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Methodology of a Natural History study of a Rare Neurodevelopmental Disorder: Alternating Hemiplegia of Childhood as a Prototype Disease

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List of Abbreviations

AHC: Alternating Hemiplegia of Childhood

IAHCRC: International Alternating Hemiplegia of Childhood Research Consortium

ERN Epicare: European Reference Network for Rare and Complex Epilepsies

ILAE: International League against Epilepsy

PDI: Paroxysmal disability index

NPDI: Non-paroxysmal disability index

NIH: National Institute of Health

ANOVA: Analysis of Variance

COS: Core Outcome Set

COMET: Core Outcome Measures in Effectiveness Trials

Abstract

Here, we describe the process of development of the methodology for an international multicenter natural history study of Alternating Hemiplegia of Childhood (AHC) as a prototype disease for rare neurodevelopmental disorders. We describe a systematic multistep approach in which we first identified the relevant questions about AHC natural history and expected challenges. Then, based on our experience with AHC and on pragmatic literature searches, we identified solutions to determine appropriate methods to address these questions. Specifically, these solutions included development and standardization of AHC-specific spell video-library, spell calendars, adoption of tailored methodologies for prospective measurement of non-paroxysmal and paroxysmal manifestations, unified data collection protocols, centralized data platform, adoption of specialized analysis methods including, among others, Cohen's kappa, interclass correlation coefficient, linear mixed effects models, principal component, propensity score, and ambidirectional analyses. Similar approaches can, potentially, benefit in the study of other rare pediatric neurodevelopmental disorders.

Keywords: neurodevelopment, genetics, children, developmental disability, disability, epidemiology, outcome, risk factors

I. Introduction

The quantitative understanding of the natural history of rare neurodevelopmental diseases is a necessity to continue to grow the field of pediatric neurology. This can provide an integrated and comprehensive approach to develop specific interventions for diseases and for counseling.[1] Rare neurodevelopmental diseases in pediatric neurology present challenges in establishing a natural history course due to the multisystem complexity of pathologies and, often, lack of availability of validated measurement tools.[2] It is imperative to have validated measures when developing interventions to be able to measure outcomes meaningfully and reliably, especially in the presence of both paroxysmal and non-paroxysmal manifestations. In addition, natural history studies are needed to help families understand the potential trajectory of their child's disease. In this article, we describe the approach we used to address these goals for a rare neurodevelopmental disorder, Alternating Hemiplegia of Childhood (AHC), that we are currently using in our ongoing multicenter multinational natural history study (OBSERV-AHC study).

This study addresses a gap in knowledge on how to study the natural history of neurodevelopmental disorders that manifest as epilepsy and movement disorders and can be applied to other diseases in the field of pediatric neurology. AHC is an appropriate prototype to learn how to study neurodevelopmental disorders due to its manifold manifestations of paroxysmal (movement disorders and epilepsy) and non-paroxysmal (developmental challenges) changes.

The approach consisted of multiple steps, which include identifying the need, performing a pragmatic literature review relevant to this initial stage, defining the questions to be answered, defining the challenges, performing another pragmatic review of the literature relevant to this stage, drafting a study design, performing a pilot study, refining the study plan based on experiences in the pilot study and on further investigator discussions resulting eventually in the final protocol. In this methodological article, when we state "study", we are referring to our "OBSERV-AHC" natural history study and when we refer

to “pilot study”, we are referring to our pilot study that has been completed and the results of which have been published.[3]

II. **Challenges (Table 1)**

There are specific challenges in planning a natural history study of rare neurodevelopmental diseases due to many factors.

Challenges are multifaceted due to the rarity of such disorders, the variable range of severity among different patients, and the lifelong nature of these diseases. Thus, not only is the duration of follow-up a challenge, but also securing enough numbers of patients is another obstacle that needs to be overcome. Therefore, establishing and working with a multi-national and/or multicenter group is of high importance.

Determining the appropriate approach to a natural history study is also a challenge. There have been many approaches proposed for developing natural history studies in the past; study models such as cross-sectional, retrospective, prospective, or a combination approach of retrospective and prospective studies (ambidirectional) approaches have been used. Some studies have used the cross-sectional approach based on case studies of patients with a certain disease.[4,5] This model can be used when there are limitations in time in performing a multicenter international or prospective study.[6] The main limitation of this approach is that it is prone to referral, recall and ascertainment bias, which can better be addressed in prospective studies. The limitations of an only prospective study are the duration of follow-up and due to the rarity of these conditions, that patients are at differing stages of the disease process. In addition, one is not sure how patients’ prior various treatments or care may have affected their current status.[7,8] Retrospective studies may provide longer periods for clinical course analysis, but, as mentioned above, raise concern about ascertainment bias about data completeness and reliability and about intervention effects.[9] The combination of the retrospective and prospective approaches, the ambidirectional approach, has been advocated as an effective option that uses the advantages of each approach to counteract the disadvantages of the other.[10] Our

approach to the natural history study of a rare disease will use an ambidirectional approach with retrospective and prospective data, in addition, we will also use a linear mixed effectors model to investigate temporal trend of scores over times along with other creative designs and statistics, detailed below, to study its manifestations over time.

Many challenges arise due to multiple types of paroxysmal and non-paroxysmal manifestations of neurodevelopmental diseases. Specifically, rare neurodevelopmental diseases often have co-existing epilepsy and paroxysmal movement disorders in addition to developmental delays and psychiatric/psychological disabilities.[11] AHC can be considered a prototypical disease as it has both the paroxysmal manifestations and non-paroxysmal manifestations. In addition, AHC can have a slowly progressive course as well as, less frequently, sudden regression or clinical worsening.[12] Challenges arise in how to accurately capture and validate paroxysmal and non-paroxysmal manifestations on both a global as well as a disease-specific scale to allow for monitoring and documentation of disease trajectories.

Other challenges include the identification of early-life clinical predictors to help prognosticate the disease trajectory that can be difficult to discern due to the heterogeneity of the disease and the difficulty of long-term follow-up. An example of this is the variability in genotype-phenotype correlations in rare monogenic neurodevelopmental disorders with high phenotypic variability, including AHC.[13] In addition, another challenge is to determine if specific treatments can also affect or change the course of natural history, which is very hard due to the difficulty of the performance of long-term controlled studies.

III. Approach used to address challenges and develop the study plan (Figure 1):

Here are the specific stepwise actions that were taken:

1) ***Identify the need:*** In developing this study, we met as a medical team and a parent-caregiver team to determine if there was a need to perform a natural history study for AHC. Based on our professional group's knowledge of the disease and the repeated input from family organizations, representing caregivers, and providers as expressed in the London 2016 ATP1A3 Disease meeting organized by the IAHCRC Consortium, it was agreed that there are urgent clinical research needs for the natural history study for AHC. These research needs were considered essential to better understand the disease, evaluate its treatment options, and plan for, expected, future controlled clinical trials.

2) ***Pragmatic review of the literature***: This was done through a pragmatic search of the PubMed and Google Scholar databases regarding articles that have been published on AHC using the terms "Alternating Hemiplegia of Childhood", "disease progression", "natural history", "therapeutics", "neurogenetic", "outcomes", "child", "neurodevelopmental", "neurodegenerative", "follow up" in various combinations. The goal was to collate methods used for the study of the natural history of various neurological and neurogenetic disorders. As compared to a systematic literature review, which is much more comprehensive and would have been exhaustively prohibitive in our case, a pragmatic literature search is targeted to specific goals and is useful in determining and finding the potentially most useful literature relevant to the specific topic in, still, a thoughtful and focused approach.[14-17]

3) ***Define the questions to be answered by the study:*** The principal investigator (PI) of the study together with the other investigators and the statistics team developed specific goals for the study based on the above steps and knowledge. The main aims of the study included determining the clinical time course of the various paroxysmal and non-paroxysmal manifestations of the disease, determining the early life and other predictors of outcome, and the effect of flunarizine therapy on long-term developmental outcome.

4) ***Define the challenges:*** Due to the rarity and complexity of the multiple manifestations of AHC, many challenges had to be addressed (**Table 1**). Defining and addressing these challenges was done through the use of the results of the above literature search which brought out methods of establishment of a natural history study in multiple neurodevelopmental disorders, such as Duchenne muscular dystrophy (DMD), spinal muscular atrophy (SMA), and Huntington's disease (HD).[1,2] Through this we also determined that for rare diseases using only retrospective or only prospective data analyses can each only provide partial answers and, thus, using an ambidirectional approach, while mostly emphasizing the prospective approach, will be helpful and, thus, we adopted this approach. Here, regarding the sequence of our approach, we would like to note that identified challenges can also contribute to defining the questions that need to be asked for a study. Thus, in those situations for other studies, the above sequence of actions maybe slightly different.

5) ***Perform another pragmatic review of the literature:*** In this step, we further focused our literature search including focus on specific suitable statistical methods. The statistical methods are discussed in detail in the relevant section below.

6) ***Draft the study design:*** This included setting up our international IAHCRC-CLOUD Platform, based on the REDCap® system provided by Vanderbilt University, as centralized online data collection service and the multicenter multinational approach with standardized data collection procedures.

7) ***Revise and finalize the pilot study plan:*** This pilot study was designed to analyze already available, before the start of the OBSERV-AHC study, data from only two centers using some of the same approaches and statistical methods that would be used in the final OBSERV-AHC study protocol. The data analyzed did not include any prospective data from the OBSERV-AHC study but did include retrospective and prospective data collected in the two centers' databases from before the start

of the OBSERV-AHC study. In addition, during this stage, we worked on how to code different elements and variables for the pilot and final study protocol.

8) ***Perform a pilot study and publish on it***: In this study, a cohort of 29 prospectively followed patients from one center was analyzed (USA). This was combined with a retrospective analysis of 36 patients from the same center and with a retrospective analysis of 52 patients from another center in a different country (France). The statistics used included the linear mixed effects models incorporating data from all the visits for all the patients, and Wilcoxon Signed Rank test comparing first and last visits of the prospectively followed patients, along with multivariable regression analyses. The results of this study were useful to refine our OBSERV-AHC study design and procedures and were published in the journal Brain Communications.[3] The experience gained in this study strengthened our final study plan.

9) ***Modify the study plan based on the prior experience of the pilot study and further discussions of the study team and statisticians:*** Based on the above pilot study, we finalized the number of patients, using power analysis, and the statistical methods that will be needed to achieve our goals. Based on this, we confirmed the need for a larger study to represent the population of AHC due to its heterogeneity and to improve its statistical power. In addition, we recognized the importance of developing and testing a spell video library, spell paper calendar, and spell electronic diary (e-Diary) to effectively distinguish and document various types of abnormal paroxysmal events as that can impact results.[18] In addition, we had added the Vineland-II test for development as it was available in multiple countries and languages and has also been used in AHC before.[19]

10) ***Final study plan:*** Several investigator meetings allowed us to finalize the study plan and procedures. Specifically, this related largely to methods of coding, for entering and proofing the data, and in the refinement of the statistical tests.

IV. Study Design and Methods (Figures 2 and 3, Table 2)

1) ***Overall study design:*** To address the challenge of securing the requisite number of patients, the design that was adopted was a collaborative prospective study over two years conducted through the International AHC Consortium (IAHCRC) on >80 AHC patients together with incorporation of retrospective data in the analyses. The IAHCRC consists of 38 centers in 13 countries of which 9 centers in 5 countries participated. The initiating-coordinating center, and site of the study PI, is the Duke University Center (Durham, USA). The centralized online data collection service, the IAHCRC-CLOUD Platform, was developed and is hosted by the Euro-Mediterranean Institute for Sciences and Technology (IEMEST, Palermo, Italy). Please see **Figure 2** for overall study organizational charts**.** The participating centers where patients were seen and followed were the following: Duke University Medical Center, NC, USA; Lyon University Hospitals of Lyon, Lyon, France; Sant Joan de Déu Children's Hospital, Barcelona, Spain; Great Ormond Street Institute of Child Health, London, UK; The National Hospital for Neurology and Neurosurgery, London, UK; Carlo Besta Neurological Institute, Milan, Italy; Istituto Giannina Gaslini, Genoa, Italy; Bambino Gesù Children's Hospital, Rome, Italy; E. Medea Institute, Lecco, Italy. The EU-based centers are all members of the European Reference Network for Rare and Complex Epilepsies, ERN EpiCARE. The timeline of the study was planned to be 2 years and 10 months **(Figure 3).**

   An initial phase of the study (4 months) was planned to ensure agreement on the details of implementation of various procedures of the study. Regarding standardization of the study procedures, we had six investigators' meetings in which we standardized the procedures of the study. This included agreement on the procedures and classification systems of spells through the development of a spell video library and methods to demonstrate inter-rater reliability of classifying-videos of different types of events and usage of a written spell diary and an electronic spell diary as well as standardization of clinic visits. The second phase

(recruitment and follow up phase) was planned for 2 years during which patients would be enrolled sequentially if they met criteria and accepted enrollment in the first year. Then they were seen for follow-up during the second year. Based on the statistical expertise available to us and on our experience with the pilot study, we ascertained that a two-year study duration while using the creative statistical analyses we described including linear mixed effects model, multiple logistical regression and ambidirectional data designs will allow us to establish the goals of the study. In the third phase (6 months), which is just starting, the data is being entered, proofed, analyzed.

Currently, we have completed recruitment and we are in the stage of data proofing and analysis phase.

Funding for this study was provided by multiple family organizations as well as by multiple funds from Duke University. The family association also provided resources in referring patients and in providing input into generating study event calendars and other related procedures.

Data Collection Forms were developed collegially, with all the variables needed to be collected at baseline and at follow ups, as indicated including the following: demographics, paroxysmal and nonparoxysmal manifestations, Flunarizine and Disability Indexes, Vineland-II, medications and Paroxysmal Event Diaries, genetic tests and physical examinations. In addition, the Spell Video Library Database and the OBSERV-AHC Study Database were uploaded on the IAHCRC-CLOUD Platform (Figure 2b). These two databases included all the variables in the Data Collection Forms as well as specific real-time automatic controls to maintain consistency of entered data with prespecified data rules. Both Databases were tested and validated for data-entry by the data-providing centers.

2) ***Correct identification of spells- Development of a Spell Video Library****:* To address our second challenge and to ensure that families could correctly identify paroxysmal spells for our analysis, we created a spell video library on the IAHCRC-CLOUD Platform, and have standardized it based on consensus among the experts on the definition of various spell types: (1) Plegia spell; (2) Dystonia spell; (3) Abnormal ocular movements spell. Different videos were chosen for the pre-training test, for the training procedure, and for the initial post-training test. Additionally, more videos for a second and third round of training (if needed) and post-training tests were also chosen.

3) ***Monitor paroxysmal spells- Development of a spell calendar and e-Diary****:* To address our third challenge, we developed a paper-based spell calendar and its electronic version (e-Diary) that could be used after caregivers were trained on distinguishing spells using the above spell video library. For patients with epilepsy, we used seizure calendars and the ILAE (International League against epilepsy) guidelines for seizure classification.[18,20] The paper-based calendar use was tested in a selected subgroup of patients on weekly basis via video conference for two months. During each video conference follow-up appointment, providers and caregivers reviewed the calendar entries through a standardized interview and completeness of filling of the calendar over time was determined. On the other hand, the e-Diary can be filled by connecting to the IAHCRC-CLOUD Platform through a computer or through a smartphone application. The e-Diary was based on the paper version of the event calendar described above. Mandatory fields for completion of events in the e-Diary included the date, time, duration, and type of the recorded spells. The e-Diary was offered to caregivers/patients during either the initial appointment or a follow-up appointment that was already scheduled for their participation in the study. To evaluate the usefulness of the e-Diary and the satisfaction of the caregivers, a questionnaire was prepared, reviewed, and approved by the OBSERV-AHC study investigators and provided to the caregivers to give their input about the e-Diary.

4) ***Use validated non-paroxysmal disability assessment tools****:* The concern is that many developmental measurement tools have not been validated or are not available for use in multiple different patient populations. Consequently, we chose to use the Vineland-II tests. This is a standardized and reliable measure of adaptive behavior that is used in the assessment of individuals with intellectual, developmental, and other disabilities from ages birth to 90 years old. It is available in multiple languages for countries participating in the study.[21-23] In addition, it has previously been used in patients with AHC prior measurement of their developmental skills.[19] The Vineland-II groups the subdomains into four domain composites: Communication, Daily Living Skills, Socialization and Motor Skills. The four domain composite scores make up the Adaptive Behavior Composite for ages birth through 6 yr 11 mo. There are three domain composite scores that make up the Adaptive Behavior Composite for ages 7 yrs through 90 yrs: Communication, Daily Living Skills and Socialization. In addition, we chose the AHC non-paroxysmal disability index (NPDI).[3,4,24,25] Higher NPDI scores correspond to more severe developmental impairments. This index has been used in patients with AHC and has undergone face validity procedures.[4,24] It also has been shown to have an inter-rater reproducibility comparable to that of the NIH Toolbox Cognitive Battery in children.

5) ***Determine appropriate measurements for paroxysmal manifestations:*** The Paroxysmal disability index (PDI) assesses the paroxysmal features of AHC (hemiplegia and dystonia, not seizures) and was developed specifically for use in patients with AHC. This index assesses both plegic and tonic/dystonic attacks and their severity. Higher PDI scores correspond to more severe paroxysmal hemiplegic/dystonic features of AHC. [3,4,24,25] Similar to the NPDI, this index has been used in patients prior with AHC and has undergone face validity testing.[24] Similarly, its inter-rater reproducibility comparable to that of the NIH Toolbox Cognitive Battery in children.[3,25]

6) ***Determine early-life clinical predictors of long-term paroxysmal and non-paroxysmal manifestations****:* The next challenge is to determine early-life clinical predictors of long-term paroxysmal and non-paroxysmal manifestations. For long-term outcome measures we will use collected prospective data regarding the scales mentioned: Vineand-2, NPDI and PDI scales. For potential predictors, we will use collected retrospective data about disease severity, NPDI and PDI scales, by the age of 18 months. The reason for choice of the landmark age of 18 months is that AHC as a rule is diagnosed from birth to 18 months of age and its diagnostic criteria include onset before the age of 18 months. Other potential predictors that we will test include the age of onset of AHC, sex, *ATP1A3* gene variant type and presence, epilepsy status classification.

7) ***Changes in paroxysmal and non-paroxysmal disability over time:*** To address this challenge, we developed unified procedures among the various centers to collect the above data and a tailored statistical approach that will be described in the next section V below.

8) ***Effects of medication on outcomes:*** Our eighth challenge that needs to be addressed is to determine whether and to what extent does flunarizine therapy, which is the most commonly used therapy for AHC, affects long-term outcome in PDI, NPDI, or Vineland-II composite scores.[17,26-28] Here we will use specifically tailored statistical tests, as described in the next section, to analyze the data of these parameters with the aim to determine whether flunarizine use has long term beneficial or detrimental effects or both.

V. Statistical methods to analyze results generated by study protocol (Table 3)

1) ***Correct identification of spells- Development of a Spell Video Library:*** To validate this spell video library, Wilcoxon matched-pairs signed-rank test will be used to compare pre- and post- training test results. The Wilcoxon matched-pairs signed-rank

test is a nonparametric method to compare the before-after of matched measurements. This test is appropriate for a repeated measure design, where the same subjects are evaluated under two different conditions (before or after training using the spell library). This is the nonparametric counterpart of the parametric paired t-test. In addition, we will use Cohen's Kappa for the degree of agreement between the expert panel and caregivers' opinions. This will be tested to compare caregivers' classifications with those of the experts. A Cohen's Kappa of > 0.8 will be considered as excellent agreement.[29,30]

2) **Monitor paroxysmal *spells- Development of a spell calendar and e-Diary:*** To help accurately monitor spells we will test the usage (percent completion of data) of a spell calendar and e-Diary. A one-way repeated measures ANOVA (Analysis of variance) test will be performed to examine if there is a change over time in this parameter of spell calendar completion (completion the required information about each spell). This statistical test is helpful in determining the sustainability of maintaining the calendar. The one-way repeated ANOVA is helpful to determine whether or not there is a statistically significant difference with repeated measurements of the same or multiple groups.[31] For these and for other analyses that require normal distribution, the Kolmogorov-Smirnov normality test will be used and if the data is not normally distributed then non-parametric tests like the Kruskal-Wallis tests will be used.[32,33] For the group of participants that have used the e-Diary, we will use the Kaplan-Meier method to analyze the "time-to-end of usage" data, since weekly "check in" visits with the providers about the completeness of filling the e-Diary are not in the plan. Kaplan-Meier is typically used to estimate the survival function, in our case "time-to-end" of usage.[34] The visual representation of this function is usually called the Kaplan-Meier curve, and it shows what the probability of an event (in this case, continued use of the e-Diary) is at a certain time interval.[35]

3) ***Validate non-paroxysmal disability index:*** To address this goal, we will calculate Spearman's correlation to assess the association between the global Vineland-II adaptive behavior composite score to with NPDI. Spearman's rank correlation

coefficient is a nonparametric measure of rank correlation, it helps assess the relationship between two variables. In addition, the interclass correlation coefficient for the NPDI and PDI scores variations will be estimated between two independent investigators each determining the scores independently is the method we chose to assess test reproducibility.

4) ***Determine change over time of paroxysmal and non-paroxysmal disability index and early life predictors of long-term outcome:*** For determination of early-life clinical predictors of long-term outcome, we will use both retrospective data about the disease severity status by the age of 18 months and prospectively obtained data, about the paroxysmal and non-paroxysmal manifestations outcome (i.e. PDI, NPDI, and Vineland-II) in an ambidirectional approach.[36,37] The response variables will be the change in PDI, NPDI, and Vineland-II from baseline to one year after and these scores at the end of follow up. The key risk factors include age at baseline, sex, *ATP1A3* mutation (E815K, D801N, G947R, other ATP1A3 mutations, no mutation), epilepsy (no epilepsy, epilepsy with history of status epilepticus, presence of epilepsy), and baseline PDI, NPDI, Vineland-II scale.

To compare baseline and one-year follow-up data regarding the paroxysmal and non-paroxysmal disability indices, we will use the Paired t-test or the Wilcoxon signed-rank test as appropriate to investigate for any evidence of deterioration or improvement during the one-year prospective follow-up period up to two years. In addition, multivariable linear regression analyses will be performed to study the association between each of the response variables and risk factors.

In addition, to investigate temporal trend of the disability scale scores that will be measured repeatedly over time, we will use the linear mixed effects models.[38] The mixed effects model will account for the temporal correlation within a patient and individual differences in disease progression by adding patients-specific random effects. The model will be adjusted by the key explanatory variables.

5) ***Effects of medication on outcomes:*** To help determine the effects of medication on the disease course, we will study flunarizine. Flunarizine is defined as a binary (0=no flunarizine, 1=flunarizine). As flunarizine was not randomly assigned in these patients, a propensity score approach will be used to estimate the effect of flunarizine. Propensity score approaches are used to balance the two groups with and without flunarizine with respect to the observed covariates to emulate a randomized controlled trial using an observational study.[39] This will help remove confounding effects when estimating the effect of flunarizine. The propensity score will be estimated by fitting a logistic regression model of treatment indicator with baseline covariates as independent variables. If the two treatment groups have a similar sample size, the propensity score weighting method will be applied.[40] If the two groups have an unbalanced sample size, the propensity score matching method will be applied. After propensity score weighting or matching is applied, univariable regression analysis will be performed to study the association between the change in PDI and the Flunarizine Index.[41] Flunarizine Index is defined as the percent calculated by dividing the duration of the intake of flunarizine by the age of the patient at the time of assessment.[17] If needed, a multivariable regression model will be fitted to, additionally, adjust for the baseline covariates. If sufficient data are collected for a continuous variable of flunarizine, a sensitivity analysis will be conducted to study the effect of flunarizine dosing.

6) ***Assess potential variability across centers or countries:*** This will be addressed by including centers or countries as "random effects" in the linear mixed effects model and multivariable linear regression analyses. The resultant variance of the random effects will determine how heterogeneous or homogenous different centers or countries are and will account for any such heterogeneity through coefficient estimation.

VI. Discussion and conclusions:

The quantitative analysis of a rare neurodevelopmental disease presents many challenges from the validity of measurement tools to the methods used to determine the natural history and determine early-life clinical predictors of outcomes.

In the planning of this study, we relied on and provided an illustration of the benefits of having a collaborative team of physicians, caregivers, and statisticians in planning a natural history study of a rare disease. While recognizing the limitations of the current tools that measure outcomes from both a paroxysmal and non-paroxysmal disability standpoint and the complicated nature of spells, we hope that we aimed to show how, for a specific disease, one can create not only a spell video library to train families, but also develop alternative methods to record those spells.

In addition, from a non-paroxysmal disability standpoint, we had the concern about measuring development appropriately in individuals with developmental delay in different countries. Currently, we are limited by using an accessible measure for development through the global Vineland-II scale. We understand that this scale may have limitations, but it is comprehensive and can be administered in multiple different countries and languages. In addition, we have disease-specific scales including the paroxysmal disability index (PDI) and non-paroxysmal disability index (NPDI) that have been used in patients with AHC. Therefore, being able to correlate the PDI with spell calendar and e-Diary and the NPDI with Vineland-II scores can represent an advantage to show that rare diseases can have both a global scale and create a disease-specific scale. Developing such scales and validating them through the above, or similar, statistical methods is important as these can be markers to measure improvement for interventions, which include therapies of various types and pharmaceutical therapeutics in the future. This is of particular importance given the prospects of gene therapy for AHC based on current studies in its mouse model carrying the most frequent mutation causing AHC in humans, the D801N mutation.[42]

Based on the methodology from this study, using the above-mentioned univariable and multivariable analyses, we can be able to determine early-life clinical predictors for

outcomes. We can note what factors (i.e. age, sex, epilepsy, and baseline scores) affect the outcomes measures NPDI, PDI, and Vineland-II. Once we can determine these effects, we can create disease-specific outcome scores and roadmaps that physicians can use when discussing the prognosis for the patient. Our approach can also be used for longer term studies that do not have to be restricted to one year follow-up data.

The statistical methods and the ambidirectional approach we decided on offer advantages. As alluded to above, the ambidirectional approach is unique and key to collect granular and hidden information describing the patients of interest.[4,5] The linear mixed effects models and the propensity score analyses are extensions of linear regression models. They use random effects to account for variability among individuals and allow for the analysis of repeated measures over time. This type of analysis has been used in previous natural history studies of neurological disorders, including Canavan disease, Friedreich ataxia, cerebral palsy, Parkinson's disease, and Alzheimer's disease.[38,43-47] Given the need for sophisticated statistical analyses in natural history studies, we would like here to emphasize that our experience highlights the importance of working with statisticians starting in the early stages of planning such studies to select the proper statistical methods in advance and to continue to be engaged with them throughout these studies. These are crucial and key steps to succeed in such studies.

The approaches we use in this study can potentially also be applied, with modifications, to the development of core outcome set (COS) for AHC along the lines of the Core Outcome Measures in Effectiveness Trials (COMET) initiative.[47] This will be important in knowing the baseline that should be measured and reported in all clinical trials for a specific condition. In addition to this, the COS can also be suitable for use in routine care, clinical audit, and research in randomized controlled trials and in other than randomized trials.[48]

In this study, we emphasized global outcome parameters since AHC, due to its underlying pathophysiology, causes such pervasive impairments.[49-52] Whereas, understandably, such global outcomes are of major interest, future studies could choose to concentrate

on specific impairments resulting from AHC which include: behavioral, sleep, psychological, growth, cardiac, gastrointestinal, epileptic, or otherwise.[19,51-62] The same methodology that we describe in this study can help develop further scales for future studies of this disease by addressing specific aspects and can also be used for other rare neurodevelopmental diseases.

We are aware of other studies such as a prior retrospective AHC natural history study, but our study is different in that we use prospective study methodologies, ambidirectional methodology, and other creative statistical analyses as mentioned above. In addition, we are aware of another study that has been investigating predominantly Rapid Onset Dystonia Parkinsonism (RDP), but our study is different as it is focused on patients with AHC, which has a different phenotype with early onset movement disorders, epilepsy, along with development delay, and is a prototype of pediatric encephalopathies with movement disorders and epilepsy while RDP is not. There have been challenges in studying the natural history of these encephalopathies. Our study and its methodologies can help others in the field of pediatric neurology who are studying the natural history course for these other neurodevelopmental encephalopathies that can manifest through movement disorders, epilepsy and developmental delay. The creative statistical methods and study design and methods to measure paroxysmal and non-paroxysmal impairment can be used, with likely some modifications, for such disorders. Examples of disorders that can potentially benefit from similar approaches we described include not only those caused by *ATP1A3* mutations (Alternating Hemiplegia of Childhood, Cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing impairment (CAPOS) syndrome, Dystonia, Dysmorphism, Encephalopathy, MRI abnormalities no hemiplegia (D-DEMO) syndrome) but also others including many forms of mitochondrial disease, Neuronal Cerebroid Lipofuscinosis, Paroxysmal kinesigenic dyskinesia, Paroxysmal nonkinesigenic dyskinesia, SCN8A developmental epileptic encephalopathy, Glut-1 deficiency, Leigh's syndrome, Allan Herndon-Dudley syndrome, DOORS syndrome, Dopa responsive dystonia, FOXG1 mutation disorder and Epileptic encephalopathy and chronic ataxia.

We hope that this methodology and its review, can be used to develop natural history studies for other disorders caused by *ATP1A3* mutations as well as potentially other rare neurodevelopmental diseases **(Table 4)** that often share overlapping manifestations with AHC and with each other including neurodevelopmental impairments, movement disorders, and epilepsy.[11,63,64]

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Table 1: Challenges facing design and performance of the Alternating Hemiplegia of Childhood natural history study

Challenge	Description
Have enough patients (Rarity of disease)	Since AHC is a rare disease with often heterogeneous manifestations, it is a challenge to secure the prerequisite number of patients required for a study.
Determine appropriate study design	There have been many approaches to developing natural history studies in the past, both prospective and retrospective each with advantages and disadvantages, a combined approach is desirable but requires more procedures than either alone.
Multiple paroxysmal manifestations of the disease	Neurodevelopmental diseases have paroxysmal spells that can range from movement disorders, seizures, abnormal ocular movements, and specifically in AHC also plegia spells. These often can be difficult to distinguish.
Monitoring paroxysmal manifestations	It is hard to accurately document spell type, severity and frequency over time particularly over long periods.
Multiple nonparoxysmal manifestations of disease	Most developmental measurement tools have not been validated for use in multiple different patient populations. Many of these tools also may not address disease-specific manifestations.
Monitoring nonparoxysmal manifestations over time	Most developmental measurement tools have not been validated for use in multiple different patient populations. Many of these tools also may not address disease-specific manifestations.
Determine natural history over time	With a rare disease, it is hard to determine natural history over time as each patient or subgroup of patients may progress differently. It is important to establish a baseline trajectory that can be followed for diseases to help with expectations, counseling, and treatment options in the future.
How to determine early life prognostic predictors	Early life clinical predictors are important as they can help stratify patients into potential trajectories that they may follow. Prospective studies would have to take decades.
How to determine if treatment affects outcomes	A long-term goal of developing these studies is to see if we can assess the effects of treatments or medications on prespecified disease outcomes.

Table 2: Study design solutions adopted to address each challenge

Challenge	Solution
Have enough patients (Rarity of disease)	Multicenter Multinational Design with unified data collection procedures
Determine appropriate study design	Review the literature and determine what study design methods will be most effective for our patient population
Distinguishing multiple paroxysmal manifestations of AHC	Correct identification of spells through the creation of a spell video library for the disease and its manifestations
Monitoring paroxysmal manifestations over time	Creation and validation of a paper-based event calendar and an electronic-based event diary
Multiple nonparoxysmal manifestations of the disease	Vineland-II (accessible in multiple countries) and nonparoxysmal disability index
Monitoring nonparoxysmal manifestations over time	Validate the global and disease specific assessment tools against each other and then use both in the study
Determine natural history over time	Determine variables as noted above for all manifestations of the disease type and follow them with baseline, 1- and 2-year data points
How to determine early life predictors	Investigate early-life disease severity measures, determined retrospectively, and non-age dependent variables like mutations status, as potential predictors
How to determine treatment effects on long term outcomes	Prospective and retrospective efficacy data analysis of responses to medical therapies

Table 3: Statistical Methods used to address each of the goals

Goal	Statistical Test/s
Recognize the multiple paroxysmal manifestations	Development of Spell Video Library and caregiver training procedure. 1. Pre versus post test results of correct identification of spells by caregiver: Wilcoxon signed-rank test. 2. Inter-rater reliability: Cohen's Kappa. 3. Caregiver Likert scale response and calculation of confidence interval.
Record paroxysmal manifestations over time	Creation of two alternative methods of recording spells: a paper-based and an electronic (smartphone and computer-based) diary. 1. Reliability of percent completion of the paper calendar over time studied by one-way repeated measures ANOVA. 2. Long-term usage of electronic diary studied using Kaplan-Meier survival analysis. 3. Determine the interclass correlation coefficient of two different investigators when measuring the paroxysmal disability index.
Validate NPDI	1. Determine the Spearman correlation between NPDI and global Vineland-II. score 2. Determine the interclass correlation coefficient of two different investigators when measuring NPDI.
Determining the changes in paroxysmal and nonparoxysmal manifestations over time and early life predictors of outcome	1. Univariable and multivariable linear mixed effect models will be fit. The response variables will be total Vineland Score, NPDI and PDI; the covariates include follow-up time, age at the initial visit, *ATP1A3* status, epilepsy status, and sex. 2. Wilcoxon signed-rank test will be used to compare total Vineland Score, NPDI and PDI at baseline and one-year follow-up. 3. For the association between scores and risk factors, we will perform the same analyses as documented above for the disease-severity scales. *

Effect of flunarizine on long-term outcome	Propensity score analysis to analyze non-experimental data.
Assess heterogeneity in results across centers or countries	Include center/country as one of the potential confounding variables in the statistical analyses. Treat center as the random effect in the linear mixed model.

* Alternative and supplementary analyses that may apply to future extensions of this study or to future studies: latent growth model. Paired t-test, sign test, Chi-Square/Fisher Exact test using Bonferroni corrections. Vector or principal component analysis to find out the one or two simple subitems that best correlate with the totality of the outcome as assessed by the above variables. Latent class analysis to group patients into different sub-categories that may have a different long-term clinical course.

*Note: Baseline is the first visit during the first year of the study and one year visit is the visit one year later

Table 4: Examples of neurodevelopmental disorders that, in the future, could potentially benefit from approaches similar to the approaches we used, in developing future natural history studies addressing various aspects of each disorder (for a review of such disorders, please see de Gusmao et al., 2021).

Neurodevelopmental diseases with epilepsy and movement disorders
Alternating Hemiplegia of Childhood
Rapid-onset dystonia parkinsonism
Cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing impairment (CAPOS) syndrome
Dystonia, Dysmorphism, Encephalopathy, MRI abnormalities no hemiplegia (D-DEMO) syndrome
Neuronal Cerebroid Lipofuscinosis
Niemann Pick disease
Paroxysmal kinesigenic dyskinesia
Paroxysmal nonkinesigenic dyskinesia
SCN8A developmental epileptic encephalopathy
Glut-1 deficiency
Leigh's syndrome
Allan Herndon-Dudley syndrome
DOORS syndrome
Dopa responsive dystonia
Epileptic encephalopathy and chronic ataxia

Figure 1: Approach showing the various steps used to develop the OBSERV-AHC study plan

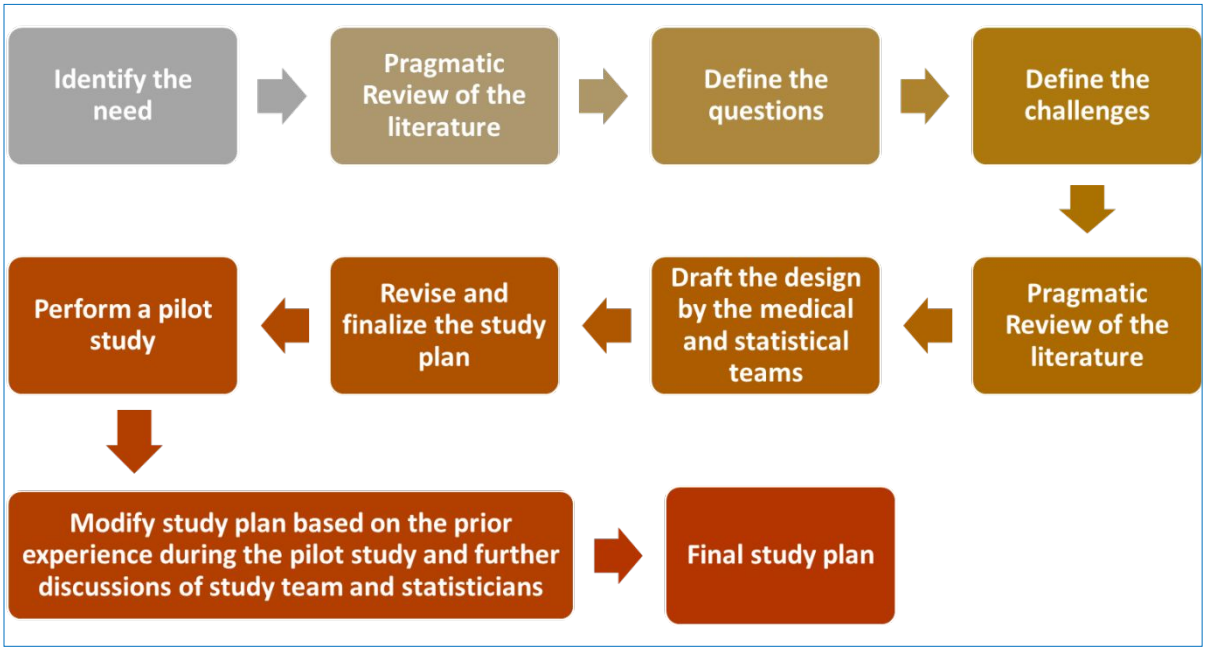
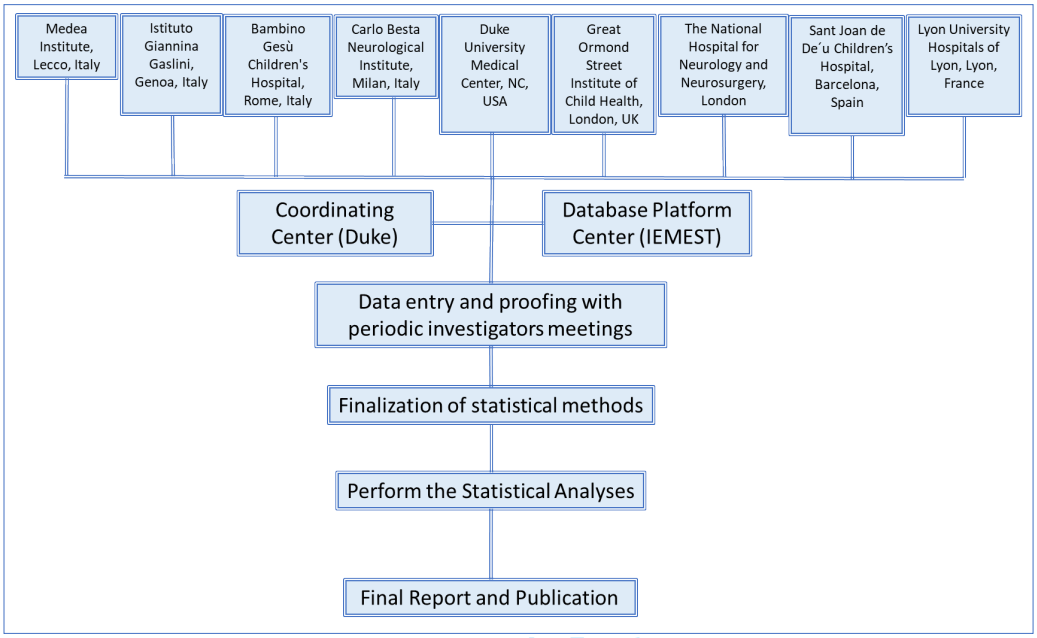


Figure 2: Organization of the Study

2a. Diagram showing the OBSERV-AHC study centers and study organization and steps of the study



2b. Architecture of the IAHCRC-CLOUD Platform Service

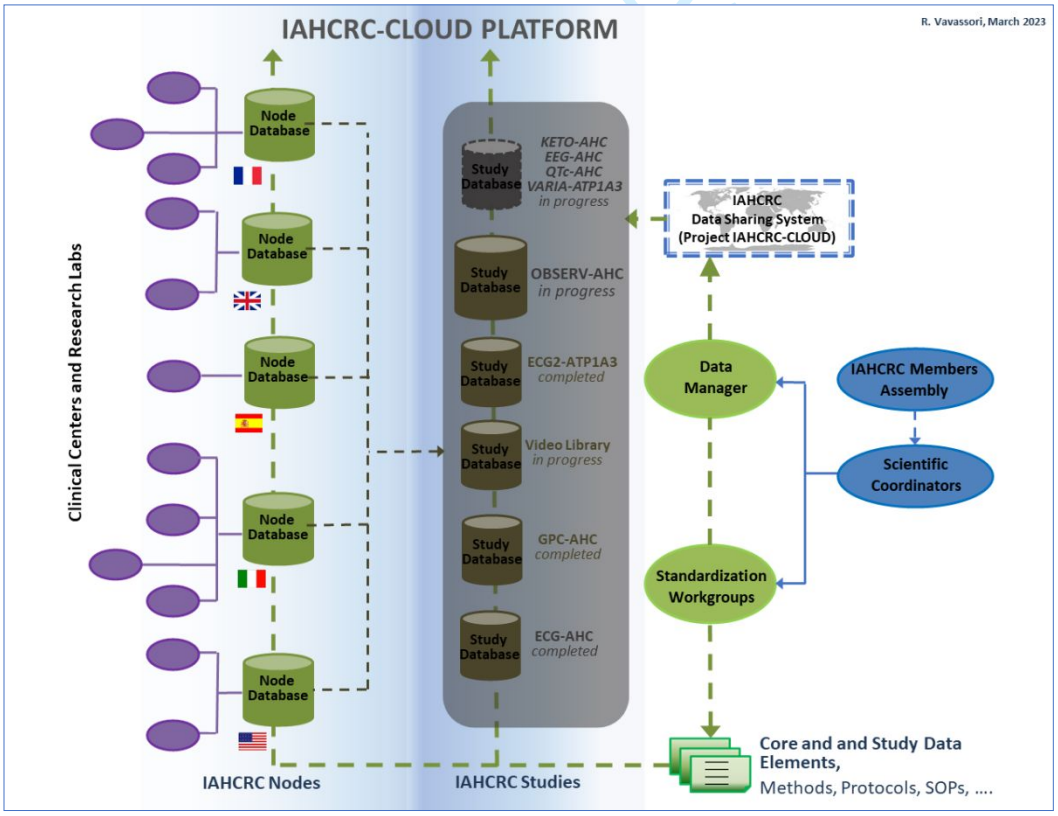
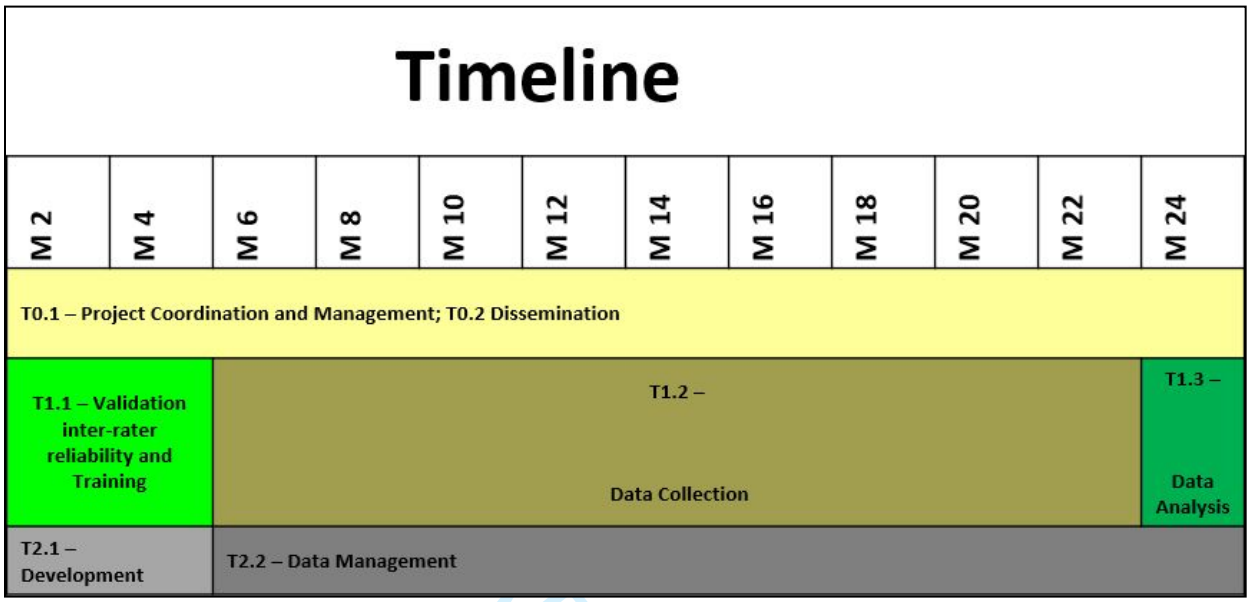


Figure 3. Timeline of the study



M=Month

T=Time period

Please note during data collection phase the following variables were collected at Baseline and one-year follow-up: Demographics, prior history of the disease and disease severity, Flunarizine Index, Vineland-II, PDI, NPDI, Paroxysmal event diaries, medications, and physical exams (as also delineated in the text). In between, additional follow-ups were done if and as clinically indicated.