Pilot Study of Modified Atkins Diet in Kabuki Syndrome

ID:00250195

NCT 04722315

Date of Last Approval: 1/31/2024

1. Abstract

Kabuki syndrome is a rare genetic disorder characterized by intellectual disability and a unique cognitive profile. Studies in a mouse model of Kabuki syndrome have demonstrated hippocampal memory defects and a disruption of adult neurogenesis in the the dentate gyrus. Interestingly, these deficits were normalized in postnatal life with agents that inhibit histone deacetylases, indicating that Kabuki syndrome may be a treatable cause of intellectual disability. Preliminary data shows that patients with Kabuki syndrome have deficits in visuospatial reasoning and memory. This proposal aims to build upon the basic science and clinical work to evaluate a potential treatment using disease-specific outcome measures. If successful, these studies will yield insights into the pathogenesis of Kabuki syndrome and lead to the first therapeutic strategy.

2. Objectives

Objective 1: Test whether HDAC inhibition through modified Atkins diet modifies the neurocognitive or neurobehavioral phenotype in individuals with Kabuki syndrome. Previously, a ketogenic diet has been shown to ameliorate cognitive and neurohistological defects in a mouse model of Kabuki syndrome. This proposal aims to conduct a pilot study of 10-15 adult patients with Kabuki syndrome given 12 weeks of HDAC inhibition through diet and determine whether performance on the cognitive assessment protocol changes.

3. Background

Kabuki syndrome (KS; MIM 147920) is a genetic disorder occurring in about 1 in every 32,000 births characterized by five cardinal features; dysmorphic facial features, skeletal anomalies, persistence of fetal fingertip pads, postnatal growth deficiency, and intellectual disability (ID). The most common variants in patients with KS are found in KMT2D – a histone methyltransferase that adds mono-, di- and trimethylation to K4, changes found at enhancers and actively transcribed promoters. Mutations in KDM6A – a histone demethylase that removes H3K27me3, a modification seen in closed chromatin – represent another known, but less common cause of KS (MIM 300867). Mutations in KMT2D or KDM6A thus result in widespread changes in normal cellular transcription due to loss of histone modifications resulting in too much closed chromatin. Kabuki syndrome is thus an excellent example of a Mendelian disorder of histone machinery (MDEM), most of which, like KS, result in intellectual disability. In addition to information about a specific rare disease, understanding more about the cellular mechanisms resulting in impaired cognition in KS gives insight into the whole group of MDEMs. Understanding these as a group, in turn, may provide a window into the mechanisms underlying normal cognition and memory and learning, and pathologic states – specifically intellectual disability.

Intellectual disability (ID) is a heavy burden on patients, family and society. Many genetic syndromes result in intellectual disability; however, although the causative genes of these syndromes are known, little is understood about how these variants lead to intellectual disability. Only recently, basic science has begun to try to characterize the mechanisms underlying cognitive impairment. This work has led to treatment trials in diseases such as Fragile X syndrome and Angelman syndrome. The Bjornsson lab has demonstrated that Kmt2d is highly expressed in the granule cell layer of the dentate

gyrus. Kmt2d +/βGeo mice have many physical features of humans with KS as well as abnormalities in visuospatial memory and deficits in neurogenesis in the dentate gyrus region of the hippocampus seen in association with a thinner granule cell layer. Importantly, these cognitive and histopathologic deficits were corrected with postnatal administration of a histone deacetylase inhibitor and with the ketogenic diet. The dentate gyrus – and postnatal neurogenesis in this region – has been linked to spatial pattern separation and thus tests that examine visuospatial reasoning and memory at least partially localize to this brain region. While problems with visuospatial areas of cognition are far from a 1:1 correlation with dentate gyrus neurogenesis, there is certainly an important correlation. Thus, we would expect this neuroanatomical region to be abnormal in individuals with KS if mouse studies are predictive. Our recently published study of 23 patients solidified the visuospatial/verbal differences in the KS cognitive profile and more specifically linked the weaknesses in the profile to the dentate gyrus region. Additionally, by comparing the KS patients to age and IQ-matched controls, we established that this cognitive phenotype is specific to Kabuki syndrome and different from what is seen in other causes of ID. In addition, impaired neurogenesis in the hippocampus has been strongly linked to anxiety and anecdotally we have noticed a significant burden of anxiety in patients with Kabuki syndrome. Thus it is important to examine emotion and behavior in totality as well as anxiety in depth in this population and determine whether anxiety moderates the cognitive performance. As a next step, a biologically relevant and evidence-based treatment must be selected as an intervention for a clinical trial.

The Bjornsson lab has demonstrated that in the Kmt2d +/βGeo mice, a ketogenic diet is equally effective in reversing the visuospatial deficits and increasing neurogenesis in the dentate gyrus. This is a treatment that is used clinically for many conditions including ones that affect the central nervous system like epilepsy. Modified Atkins diet has been shown to be effective in these conditions in place of stricter ketogenic diet and is easier for patients to tolerate. The pilot treatment trial we propose here could lead to a larger trial which could have immediate benefit to patients and change clinical practice. The Bjornsson lab demonstrated in 2016 that neurogenesis and memory deficits in the mouse model of KS was not only rescued by administration of HDAC inhibitor AR-42, but that it was also rescued with the ketogenic diet (Figure 2). Beta hydroxybutyrate, a ketone body that is actively transported into the central nervous system and enters the hippocampus during ketosis, is a potent HDAC inhibitor. Modified Atkins diet (MAD) has been shown to be an effective, safer to initiate, and easier to comply with substitute for the ketogenic diet in other CNS processes like epilepsy. Thus we believe the rationale is there to attempt a low-risk pilot study using this intervention in patients with KS. The Neurology teams at Johns Hopkins include nutritionists, clinicians, and researchers who have extensive experience with MAD and they will be available for consultation and for any diet-related needs. 12 weeks was chosen because that is the minimum amount of time used to see effect in studies on the ketogenic diet or MAD. Additionally, 8 weeks is the approximate length of time of neurogenesis of a new neuron. Since we suspect neurogenesis plays a role in cognitive performance in KS, the 12 week duration allows for neurogenesis to take place. To date, no study of treating epilepsy in KS with ketogenic diet or MAD exists.

4. Study Procedures

a. Participants with Kabuki syndrome 18 years and older will be recruited to participate. Recruitment will stop once fifteen participants are consented. This number allows for the ~30% rate of dropout expected based on difficulty complying with the diet in order to have data from 10 participants. Exclusion criteria would be inability to tolerate or comply with modified Atkins diet, inability to do repeat blood and urine testing, and inability to travel to Baltimore for 2 study visits 3 months apart. Participants will begin whenever they have their initial visit and conclude 12 weeks later. See Figure 1.

Figure 1: Study Flow

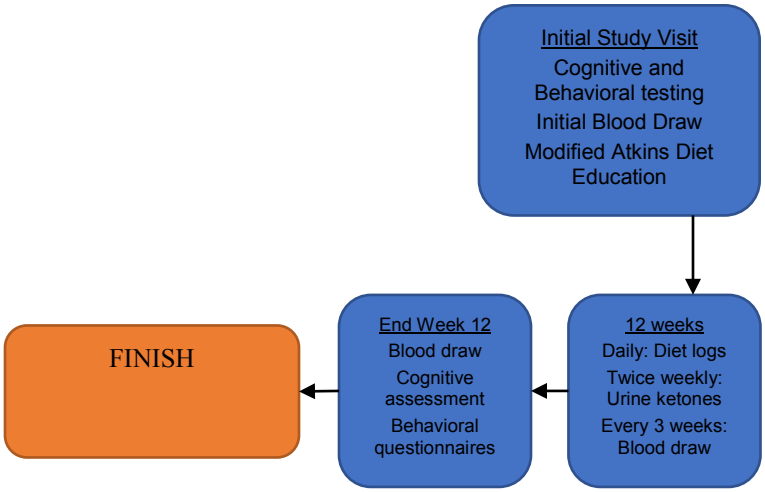

Table 1: Neurocognitive and Neurobehavioral Testing

Protocol (Repeated Day 1 and Week 12)	Purpose of Test	Area of Cognition or Behavior Evaluated	Neurocognitive Subtest Current Battery
Cognitive Assessment Protocol	Outcome Measure Development	Visuospatial reasoning	Benton Judgement of Line Orientation
		Visuospatial perception, construction, and memory	Brief Visuospatial Memory Test, NIH Toolbox Picture Story Memory
		Language	Wechsler verbal portion of IQ testing
		Visual motor integration	Beery Developmental Test of Visual Motor Integration
		Visual perception	Beery Developmental Test of Visual Perception
		Executive function – inhibition, attention, and switching	NIH Toolbox Flanker Inhibition and Card Sorting
		Visuospatial perception and construction	Wechsler Intelligence Scale for Children -V Block Design
Neurobehavioral Questionnaires	Characterize Cognitive Phenotype and Brain-Behavior Relationship	Anxiety and Behavioral Phenotype	Caregiver Rating Scales: SCARED **and** Child Behavior Checklist (CBCL) **or** Adult Behavior Checklist (ABCL) **and** NIH Toolbox Emotion Measures
Verbal Hippocampal Measure	Brain-Behavior Relationship	Verbal memory	Hopkins Verbal Learning Test

The study flow is outlined in Figure 1. These consented adult participants will participate in an initial study visit. These participants will undergo cognitive and neurobehavioral testing and also will have baseline labs drawn and will meet with a study team member trained in modified Atkins diet for education. The labs are standard of care for diet initiation to rule out contraindications (CBC, CMP, lipids, UA, urine amino acids, free and total carnitine, Vitamin D, selenium). Blood will also be taken for genome-wide DNA methylation analysis to look for the known abnormal signature in Kabuki syndrome and see if it changes over time with the diet. Participants and/or their caregivers will keep a daily diet log and/or will be asked to download the free Carb Manager application onto their smartphone. They will send the logs and data back weekly. The application is optional, only if participants prefer it to keeping their own logs. Participants will be given urine ketone strips and asked to use and record in their diet log twice weekly. Participants will have blood ordered and drawn remotely every 3 weeks and sent securely to the study team. Beta-hydroxybutyrate will be measured on this sample as well as genome-wide DNA methylation measured every 3 weeks to see if it changes over the course of the trial. At the 6 week mark participants will also securely send a urine sample for measurement of urine ketones and amino acids to investigate metabolism while on the diet. Participants will return to Baltimore at the end of 12 weeks for a one day visit to repeat the cognitive assessment protocol, neurobehavioral measures, and repeat the initial lab tests including genome-wide DNA methylation (Figure 1).

b. The study will last for 12 weeks for each participant. There will be 2 on site study visits. Participants will then return home for the 12 week diet. They then will return to Baltimore for a 1 day study visit.

c. This study is not blinded for participants or investigators because that would be too difficult logistically since the participants have to keep the diet. Additionally, because this is just a very small pilot study, blinding is not necessary.

d. Participants will receive all standard of care and treatments. None will be stopped.

e. No placebo group will be used because this is only a pilot study and placebo groups will come in later studies.

f. If participants cannot tolerate and/or adhere to the diet during the 12 weeks by their own self report and diet logs, they will be removed from the study.

g. Participants can go back to their typical diet after the study ends or if they end the study early.

h. Participants will have blood drawn 5 times and urine collected 2 times. Blood and urine collection at the initial and final study visits will be done at KKI. Most of the blood and urine will be sent to JHH core lab and are standard of care for diet

initiation and monitoring to rule out contraindications or severe side effects (CBC, CMP, lipids, UA, urine amino acids, free and total carnitine, Vitamin D, selenium). Another 2 purple top tubes of blood will also be taken and transported to the Bjornsson lab by a study team member to run a genome-wide DNA methylation array to look for the known abnormal signature in Kabuki syndrome and see if it changes over time with the diet. The total additional blood volume taken beyond the standard of care labs for the diet will be 6 mL. In addition, participants will have blood ordered and drawn remotely every 3 weeks and sent securely to the study team. Once the study team receives it, one vial will go to JHH core lab and one to the Bjornsson lab. Beta-hydroxybutyrate will be measured on this sample by JHH core lab every 3 weeks and the other tube will again be taken to the Bjornsson lab to perform genome-wide DNA methylation arrays at 6 weeks to see if it changes over the course of the trial.

5. Inclusion/Exclusion Criteria

We will recruit 15 total participants with clinically-definite and genetically-confirmed Kabuki syndrome type 1 age 18 years and older. Clinical diagnosis of KS will be made based on recently published consensus diagnosis criteria. Genetic confirmation of a pathogenic mutation in *KMT2D* will also be required. Exclusion of participants younger than 18 years old is based on: 1) adults being able to consent to their own diet therapy, 2) ability to adhere to the diet, and 3) ability to standardize one cognitive battery to compare across and between participants. Other exclusion criteria include presence of another known genetic syndrome, a health problem that would make a modified Atkins diet harmful, inability to travel to Baltimore for 2 visits separated by 12 weeks. Recruitment of participants will be done solely by asking adults already participating in IRB00240569 to participate in this study as well. No recruitment will be done of participants not already enrolled in the other study.

6. Drugs/ Substances/ Devices

a. The Bjornsson lab demonstrated in 2016 that neurogenesis and memory deficits in the mouse model of KS was not only rescued by administration of HDAC inhibitor AR-42, but that it was also rescued with the ketogenic diet (Figure 2). Beta hydroxybutyrate, a ketone body that is actively transported into the central nervous system and enters the hippocampus during ketosis, is a potent HDAC inhibitor. Modified Atkins diet (MAD) has been shown to be an effective, safer to initiate, and easier to comply with substitute for the ketogenic diet in other CNS processes like epilepsy. Thus we believe the rationale is there to attempt a low-risk pilot study using this intervention in patients with KS. The Neurology teams at Johns Hopkins include nutritionists, clinicians, and researchers who have extensive experience with MAD and they will be available for consultation and for any diet-related needs. 12 weeks was chosen because that is the minimum amount of time used to see effect in studies on the ketogenic diet or MAD. Additionally, 8 weeks is the approximate length of time of neurogenesis of a new neuron. Since we suspect neurogenesis plays a role in cognitive performance in KS, the 12 week duration allows for neurogenesis to take place. To date, no study of treating epilepsy in KS with ketogenic diet or MAD exists. AR42 and Vafidemstat were chosen as in vitro comparisons (no in vivo use of these drugs) because of the previous evidence from the Bjornsson lab that

both HDAC inhibition and LSD1 inhibition also ameliorate the phenotype in mice like ketogenic diet does.

7. Study Statistics

a. Primary outcome variable: Change in cognitive testing protocol before and after 12 weeks of the diet
b. Secondary outcome variables: 1) Change in anxiety measure scores after 12 weeks on the diet. 2) Change in genome-wide DNA methylation array signature while on the diet.
c. Statistical plan including sample size justification: We will recruit 15 participants to this study with a goal of having 10 participants who complete the whole 12 weeks. We will only analyze data from participants who complete the whole 12 weeks. This number is completely generated based on the rarity of the disease model and therefore how many participants were felt were feasible to recruit. This is a very early stage, non-masked trial with no control group so no power analysis was done. To analyze the data, we will do intra-subject comparisons of the testing measures before and after 12 weeks of the diet, controlling for practice effects and for the fact the cognitive assessment protocol will be repeated 3 times in these participants. We will perform regression analysis to determine if the change is dependent on BHB levels. We will investigate whether scores on a measure of anxiety and if genome-wide DNA methylation analyses change after 12 weeks of diet and also if these potential changes moderate any change seen on cognitive assessment protocol.
d. Participants will only stop early based on inability to tolerate the diet. With only 15 participants, there will be no interim data analysis to stop early.

8. Risks

a. The main risk of this study is the modified Atkins diet. Potential risks of the diet include kidney stones, constipation, acidosis, diminished growth, weight loss, and hyperlipidemia. The rest of the proposed research presents no more than minimal physical, psychological, legal, or financial risk to participants. Neurological examination is part of a routine physical examination and involves no more than minimal risk to the participants. Neuropsychological testing can take a prolonged amount of time and can cause anxiety and discomfort in participants. Venipuncture can cause discomfort and mild bruising or bleeding at the site. Risk of infection is minimal. Patients may be disturbed by seeing their blood drawn. Risk of using the CarbManager application is minimal. Participants do not enter name or date of birth. They do enter a username (which will be their subject ID), sex, and year of birth. However sex and year of birth can be skipped. Other than that, participants only enter diet logs daily. The participant then generates weekly reports through the app that will be saved as a pdf to their device which they then will securely email to the study team. Use of the app is optional for participants and they can opt to just type their diet logs and securely email them

b. Labs and an exam will be done at the initial study visit to ensure that participants are healthy enough for 12 weeks of diet. Additionally, participants will be thoroughly educated about the diet at their initial study visit including potential adverse effects. Weekly check-ins with participants will be done to ensure they are complying with the diet and hear about any adverse effects. Subjects will be allowed to take breaks during Neuropsychological testing if significant anxiety or discomfort occurs. We will reinforce at each study visit that subjects can stop study procedures at any time if needed.
c. Any problems should be reported directly to Dr. Harris and she will be responsible for reporting the adverse events and responding to them. This study does have a DSMB consisting of 4 JHH faculty members.
d. Any physical data forms will be kept in a locked office in a locked cabinet to which only the PI has the key. The linking document with patient identifiers and study ID numbers will be kept on a password protected computer in the locked office, within a password protected file. This file will be kept separate from all data files including imaging data and testing results. These files will be deidentified and marked with a study identifier only and stored on a secure server - JHH OneDrive. Data collected through the CarbManager app, for those who choose to use it, will not have any identifiable information in it and will be emailed securely to the study team.
e. Participants may incur a financial toll by having to travel to Baltimore. We will minimize this by making the needed travel clear in screening.

9. Benefits

a. There is a possibility that patients may experience improvement in their cognition after 12 weeks on the diet. Other benefits of the study include furthering the scientific knowledge about Kabuki syndrome specifically and brain-behavior relationships in general

10. Payment and Remuneration

a. Participants will not receive any payment as part of this study but will be provided lunch and parking during the study visits.

11. Costs

a. Participants will not incur significant costs as part of this study. Neurobehavioral testing and laboratory costs will be paid for by PI's grant funding. Participants will be responsible for their own travel costs to Baltimore. Participants will also be responsible for the cost of their own food during the diet period.