A Therapeutic Trial in AS

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1. Title of Protocol

Molecular genetics of Prader Willi Syndrome (PWS) and Angelman Syndrome (AS): Part II - A therapeutic trial in Angelman Syndrome (AS)

2. Background

(Identical for Parts I and II)

Prader Willi Syndrome (PWS) is caused by paternal genetic deficiency for a gene or genes mapping to chromosome 15q11-q13. Prader Willi Syndrome is characterized by neonatal feeding difficulties (often requiring tube feeding), infantile hypotonia, moderate mental retardation, excessive appetite leading to obesity in childhood, and hypothalamic defects leading to hypogonadism and growth hormone deficiency. All or almost all patients with a typical Prader Willi phenotype have an identifiable molecular defect.

Angelman Syndrome (AS) is caused by maternal genetic deficiency for a gene that encodes E6-AP ubiquitin-protein ligase (gene symbol UBE3A) mapping to chromosome 15q11-q13. The genes causing PWS and AS are subject to genomic imprinting; expression of the relevant gene(s) from the paternal chromosome is essential to prevent PWS, and expression of UBE3A from the maternal chromosome is essential to prevent AS. PWS is caused by large deletions of ~4 Mb of 15q11-q13, by uniparental disomy (UPD) with two maternal copies of 15q11-q13 and paternal deficiency for 15q11-q13, and by imprinting defects such that the paternal chromosome has the methylation and gene expression pattern normally expected for a maternal chromosome (Table 1).

Angelman Syndrome is caused by large ~4 Mb deletions of maternal 15q11-q13, by UPD with two copies of paternal 15q11-q13 and deficiency of maternal 15q11-q13, by imprinting defects such that the maternal chromosome has the methylation and gene expression pattern of a paternal chromosome, by loss-of-function point mutations in UBE3A, and by unknown molecular abnormalities in a fraction of patients. The number of genes involved, the phenomenon of genomic imprinting, the diversity of molecular mechanisms bringing about genetic deficiency, and the tissue-specific aspect of gene expression makes the background information particularly complex, reviews are available (see first 3 references). There is extensive methylation of DNA on the maternal chromosome associated with silencing of the large domain of paternally expressed genes.

The paternal chromosome is for the most part unmethylated with most of the genes in the region being expressed. The Angelman gene (UBE3A) is expressed from both the paternal and maternal chromosome in somatic tissues and in many parts of the brain, while only the maternal gene is expressed in certain parts of brain, probably Purkinje cells and hippocampal neurons based on analogy to mouse data. In part II of this study, it is

hypothesized that increased methylation of DNA on the paternal chromosome might increase the expression of UBE3A and ameliorate the phenotype in Angelman Syndrome.

Table 1. Molecular findings in AS and PWS.			
Type	**Angelman**	**Type**	**Prader-Willi**
I	~4.0 Mb deletion (65-75%)	I	~4.0 Mb deletion (65-75%)
II	Maternal uniparental disomy (UPD) (3-5%)	II	Paternal UPD (20-30%)
III	Imprinting defect (6-8%)	III	Imprinting defect (1-5%)
IV	Point mutation UBE3A (4-6%)	IV	No point mutation known
V	No defect identified (10-14%)	V	

3. Purpose of the Protocol (Part II):

This study is based on the hypotheses 1) that dietary manipulation may increase DNA methylation; 2) that increased methylation of the paternal chromosome in Angelman patients may increase expression of the Angelman gene; and 3) that dietary intervention with folic acid, betaine, vitamin B12, methionine and zinc would represent little risk, but some chance for benefit. The purpose of Part II of this study is to determine if there would be 1) any change in methylation of DNA from peripheral leukocytes and 2) any clinical benefit to patients with Angelman Syndrome if placed on a dietary regimen designed to promote methylation of DNA. There is some evidence from studies in mice and humans that methylation of DNA can be increased by a dietary regiment of this type. The dietary regimen is felt to be quite benign and safe. There will be some advantages in enrolling AS patients in a therapeutic trial as a baseline for more aggressive therapies that may be justified after further studies in mouse models. The purpose of the current trial is to evaluate the effect of a regimen that includes betaine and folic acid in high dose and assurance of adequate intake of vitamin B12, zinc, and methionine on the clinical symptoms of Angelman Syndrome. The biochemical response will be measured including levels of homocysteine, methionine, and S-adenosylmethionine. The molecular response will be measured by methylation analysis of DNA.

4. Description of Study

4.a. Study Design

Studies will be completed during four admissions to the Texas Children's Hospital GCRC at baseline, 3 months, 6 months, and 12 months. Families will be contacted monthly by phone to review response. We will study the effects of combination therapy of folate and betaine in two groups of Angelman Syndrome patients: (1) 40 AS patients, ages less than 3 years, and (2) 40 older AS patients, ages 3 years or greater. The drug trial will be a blinded placebo-controlled study. Patients will be randomized to folate-betaine combination or placebo. Patients will complete a 12-month trial of folate-betaine or placebo. The dose of folate will be 15 mg p.o. and the dose of betaine will be 6 grams if weight < 30 kg, or folate 15 mg. p.o. and 12 grams betaine p.o. if weight > 30 kg.

Stadiometry, anthropometry, physical and neurological examinations, motor function assessment, and developmental assessment, will be completed at baseline, 3 months, 6 months, and 12 months. General behavior and EEG characteristics including background and epileptiform activity will be measured for one hour using standard video-EEG-polygraphic monitoring previously developed by Dr. Glaze for evaluation of Rett Syndrome, but now to be applied to AS patients. The monitoring sessions will be time synchronized and videotaped to allow for review of the polygraphic record for precise characterization of the EEG and quantification of seizure activity and general movements. The one-hour monitoring will be done at baseline, 6 months, and 12 months for each patient. Parent questionnaire will be completed at the end of each month of the 12-month study period by in-person or phone interview. Blood work will include CBC, BUN/creatinine, and anticonvulsant levels at baseline, 3 months, 6 months, and 12 months. Plasma levels of homocysteine, methionine, folic acid, betaine, dimethylglycine, S-adenosylmethionine, and S-adenosylhomocysteine will be done at baseline, 3, 6, and 12 months. DNA extraction for methylation studies will be performed on blood samples drawn at baseline, 6 months, and 12 months. Urine analysis will be done at baseline, 6 months, and 12 months.

Table: Study Design

Medication/Event	**Time 0 Base Line**	**3 Months**	**6 Months**	**12 Months**
CBC BUN Creatinine	**X**	**X**	**X**	**X**
Urine Analysis	**X**	**X**	**X**	**X**
Folate/ Betane Homocysteine Methionine Dimethylglycine S-ad-methionine S-ad-homocysteine	**X**	**X**	**X**	**X**
Polygraph	**X**	**X**	**X**	**X**
Stadiometry Anthropometry PE/Developmental Motor	**X**	**X**		**X**
Parent Question	**X**	**X**	**X**	**X**
DNA Methylation Studies	**X**	**X**	**X**	**X**

4.b. Drugs

Betaine: The usual dosage used in adult and pediatric patients with certain inborn errors of metabolism is 6 grams per day administered orally in divided doses of 3 grams b.i.d. Dosages of up to 20 grams per day have been used in some children to control homocysteine levels. We will initiate a dose of 6 grams per day for AS patients < 30 kg weight (given 2 grams t.i.d. p.o.), and 12 grams for AS patients > 30 kg weight (given 3 grams q.i.d. p.o.). A therapeutic threshold for treating homocystinuria has been suggested to be a blood level for betaine of 400-500 mM. We will measure betaine plasma levels at baseline, 3, 6 and 12 months.

Folate: 5 mg/day is the usual dosage reported in the literature to control homocysteine levels in homocystinuria, though dosages of 30-120 mg/day have been used in children ages 3-5 years. A plasma folic acid cut-off value, derived from its relationship with homocysteine, has been reported to be 10 nM. We will measure plasma folate levels at baseline, initiate a folate dose of 15 mg/day, and at 3, 6, and 12 months, measure plasma folic acid levels.

Adequacy of vitamin B12, methionine, and zinc intake: Each patient will have a dietary and nutritional history. The intake of vitamin B12, methionine, and zinc will be calculated. If the intake for these three nutrients is below twice the minimum daily requirement, the diet will be supplemented to achieve at least those levels of intake. Patients will receive betaine/folate or placebo.

4.c. Polygraphic Study

The percent time awake occupied by various behaviors and movements, and EEG characteristics will be measured for one hour using standard video-EEG-polygraphic monitoring. The monitored parameters will include 10 channels of electroencephalogram (EEG), 1 channel of electro-oculogram (EOG), 1 channel of electrocardiogram (ECG), 1 channel electromyelogram (EMG), and body movement via a triaxial accelerometer. Additional parameters will include monitoring of respiratory effort (abdominal and thoracic strain gauges), end tidal CO2 (nasal catheter) and oxygen saturation (pulse oximeter). The monitoring sessions will be time synchronized and videotaped to allow for review of the polygraphic record for precise characterization of the EEG and quantification of the breathing patterns and hand movements. This methodology has been previously developed by our Center for a previous drug trial and for characterization of Rett Syndrome.

4.d. Motor Scale Assessment

The motor-behavioral scale will provide objective criteria for evaluating the effects of folate and betaine in AS. Each subject will be rated in three areas: behavioral/social, orofacial/respiratory, and motor assessment/physical signs. The rating scale is as follows: 0 = absent or normal; 1 = mild, rare; 2 = moderate, occasional; 3 = marked, frequent; and 4 = very severe, constant. Each subject will receive a numerical score for data analysis. Assessments will be done at baseline, 3, 6 and 12 months.

4.e. Nutritional Status

The nutritional status of the patients will be determined using standard stadiometric (height, weight) and anthropometric (multiple skin fold thicknesses and body circumferences) techniques. These measurements will be converted to z-scores and degrees of malnutrition using Waterlow"s criteria and body fatness using Durnin's criteria. The sum of four skinfold thicknesses will be used to estimate lean body mass and body fat. The use of multiple skinfold thicknesses has shown a good correlation with other measures of body composition such as whole body potassium (40K) and deuterium (D2O) isotope techniques.

4.f. Parental Behavior Questionnaire

At the end of every month of each drug trial, the AS patient's family will be interviewed (in person or by phone) and a questionnaire will be completed by the program co-ordinator (to be recruited). The parents/caretaker will be asked to indicate if the AS patient has experienced worsening, no change, or improvement (0-25%, 25-50%, 50-75%, 75-100%) in each of the following:

(1) Sleep	(4) Feeding	(7) Alertness
(2) Hyperactivity	(5) Walking	(8) Communication
(3) Drooling	(6) Hand use	(9) Mood
		(10) Other

4.g. DNA and Other Laboratory Testing

Plasma levels of folate, betaine, and the amino acids will be done in the Baylor College of Medicine Biochemical Genetics Laboratory (Dr. William E. O'Brien).). DNA methylation studies for the PWS/AS region of chromosome 15 will be done in Dr. Beaudet's laboratory. The methodology for these is well established.

4.h. Dispensation of Drugs

Randomization, dispensing of Folate-Betaine and placebos will be done by the Texas Children's Hospital Pharmacy, Investigational Drug Section.

4.i. Review & Investigation

We propose to establish a small group of 2-3 investigator colleagues who will review results every three months. Candidates to participate in this group might be Dr. Huda Zoghbi, Dr. Marvin Fishman, and Dr. James Lupski. If any of this group are unable to participate, alternates will be identified. These investigators will see the unblinded data, and they will be responsible for deciding whether this study should be terminated because of convincing benefit to patients receiving treatment vs. placebo. This group of investigators will be asked to terminate the study if they believe that the evidence available is unequivocal and overwhelming, and that publication of the available data would provide adequate documentation for the scientific, medical, and family groups that the treatment is beneficial in Angelman Syndrome. In the absence of such convincing data, the study will continue.

5. Subject Population

A maximum of 80 subjects (40 per year over 2 years) with Angelman Syndrome will be studied. It is expected that approximately equal numbers of males and females will be enrolled. All patients will meet the diagnostic criteria for AS and must have laboratory confirmation of diagnosis for common deletion, UPD, imprinting defect, or UBE3A mutation. Patients with a clinical diagnosis of AS but no identifiable molecular genetic abnormality will not be eligible to enroll. Patients will be categorized as (1) "infant" AS if <3 years of age or (2) "older" AS if > 3 years of age. For each group, 40 subjects will be enrolled. All AS patients will be clinically stable; any subject with acute deterioration or physical illness will be provided appropriate medical care and excluded from the study. The ages of the patients to be studied will likely range from birth-21 years of age. All ethnic and racial groups will be eligible for study. The estimated racial and ethnic distribution of the Rett Center at Baylor/Texas Children's is 50% Caucasian, 8% Afro-American, 28% Hispanic, 2% Asian, 0% American Indian, and 12% unknown or mixed. We anticipate that the racial/ethnic distribution of the present proposal for AS patients will be similar.

6. Statistical Design & Analysis

Repeated measures ANOVA will be used to test for differences between groups with respect to change across time from baseline through 3, 6 and 12 months. Multiple comparison

procedures as outlined in Milliken and Johnson (1986) will be applied as indicated in the analysis of each outcome variable. The main outcome variables will be development of any new word usage, any other developmental progress, parental blinded reports of items in 4.f. above, physician blinded reports, frequency and type of seizures, change in EEG on blinded reports, and formal blinded developmental assessments. Other outcome variables include folate and betaine, methionine, S-adenosylmethionine, and homocysteine plasma levels, and the sum scores of behaviors. Nutritional factors (age, height, weight, BMI, etc.) will also be used as outcome variables. A baseline comparison of variables such as age, height, weight and BMI will be used to identify potential confounding variables which will be included as covariates as indicated. Forty subjects will be randomly assigned to treatment and placebo groups (total 80) in a double blind fashion. The randomization will be stratified according to age with equal numbers (20 per cell) being assigned for < 3 and > 3 year age groups. This sample is sufficient to detect a 20% difference between treatment and placebo groups. It assumes a standard deviation (cv) of approximately 30% in the main outcome variables, a type I error of 0.05 and power of at least 0.80. The equation used for sample size calculation is n = [2(Za_+_Zb)2SD2]/ D2, where Z is the standard normal value associated with type I error (a = 0.05) and type II error (b = 0.20) and D is the difference to be detected.

7. Potential Risks/Discomforts.

This study presents minimal risk to the child. Some discomfort will be experienced in the venipuncture. A total of 20cc (4 teaspoons) of blood will be drawn at baseline, 3 6, and 12 months. The total over one year will be 80cc. The amount of blood to be withdrawn is minimal in that the patients will be at least 1 year of age and sampling occurs over a 12-month period. Some discomfort may be experienced during placement of the EEG electrodes and sensors due to gentle but vigorous rubbing of the skin to prepare the site for electrode placements. Some people may have local skin reactions to the agent used to keep the electrodes in place. This is rare in our experience and has never represented a severe problem. No serious side effects have been reported for the dosages of betaine (6-20 grams/day) or folate (5-30 mg/day). Rarely patients receiving betaine have reported nausea, GI distress, diarrhea, or odor. Folic acid administered to rats in dosages of 100-400 mg/kg have resulted in alterations in renal tubular morphologic features and renal function (Calvert and Chadwick, 1994). These dosages far exceed the dosage of folate that we will administer to these AS patients. Because of the severity of neurologic impairment in AS, pregnancy and potential effects on the fetus are not issues except in the case of sexual abuse.

8. Benefits.

The potential benefits to be gained by the subject outweigh the potential risks of this study. Currently, there is no known treatment for Angelman Syndrome. Ultimately, this study could benefit the population of patients with AS by allowing us to develop a therapeutic intervention to minimize symptomatology, thus maximizing their developmental potential and enhancing quality of life.

9. Risk-benefit Ratio.

The risks to the patients are minimal and the potential benefits to them and their parents are significant. The risk benefit ratio is considered to be favorable.

10. Consent Procedures.

Informed, written consent will be obtained after the objectives and procedures have been explained thoroughly to each subject's parent by one of the investigators, (usually Dr. Glaze), or his designee. The assent of the subject will be waived because of the neurodevelopmental status of the subject.

11. Confidentially Procedures.

The data collected from each individual will be collated into computer files and backed up for security and retrievability. All files will be safeguarded using password protection to insure that only authorized users have access to these files.

12. Costs.

The subjects will not incur any costs beyond travel costs.

13. Payments.

The parents/caretakers of the subjects may receive reimbursement for parking, local travel, and meals for participation in this study.

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