RCTs) for advancing treatment standards. Observational studies, retrospective analysis or even phase II studies are afforded little weight in medical decision-making. This is clearly justified in common conditions, where large cohort studies are possible, but poses a problem in rare diseases where the sample size needed to conduct statistical analysis in a RCT is unrealistic. With rapid genetic discovery that makes promising novel treatments a realistic prospect, it is paramount that alternative strategies are developed to facilitate drug development in rare diseases. Natural history studies generate greater knowledge about the progress of these diseases, allowing the effects of novel treatments and their effects on disease progression, longevity and quality of life to be evaluated. Relying on observational studies does not automatically result in a compromise in the quality of the data; there are examples where a well-designed observational study has been able to provide information an underpowered RCT could not[1].

BACKGROUND TO PROPOSAL

Mutations in *ATP1A3*, the gene that encodes the Na+/K+-ATPase α3 subunit, have been identified as the cause of alternating hemiplegia of childhood (AHC)[2], rapid-onset dystonia-parkinsonism (RDP)[3], CAPOS syndrome (cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss)[4] and an increasing range of intermediate phenotypes. These

rare conditions are on a spectrum of *ATP1A3*-related disease, and share common features[5]. For example in both AHC and RDP, a strikingly asymmetric, predominantly dystonic movement disorder with a rostrocaudal gradient of involvement is observed, while all three phenotypes share physical, emotional, or chemical stressors as triggers. Even though case reports and small case series of distinct and intermediate *ATP1A3* phenotypes are frequently published[6,7], larger cohort studies illuminating the anticipated course of the disease, ideally further divided into its genetic subpopulations, are lacking. The one natural history study that has been published including only AHC patients relied solely on questionnaires and the conclusion reached, that AHC is a non-progressive disease,[8] seems to be disputed as further cases[9] and phenotypes emerge. A more systematic approach is needed to better understand this complex phenotypic spectrum[10].
Even though the genetic basis is clear, the pathophysiology of *ATP1A3* diseases remains elusive and, consequently, no rational treatments are available. Multiple symptomatic therapeutic approaches have been trialed, but, except for flunarizine showing some benefit for hemiplegic episodes, results have been largely disappointing[11]. Furthermore the role of flunarizine in changing the course of the disease, and especially the cognitive outcome, is still uncertain[11], not least because of the lack of available biomarkers marking disease progression. Such biomarkers, together with solid natural history data from a carefully phenotyped cohort, would be invaluable to assess efficacy of novel treatments that may start emerging soon[12].

RATIONALE FOR PROPOSAL

We propose a natural history study of *ATP1A3*-related disease combining retrospective (questionnaires; home videos) and prospective design. This will include deep phenotyping and genotyping, by utilizing traditional and novel methodology.

We thus aim to:

- Elucidate the natural history of the disease and its phenotypic and genetic subpopulations.
- Better understand pathophysiology by utilizing novel investigations and identify potential biomarkers to monitor treatment efficacy and disease progression.
- Provide the basis to support novel treatment options.

STUDY DESIGN

We are planning an observational cohort study combining retrospective (questionnaires; home videos) and prospective design. This will include deep phenotyping and genotyping of included patients by utilizing traditional (detailed history taking, examination, EEG, MRI) and novel (broadband-NIRS, TMS, genomics) methodology.

STUDY SETTING/LOCATION

The children participating in the study will be seen at Great Ormond Street Hospital for Children, whilst the adult visits will take place at the National Hospital for Neurology and Neurosurgery or the affiliated Epilepsy Society. Patients that are treated in other centers throughout the UK and are not able to travel to London for participation in the study will partake in the study activities that can be administered remotely (questionnaires, home videos, clinical history, collection of clinical data).

STUDY POPULATION

Between Great Ormond Street Hospital and the National Hospital for Neurology and Neurosurgery, we already have 30 patients with *ATP1A3*-related disease. We will utilize the British Paediatric Neurology and British Neurology Surveillance Units in collecting all further cases from around the UK: based on previous prevalence estimates we would expect to at least double our cohort to 60[13].

ELIGIBILITY CRITERIA

Inclusion criteria

- Children and adults of any age carrying a mutation in the *ATP1A3*-gene.
- Children and adults of any age matching an *ATP1A3*-related disease phenotype without a mutation in the gene.
- Written informed consent given by patient and/or parent/guardian.

Exclusion criteria

- Patients with a phenotype not fitting *ATP1A3*-related disease and no mutation in the *ATP1A3* gene.

METHODOLOGY

Recruitment of participants

We will perform a medical records search at Great Ormond Street Hospital for Children and the National Hospital for Neurology and Neurosurgery to identify all patients with ATP1A3 mutation or relevant phenotype already in our care. A member of the research team will then approach the families for a face to face or telephone consultation with an adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. They will then be provided with the REC approved version of the patient information sheet. Written informed consent will be obtained from each patient or, if applicable, parent/guardian prior to participation in the trial and can be withdrawn at any time. We will also approach new patients coming through the outpatient clinics or inpatient route and meeting the eligibility criteria for

potential recruitment. Further we plan to utilize the British Paediatric Neurology and British Neurology Surveillance Units and approach genetic centers throughout the UK in order to collect further cases. We will further inform the UK patient support group AHCUK and other relevant patient support groups of our study and ask them to disseminate the information in their forums and relevant meetings.
Our goal is to collect all cases within the UK meeting eligibility criteria during the duration of the study (3 years).

Study procedure

Clinical History
A detailed clinical history will be taken from all consented patients. To ensure consistency a clinical proforma will be designed to include standardized questions covering all aspects of ATP1A3-related disease.
For patients unable to travel to London for a full baseline assessment an option of a Video-chat based interview (Skype/Facetime) will be provided. During this the same clinical proforma will be employed.
Clinical History will be updated annually. Diaries of seizures and other paroxysmal will be continuously collected from the families for the duration of the study.

Neurological examination
A full neurological examination will be recorded at baseline and then in yearly intervals for the duration of the study.
Taking advantage of contemporary smart phone culture, home videos alongside clinical videos will be collected for semiological review, categorization of paroxysmal events and documentation of early neurodevelopment to determine possible early markers of disease.

Neurodevelopmental data/ Behavioural data/ Quality of life (QoL)
We will collect all available neurodevelopmental data for the participating patients. For patients that have not had a neurodevelopmental assessment we will organize an age-appropriate testing at our sites. Behaviour and mental health will also be assessed using standardized parent and patient questionnaires (DAWBA). Standardized QoL questionnaires will be used to assess outcome over time.

Electroencephalography
EEG data will be reviewed to identify electroclinical features specific to this patient population both within and without events, applying quantitative measures such as spectral analysis and measures of symmetry to better characterize the electrical phenotype. For this we will use our inhouse EEG-data, but also request reports and raw data from EEGs done at local hospitals.

Peripheral Nerve Conduction Studies / EMG
In patients with pes cavus and areflexia nerve conduction studies and EMGs are warranted and we will collect those results. We will not conduct these tests in patients with no clinical indication due to the pain related to the procedures.

Imaging studies
We will further collect all MRI studies of our patients. Voxel-based morphometry[14,15] will be utilized to look for disease-specific patterns of reduced grey matter density in Magnetic Resonance Imaging (MRI) data. We will initiate an *ATP1A3* working group on the ENIGMA platform[16] allowing us access to their vast MRI control cohort and meta-analyzing tools for MRI data. International collaborators will be invited to participate.
Transcranial Magnetic Stimulation (TMS) can be used non-invasively to stimulate motor cortex, and probe function in detail. Our pilot adult AHC study showed fluctuations in motor cortex excitability, not seen in controls, especially marked during hemiplegic attacks[17].
Scientists at UCL have developed an innovative Broadband Near-infrared Spectroscopy (broadband-NIRS) method to measure activity of cytochrome-c-oxidase (CCO), an enzyme in the mitochondria reflecting brain metabolism[18,19]. We propose this will be an effective biomarker to assess dynamic changes in brain metabolism in *ATP1A3*-related disease.
TMS and broadband-NIRS may act as rapid readouts for novel therapies.

Opthalmology
Patients with CAPOS typically have optic atrophy and a thorough ophthalmologic assessment, including OCT and VEP/ERGs is recommended. We will collect these results and extend the ophthalmologic assessment to other *ATP1A3*-related phenotypes as early detection of a possible associated vision deficit is important for optimal patient management.

Hearing assessment
We recently learned that *ATP1A3* mutations can cause progressive auditory neuropathy[20]. This has so far been established for the mutation causing CAPOS and patients profit from cochlear implantations[21]. We will perform hearing assessments including otoacoustic emissions (OAEs) and auditory brain stem responses (ABRs) to assess hearing across phenotypes and ensure timely intervention.

Cardiac assessment
Building on our prior collaboration with paediatric cardiology, 12-lead ECGs will be analyzed for cardiac axis, cardiac interval, repolarization pattern and J-point analysis with a view to further explore our published theory of periodic systemic decompensation in *ATP1A3*-expressing organs[22].

Sleep studies
Recent studies of the Myshkin mouse, a mouse with a heterozygous pathogenic *ATP1A3* mutation (I810N), show profound circadian behavioral alterations that are light-responsive[23], consistent with parent reports of poor sleep quality in children with *ATP1A3*-related disease. Sleep questionnaires and sleep diaries combined with data from actigraphs will be used to assess sleep quality and the impact of environmental measures (light restriction) in our patients, the results of which may provide immediately applicable sleep suggestions for our families.

Genomics
Genetic data will be correlated with clinical phenotype to identify subpopulations. In *ATP1A3* mutation negative patients we will undertake high-coverage whole exome or genome sequencing to identify mosaicism or other causative genes. In patients with an ATP1A3-

mutation associated with an atypical phenotype[24] we will initiate triome high-coverage whole exome or genome sequencing to confirm the mutation is causative in the patient and no non-*ATP1A3*-related aetiology has been missed. This is important to correctly delineate the phenotypes associated with the gene. Finally, we will look for modifiers in all ATP1A3+ patients, to help explain the heterogeneity in phenotypes.
The DNA samples will be stored in dedicated freezers at UCL. Only the research team will have access to the samples. DNA sequencing will be provided by UCL genomics or an external contract sequencing service provider. At the end of the research project the samples will be disposed off unless the participants agree to their further storage for use in further relevant ethically approved projects by our research team.

ATP1A3-antibodies
Antibodies against the ATP1A3 protein have recently been proven responsible for paraneoplastic neurologic syndrome[25]. As this could be the mechanism underlying ATP1A3-related phenotypes in mutation negative patients, we will sent their serum to measure ATP1A3-antibody levels in a laboratory in Germany providing ATP1A3 antibody analysis. After processing they will store samples for further use should novel essays for better analysis become available. A material transfer agreement (MTA) will be put in place.

Safety considerations/Patient safety

The safety of research participants is foremost. You will need to provide adequate information on how the safety of research participants will be ensured. This can include procedures for recording and reporting adverse events (and serious adverse events) and their follow-up (mandatory requirement for studies involving intervention or treatments). Remember that even administering a research questionnaire may have adverse psychological effects on susceptible individuals.
The safety of our research participants is foremost. With the exception of TMS and broadband-NIRS all other investigations are part of the patients' routine care. There is no apparent risk associated with the application of the NIRS probes. This is done at the bedside and has been performed in infants and adults in previous studies with no adverse events. Nevertheless should any discomfort occur this will be recorded and the monitoring stopped. TMS has long been used in the diagnostic and therapeutic setting. A recent systematic review shows that use in children is as safe as it is in adults[26]. We will be following published guidelines to ensure the safety of our patients.

Data monitoring

All data will be handled in accordance with the General Data Protection Regulation (GDPR) (EU) 2016/679. The Case Report Forms will not bear the subject's name or other personal identifiable data. The subject's initials, date of birth and trial identification number will be used for identification. The investigator will ensure the accuracy of all data entered in the CRFs. Medical notes will form the source data for the trial and be completed as per routine practice. All clinical data will be processed and stored as per trust policies. Broadband-NIRS and TMS data will be collected under the trial identification number not allowing connection to the research participant. All identifiable data, including home videos, will be stored within UCL Data

Safe Haven (DSH). DSH provides a technical solution for storing, handling and analysing identifiable data. It has been certified to the ISO27001 information security standard and conforms to NHS Digital's Information Governance Toolkit. Identifiable data will be stored for a maximum of three years to enable analysis of the results. Anonymised research data will be stored for 15 years on password protected UCL computers and in locked file cabinets within the UCL Institute for Child Health. Only the research team of the PI Prof J Helen Cross will have access. After the end of the 15 year period paper data will be shredded, electronic data deleted.

STATISTICAL CONSIDERATIONS AND DATA ANALYSIS

Sample size and statistical power
Between Great Ormond Street Hospital and the National Hospital for Neurology and Neurosurgery, we already have 30 patients with ATP1A3-related disease. We will utilize the British Paediatric Neurology and British Neurology Surveillance Units in collecting all further cases from around the UK: based on previous prevalence estimates of 1/1000000 and a UK population of 60000000 we would expect to at least double our cohort to 60.

Statistical methods
Cluster analysis will be performed on clinical data acquired to determine clinical groups of presentation and relationship to genetic results. Multivariate regression analysis will be used to determine relationships between clinical, EEG, neuroimaging, broadband-NIRS, TMS and genetic analysis with outcome.

ETHICAL CONSIDERATIONS

The study will be conducted in full conformance with principles of the "Declaration of Helsinki", Good Clinical Practice (GCP) and within the laws and regulations of the United Kingdom.
At no point will study procedures be allowed to interfere with the standard clinical care of the patients. It will be emphasized that study participation is entirely voluntary and can be withdrawn at any time without giving reasons.
We will use interpreters where necessary to recruit families. Funds for translation of the patient information and consent forms are not available, but we will ask the interpreter to sign as a witness that the consent process has been carried out without coercion.

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