Preliminary Results from double blind treatment trial using betaine and folic acid in children with Angelman Syndrome

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Angelman Syndrome

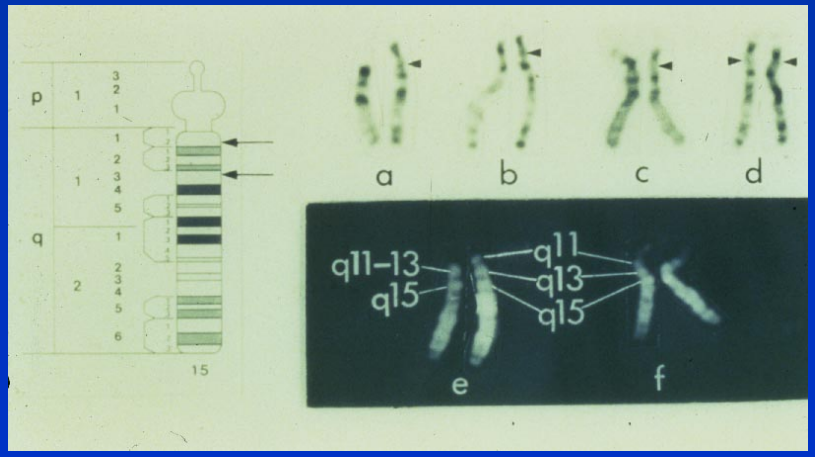
- Microcephaly with normal head size at birth, brachycephaly (flattening of the back of the head)
- Severe retardation, IQ often below 40
- Seizures, abnormal EEG's
- Midface retrusion, prognathism, wide spaced teeth, drooling, macrostomia
- No speech or limited speech
- Unprovoked bursts of laughter
- Ataxia, wide base gait with upheld arms, poor coordination, tremors

Angelman Syndrome

- Ataxia
- Midface retrusion, prognathism

Angelman Syndrome

- Microdeletions at 15q11-13 in 70-75% of the patients
- 5% have mutations in the *UBE3A* gene
- 2-3 % paternal UPD (maternal deficiency)
- 3-5 % mutations of deletions of imprinting center



Angelman Syndrome

- Mutations in *UBE3A*, gene encoding the E6AP-3A ubiquitin protein ligase
- The E6AP ubiquitin protein ligase 3A protein is involved in the ubiquitination pathway

DEFINITIONS

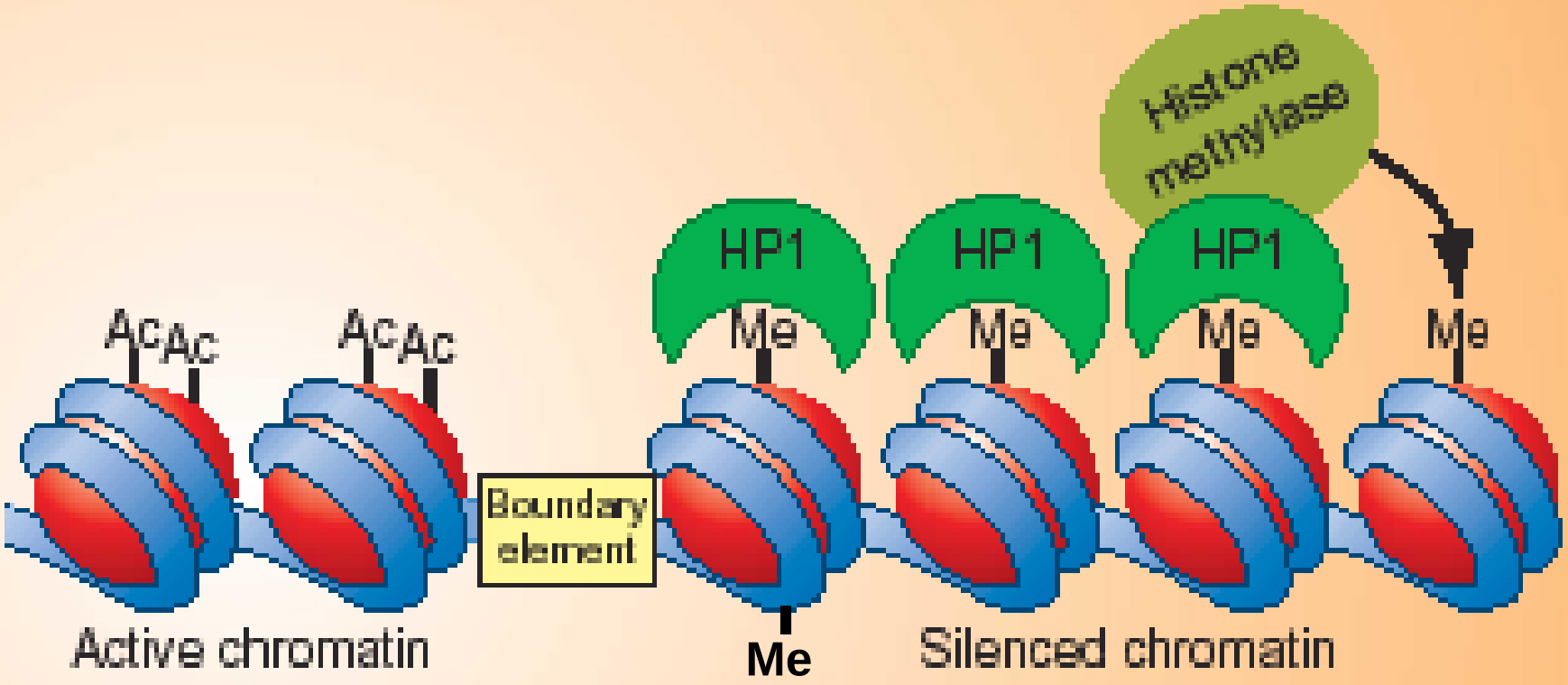
- DNA is the genetic material made up of 4 chemical bases that function like a 4 letter alphabet that makes up the “text” of the human genome.
- The “text” of the human genome is 3 billion letters in length and has 30-50 thousand genes of which AS gene is one.
- Each gene has a job to do.

DEFINITIONS

- A chromosome is a string of 1 to 4 thousand genes.
- A chromosome is made up of DNA wrapped in its chromatin proteins.
- Humans have 46 chromosomes; pairs numbered 1-22 plus two X chromosomes for females and X & Y for males.

DEFINITIONS

- Histones are proteins that bind to DNA and can influence its form and activity.
- Chromatin is DNA with histones and other proteins bound to the DNA.
- Methylation is a chemical modification of DNA or histones or other molecules.
- Acetylation is a chemical modification of histones or other proteins.



The blue ribbon is DNA. Red is histone protein. Me on red is histone methylation. Me on blue is DNA methylation.

From Pennsi, Science 293:1064, 2001

DEFINITIONS

- The *AS gene* is the gene in the chromosome 15q11-q13 region that is abnormal in most AS patients.
- Ubiquitin-protein ligase 3A or *UBE3A* is the technical name for the AS gene.
- E6-associated protein or E6-AP is the protein made by the AS gene; lack of this protein in certain brain cells causes AS.

EPIGENETICS

- The study of changes in gene function (e.g., how well the Angelman gene is working) that can be passed from cell to cell in the body or from parent to child that do not involve a change in DNA sequence, i.e., the letters making up the gene.

EPIGENETICS AS THE FONT OF DNA SEQUENCE (LETTERS)

CAGT

CAGT

CAGT

~~CAGT~~

CAGT

cagt

CAGT

CAGT

CAGT

CAGT

CAGT

CAGT

•CAGT

•CAGT

$C^{M}GATC^{M}GATC^{M}GAT$

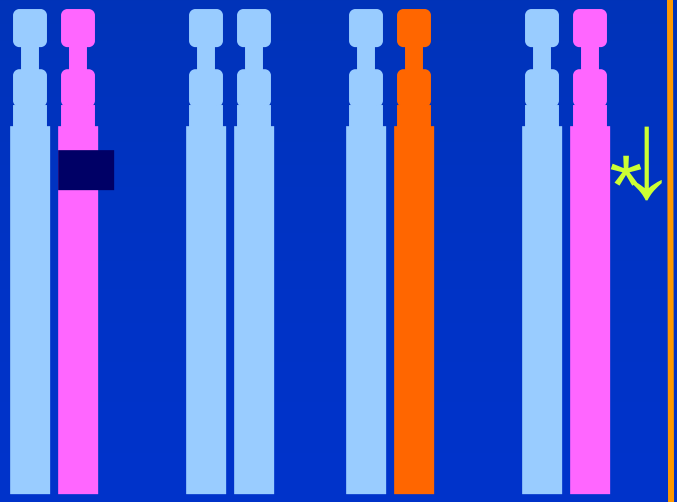
$C^{\cdot}GATC^{\cdot}GATC^{\cdot}GAT$

Epi-genetics

on top of genetics

Angelman

Deletion	UPD	Imprint Defect	*UBE3A* Null
Genetic	Epi-genetic	Mixed	Genetic



An epigenetic defect can give the same disease as a genetic defect

Many different types of defects causing one disease

GENOMIC IMPRINTING

- An epigenetic phenomenon in which the activity of a gene is reversibly modified depending on the sex of the parent that transmits it. This leads to unequal function from the copy of the gene that came from the mother compared to the copy that came from the father

EPIGENETICS GENERALLY

- Any change in the “font.”
- All genes involved
- Makes a brain cell different from a liver cell

GENOMIC IMPRINTING

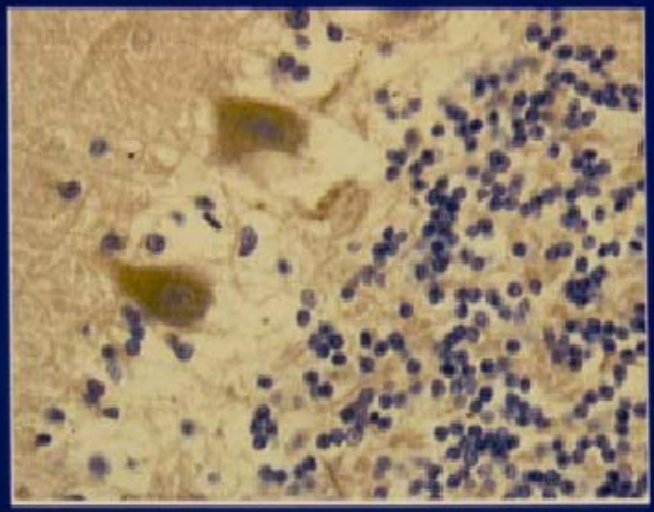
- Mom’s on & Dad’s off or vice versa
- Only a few genes involved
- Angelman gene

UBE3A AND E6-AP

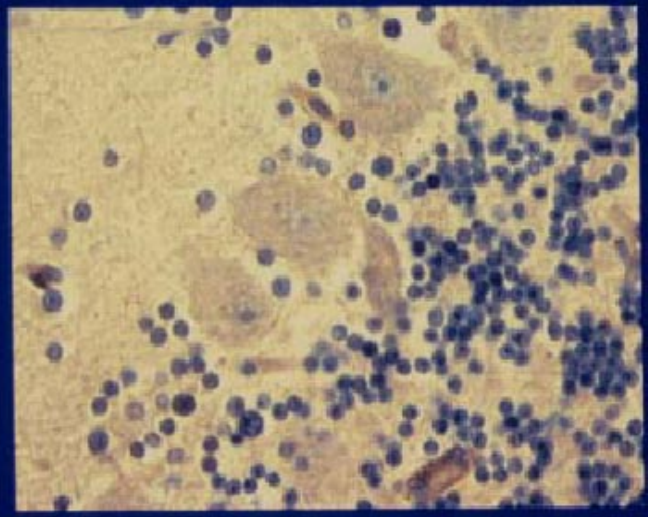
- The *UBE3A* gene makes a protein called E6AP
- The *UBE3A* gene is the Angelman gene
- The copy of the *UBE3A* gene inherited from the father is turned off in the brain, so the brain is very dependent on the copy from the mother

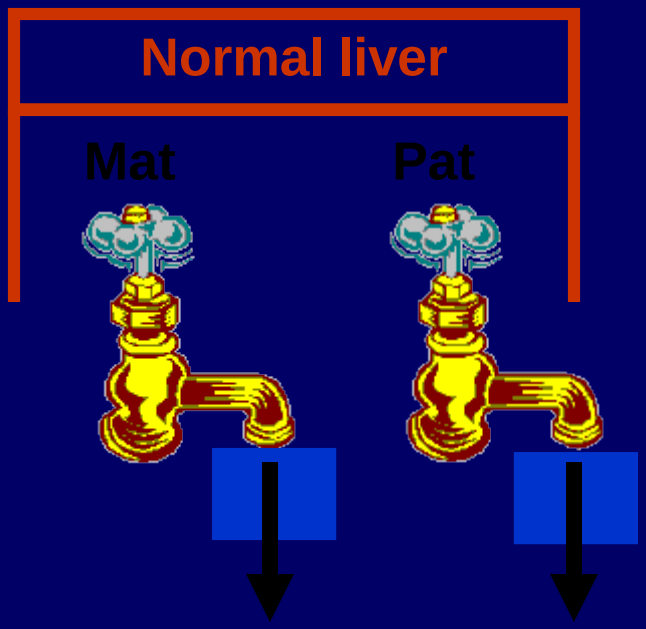
Immunohistochemistry for p53 in AS

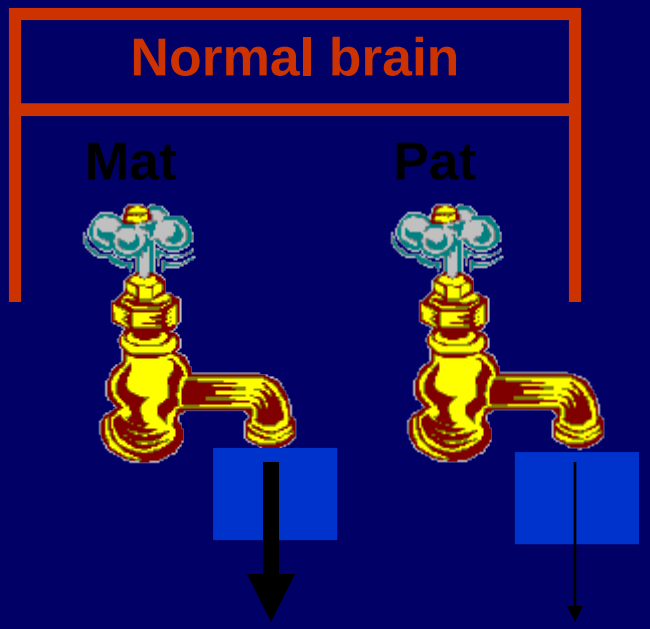
AS

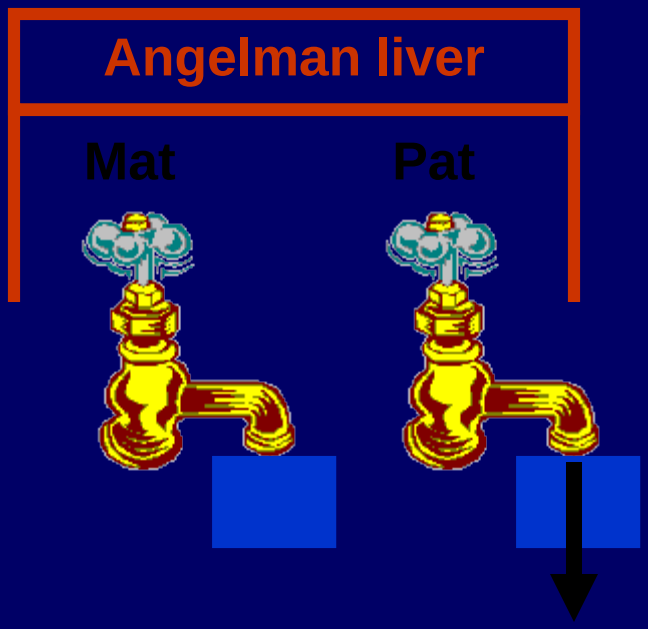


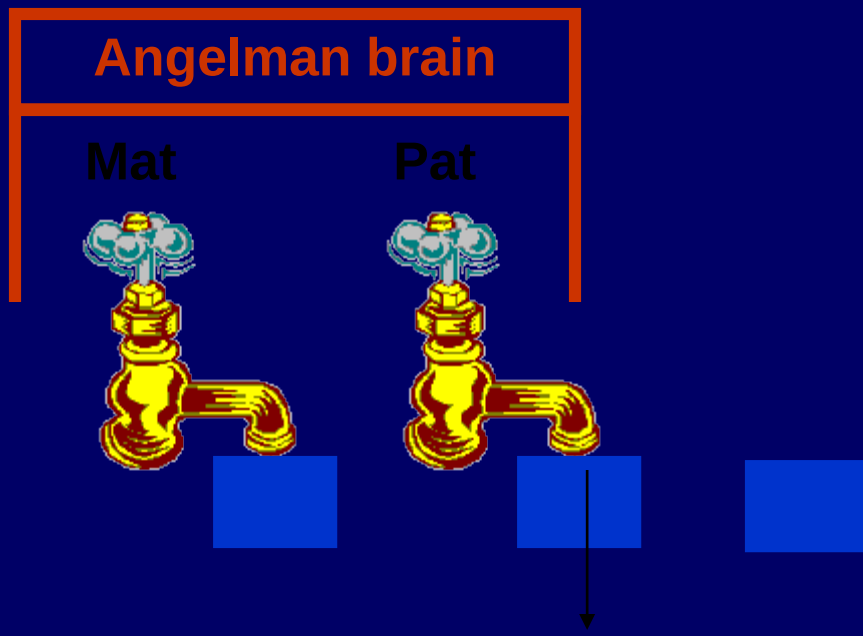
Normal




Normal liver
Mat
Pat


Normal brain
Mat
Pat


Angelman liver
Mat
Pat

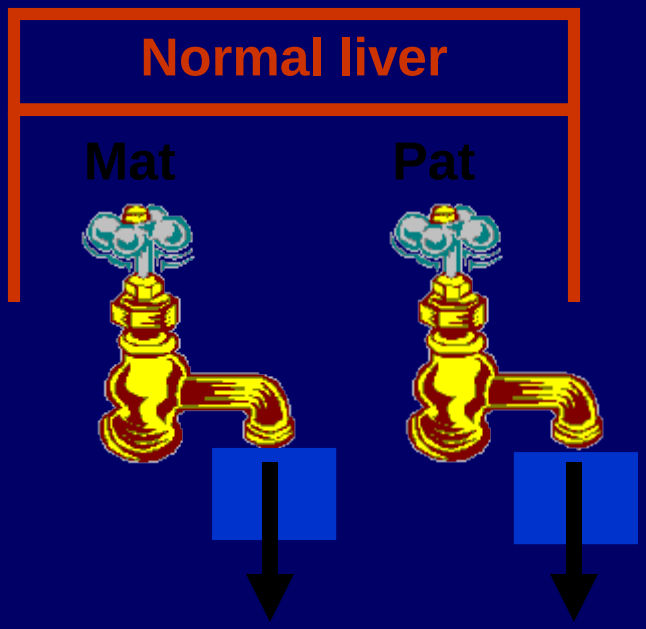

Angelman brain
Mat
Pat

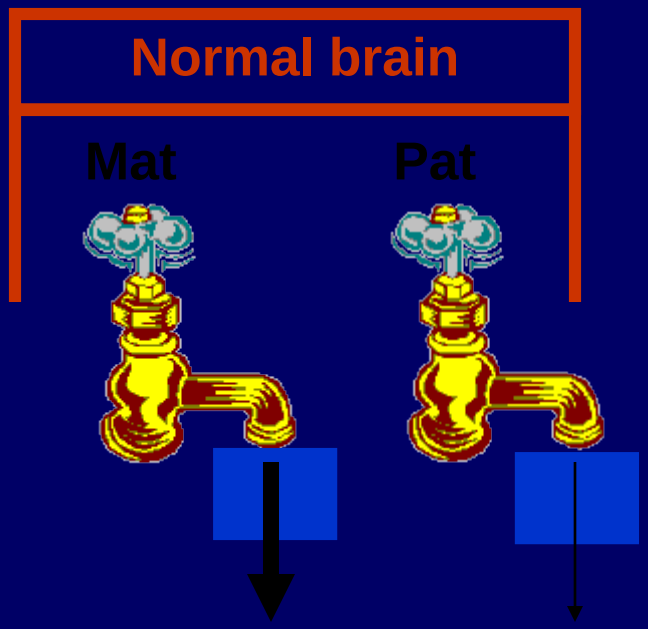
METHYLATION OF DNA USUALLY TURNS GENES OFF

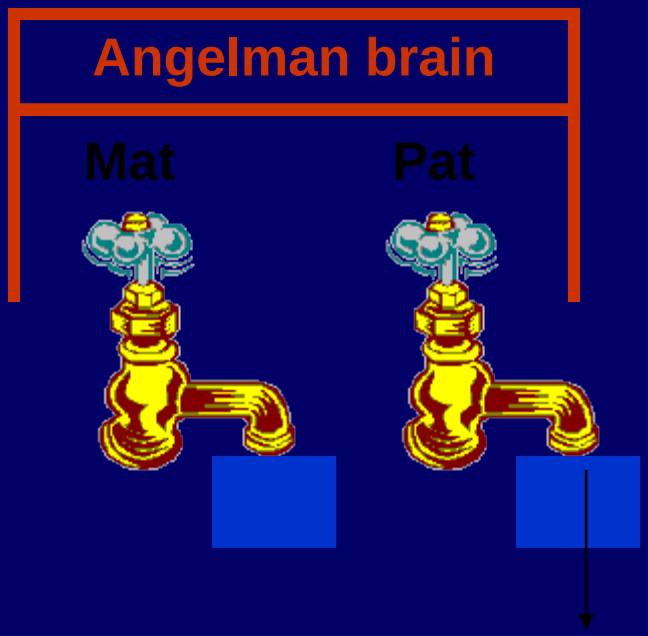
In general, methylation of DNA turns genes off and lack of methylation is associated with genes being turned on. However, there are exceptions, and for some genes, methylation may be involved in turning genes on. The AS gene may be in the exception category.

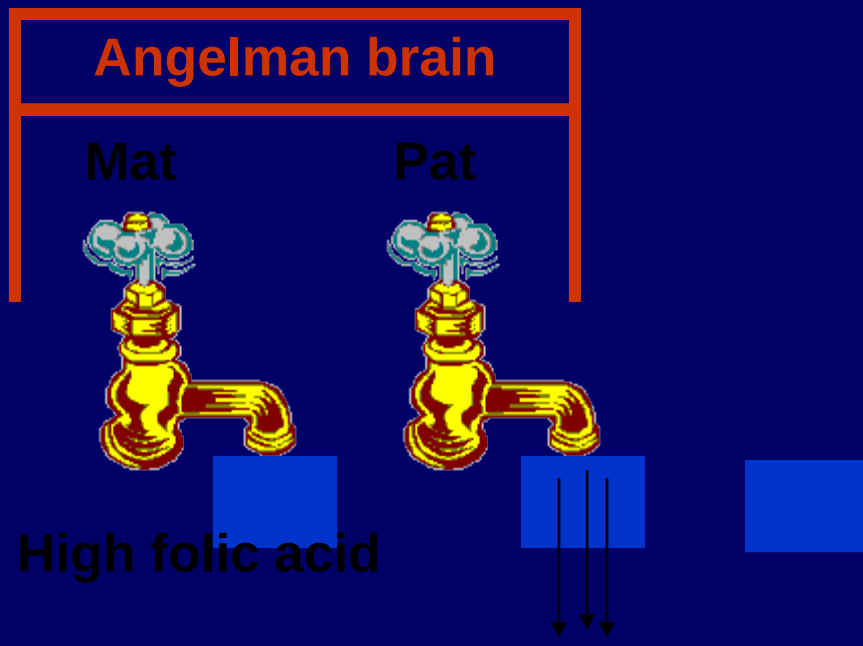
Protocol Rationale

- Diet and drugs can increase or decrease DNA methylation, but there is limited information about how effective this might be in humans
- Increasing DNA methylation can be attempted using relatively safe dietary changes


Normal liver
Mat
Pat


Normal brain
Mat
Pat


Angelman brain
Mat
Pat


Angelman brain
Mat
Pat
High folic acid

- **Methylenetetrahydrofolate Reductase Deficiency in a Patient With Phenotypic Findings of Angelman Syndrome**

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- *[2] R.C. Philips Research Unit, Gainesville, Florida*
- *[3] Division of Medical Genetics, Department of Medicine, McGill University, Montreal, Canada*

American Journal of Medical Genetics 77:198–200 (1998)

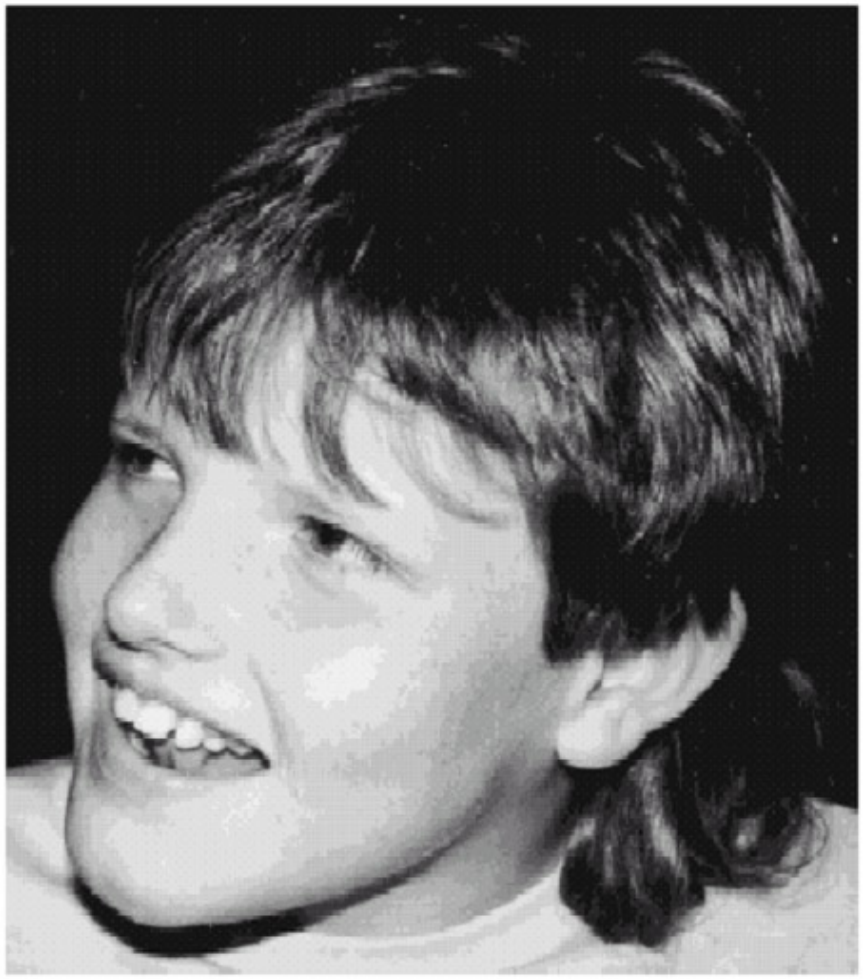


Fig. 1. The patient at 12 years of age.

- Low methionine
- + urine nitroprusside
- ↓ folic acid

TABLE I. Plasma Amino Acid Results (in μmol/L)*

Amino acid	Normal range	A	B	C
Cystine	(44–96)	21	11	26
Methionine	(7–43)	11	8	12
Homocystine (free)	(Undetectable)	23	41	3

*A = Prior to treatment; B = Treatment with folinic acid, B_6, and B_{12}; C = Treatment B with betaine added.

Hypothesis

- MTHFR defect (folic acid deficiency) causes decreased methylation of DNA on mother's chromosome and thereby causes it to be turned off when it should be turned on

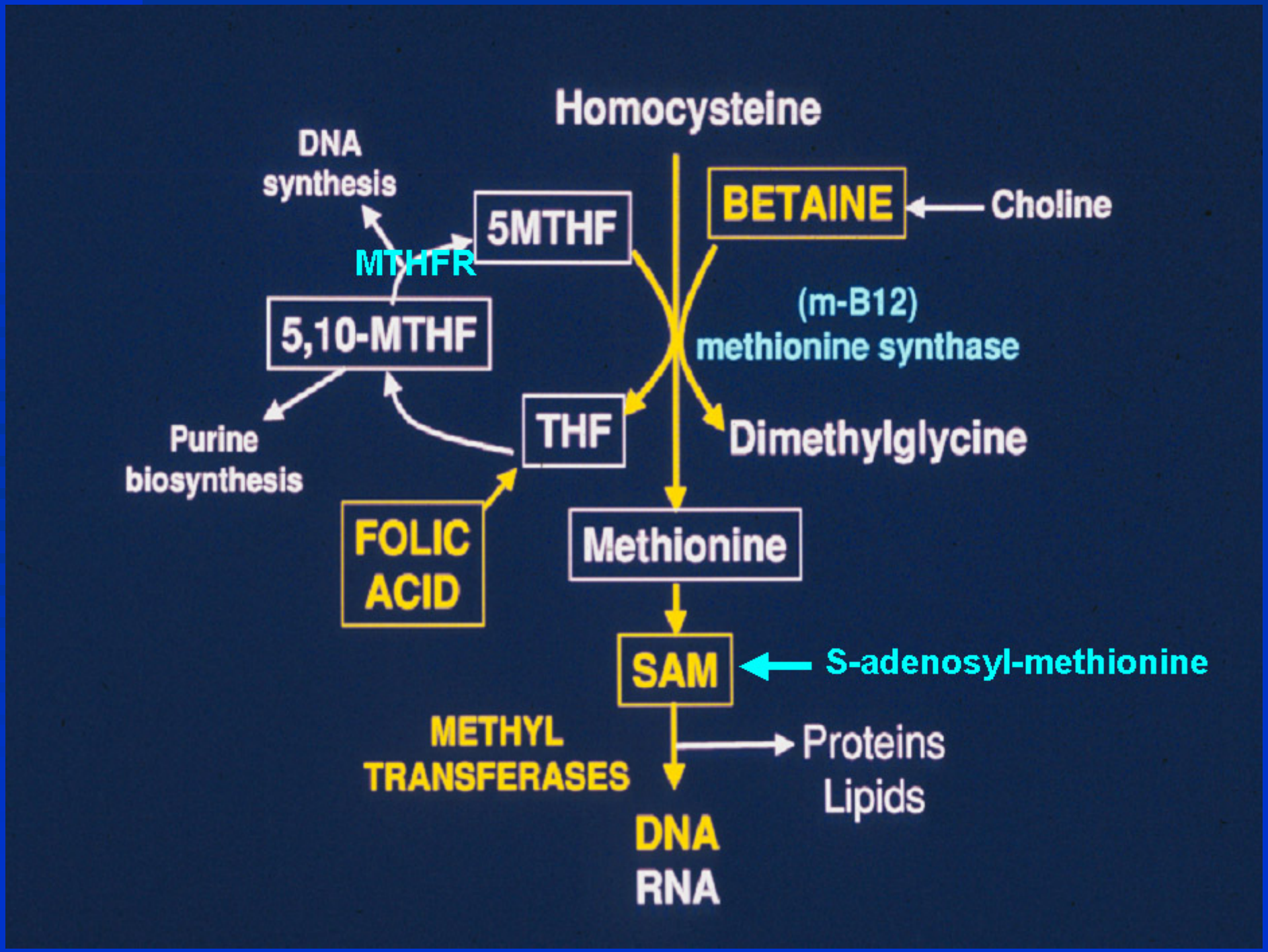
- Perhaps more DNA methylation will turn on the *UBE3A* gene, and less methylation will turn it off

Dietary Treatment?

- A diet enriched for methyl donors (high folate and high betaine) might increase DNA methylation
- Adequate intake of vitamin B_{12}, zinc, and methionine should be assured, because they might help the folate and betaine to work

Betaine and Folate Trial

- Since attempting to increase DNA methylation could be beneficial in AS, and since this can be attempted with a relatively safe diet, we proposed:
- Trial of a high folate, high betaine regime
- Double- blind protocol; half of patients on placebo


Homocysteine
DNA synthesis
5MTHF
BETAINE
Choline
MTHFR
5,10-MTHF
(m-B12) methionine synthase
Purine biosynthesis
THF
Dimethylglycine
FOLIC ACID
Methionine
SAM
S-adenosyl-methionine
METHYL TRANSFERASES
Proteins
Lipids
DNA
RNA

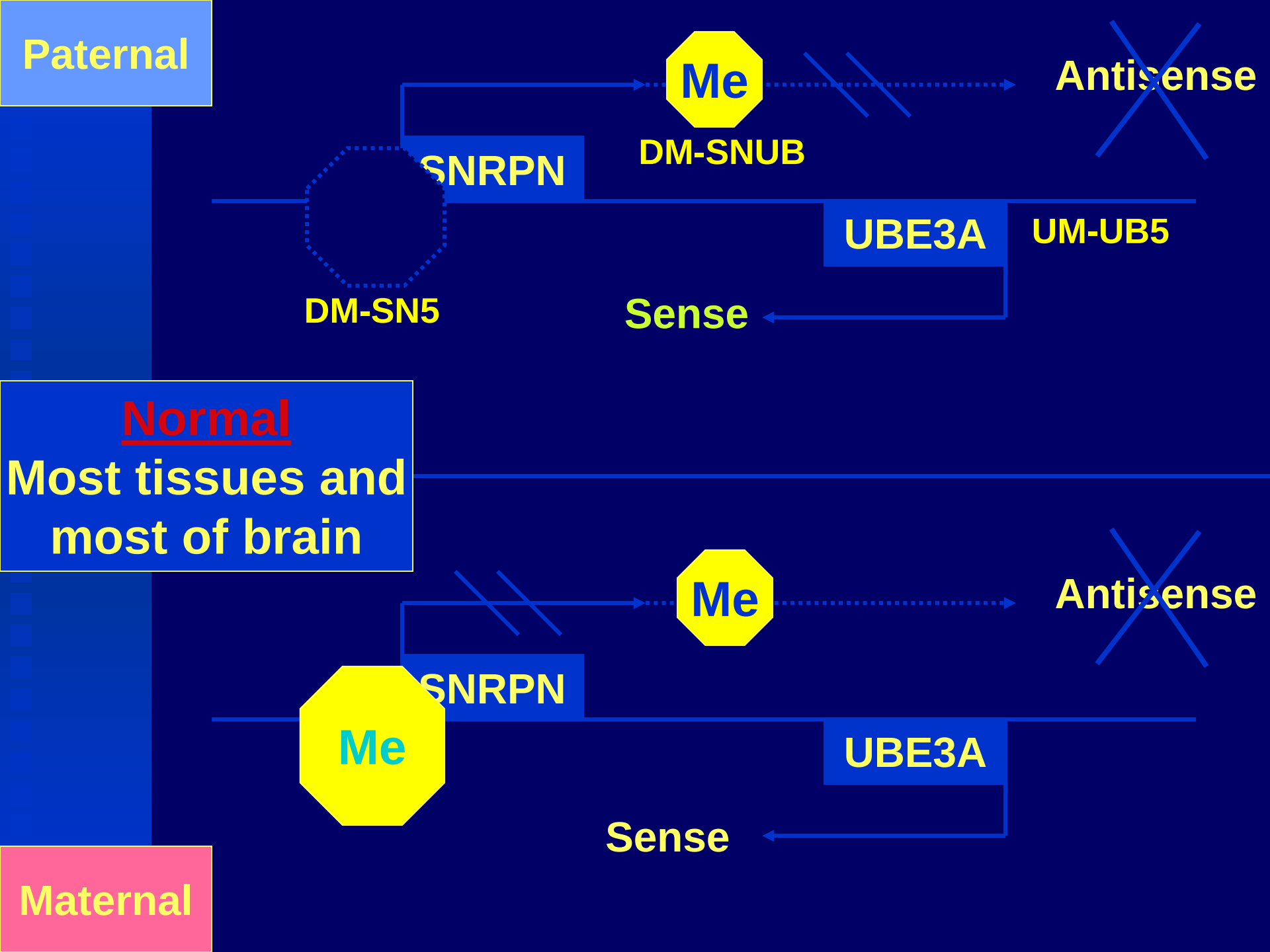
Hypothesis

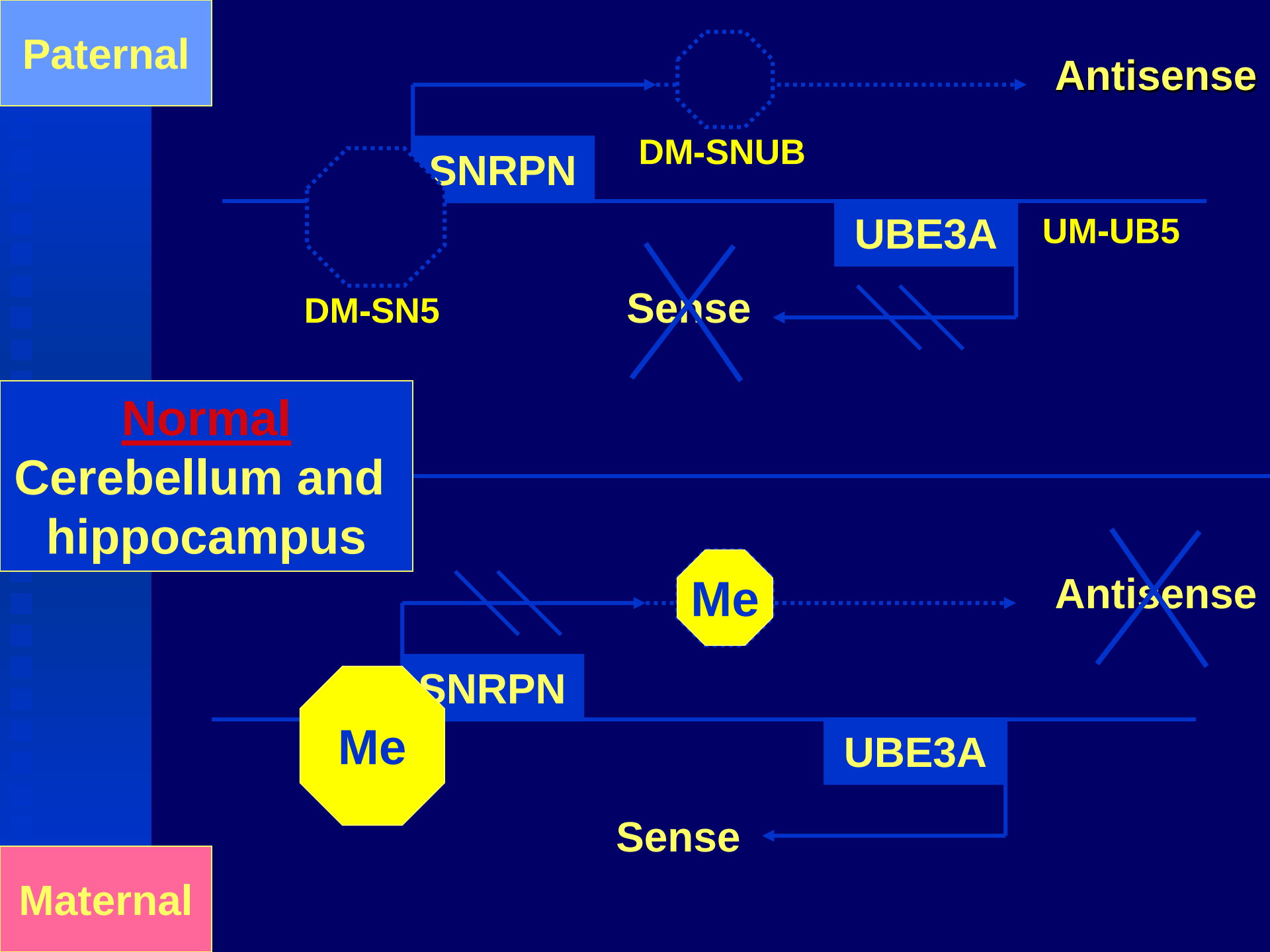
- Increased folic acid and betaine will increase methylation of DNA and make the father's copy more like a maternal copy
- Leaky expression of the father's copy may be able to reduce symptoms of AS

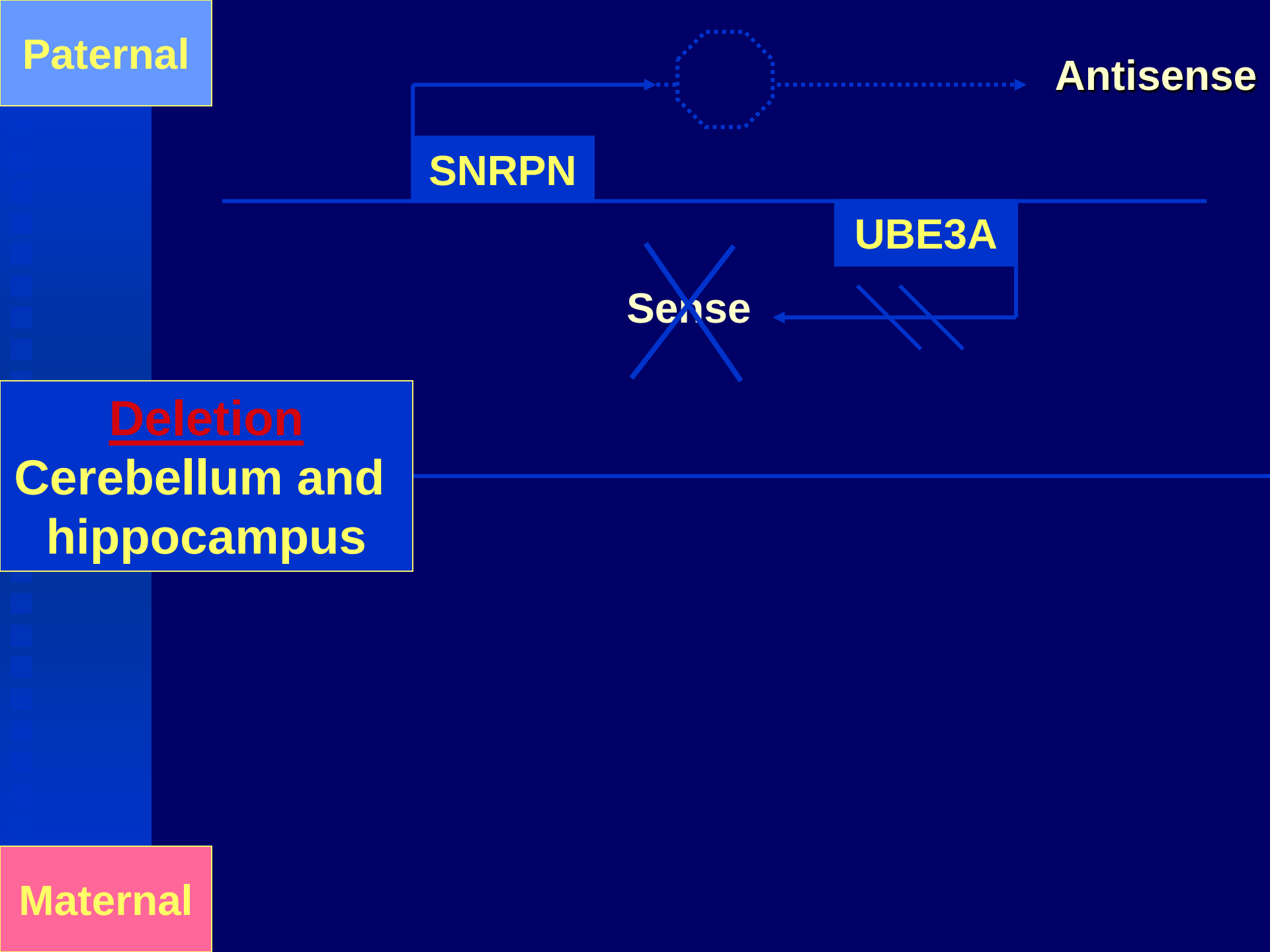
Hypothesis

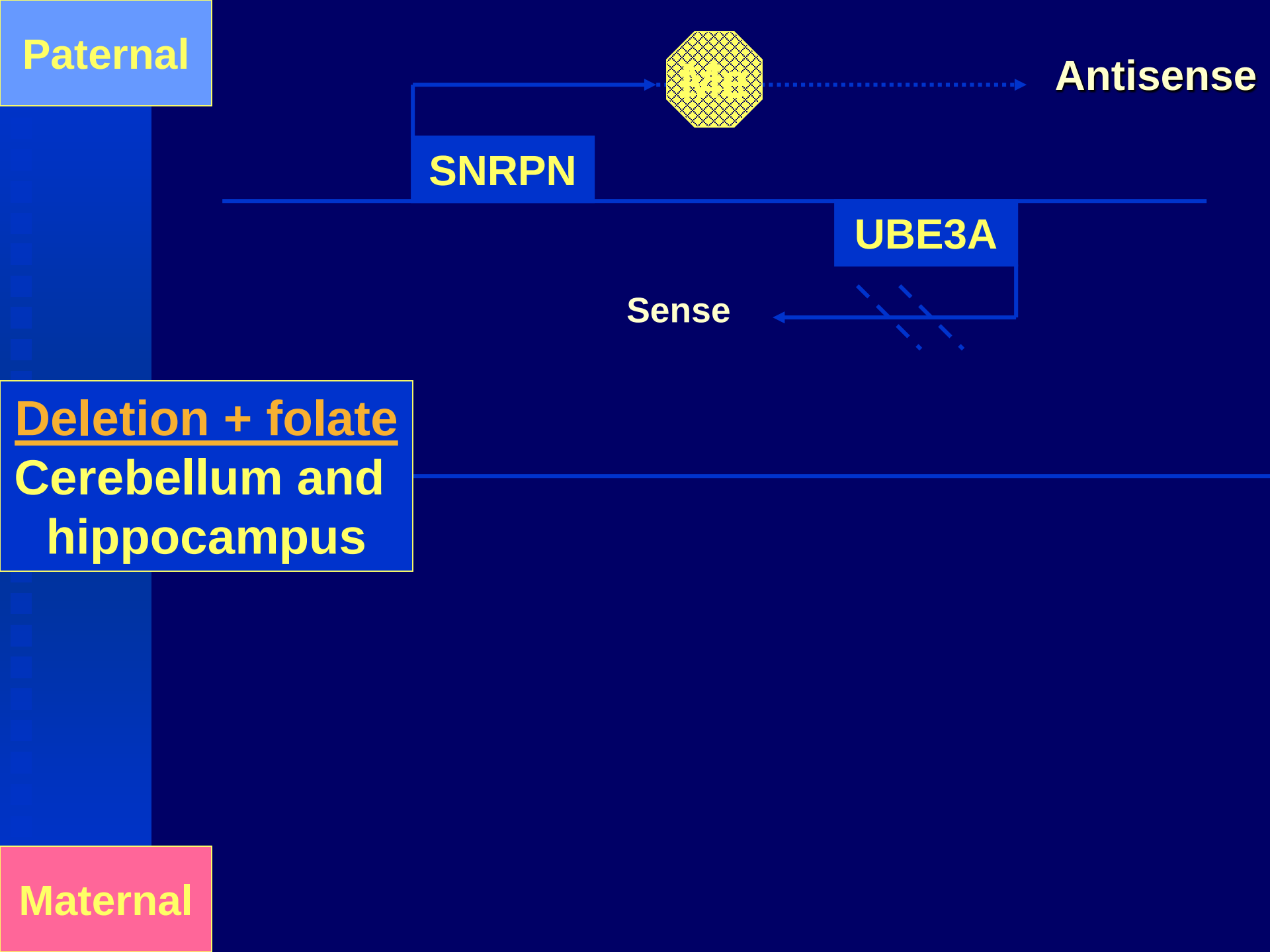
- In the key brain cells, the copy of the AS gene from the father may not be completely shut off, but may be “leaky”

- That is, it might make a tiny amount of the AS protein (E6-AP)


Paternal
Me
Antisense
SNRPN
DM-SNUB
UBE3A
UM-UB5
DM-SN5
Sense
Normal
Most tissues and most of brain
Me
Antisense
SNRPN
Me
UBE3A
Sense
Maternal


Paternal
Antisense
DM-SNUB
SNRPN
UBE3A
UM-UB5
DM-SN5
Sense
Normal
Cerebellum and hippocampus
Me
Antisense
SNRPN
Me
UBE3A
Sense
Maternal


Paternal
Antisense
SNRPN
UBE3A
Sense
Deletion
Cerebellum and hippocampus
Maternal


Paternal
Antisense
SNRPN
UBE3A
Sense
Deletion + folate
Cerebellum and hippocampus
Maternal

The Protocol

- Medications or placebo 3 to 4 times/day
- Three 2 day inpatient visits per year for one year (0, 6 and 12 months)
- Developmental assessment at 0, 6 and 12 months
- Continuous questionnaire monitoring (every 4-6 weeks)
- Blood draw and urine sample
- EEG (electroencephalogram) at 0, 6 and 12 months

Protocol and Inclusion Criteria

- Enroll 80 patients in 4 sites
- 20 in Houston: 10 <3yr and 10>3yr
- Laboratory confirmed deletion positive, paternal UPD, imprinting defect, or UBE3A mutation
- Medically stable

Protocol: Clinical Evaluation

- The clinical evaluation includes a comprehensive clinical exam, medical history and questionnaires
- Neurological exam: gait, station, abnormal movements, and progression of neurological signs over time are assessed
- Electroencephalogram (EEG)

Protocol: Clinical Evaluation

- Inpatient visits at 0, 6 and 12 months: developmental, clinical evaluation, EEG and laboratory workup
- Outpatient visit at 3 months for local patients for blood draw and questionnaire
- For out of town patients blood draw is arranged and questionnaire obtained over the phone

Protocol:
Developmental evaluation

- The Bayley Scales of Infant Development, Second Edition are used to provide:
  - Mental Developmental Index (MDI)
  - Psychomotor Developmental Index (PDI)

Protocol:
Developmental Evaluation

- Vineland Inventory (Vineland Adaptive Behavior Scales): Parental questionnaire to assess behavioral and emotional development
- Preschool language scale (PLS-3)

Laboratory Studies

Blood levels for:

- Betaine
- Homocysteine
- Methionine
- Dimethylgycine
- Folate in red blood cells and CBC
- DNA methylation studies
- Urine analysis
    - MRS

Protocol Medications

- Betaine:
- 6 grams for children less than 30 kg: 2 grams 3X a day
- 12 grams for children greater than 30 kg: 3 grams 4X a day
- Folic acid:
- 15 mg per day (7.5 mg twice a day)

Other Medications and Intake

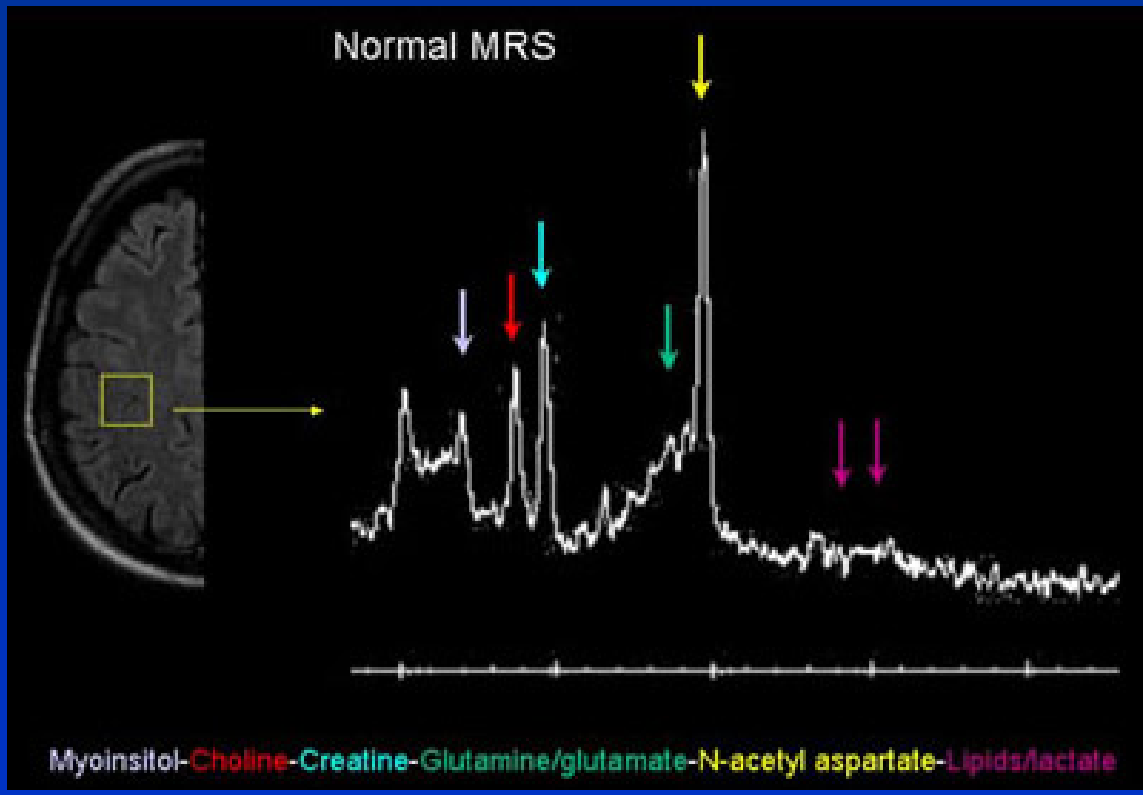
- Pediatric multivitamins (source for vitamin B12, folic acid and zinc).
- Nutritional evaluation:

  3 day dietary history to evaluate intake of folic acid, methionine, B12 and Zinc

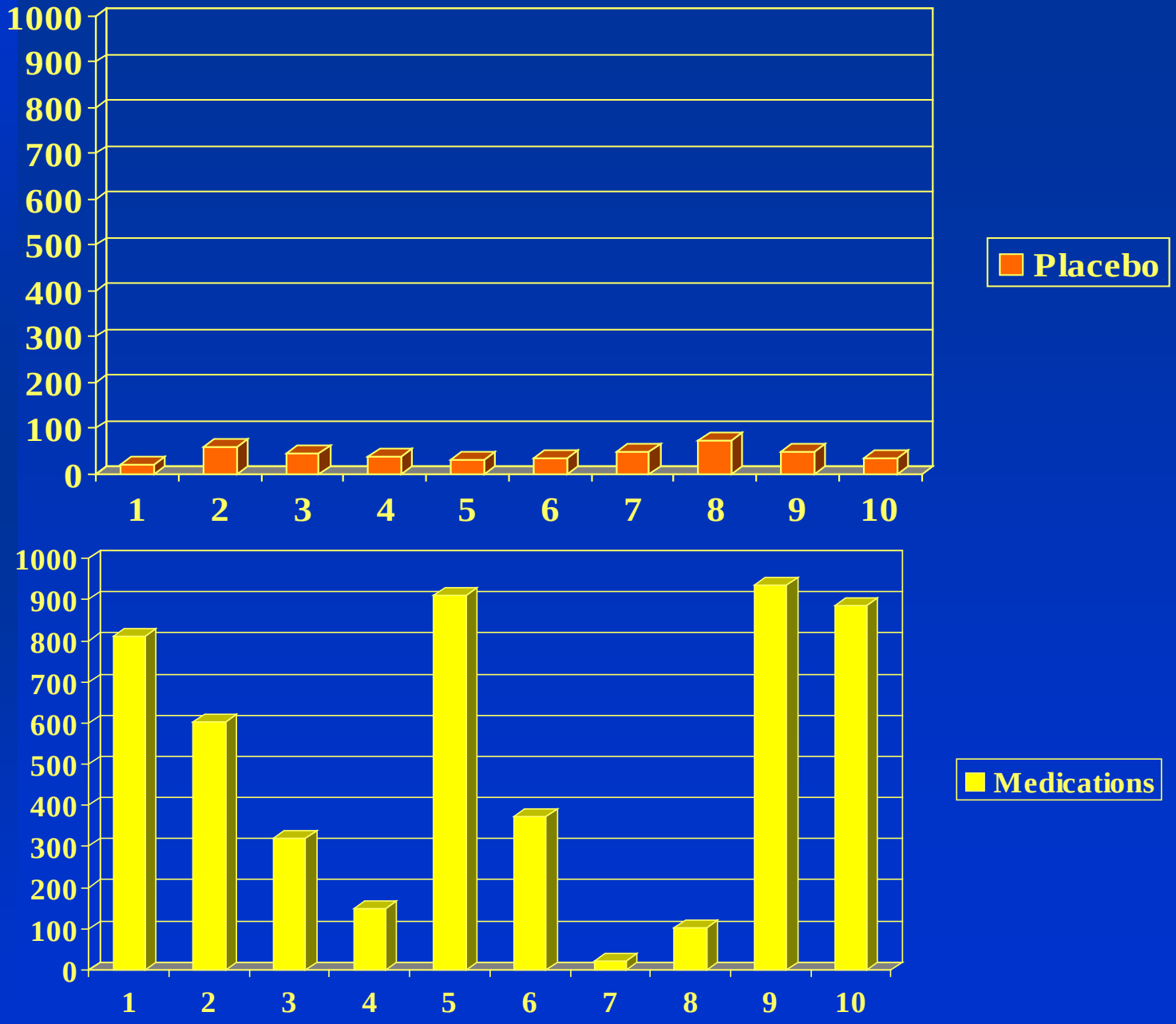
Preliminary Results

MRS (Magnetic Resonance Spetcroscopy)

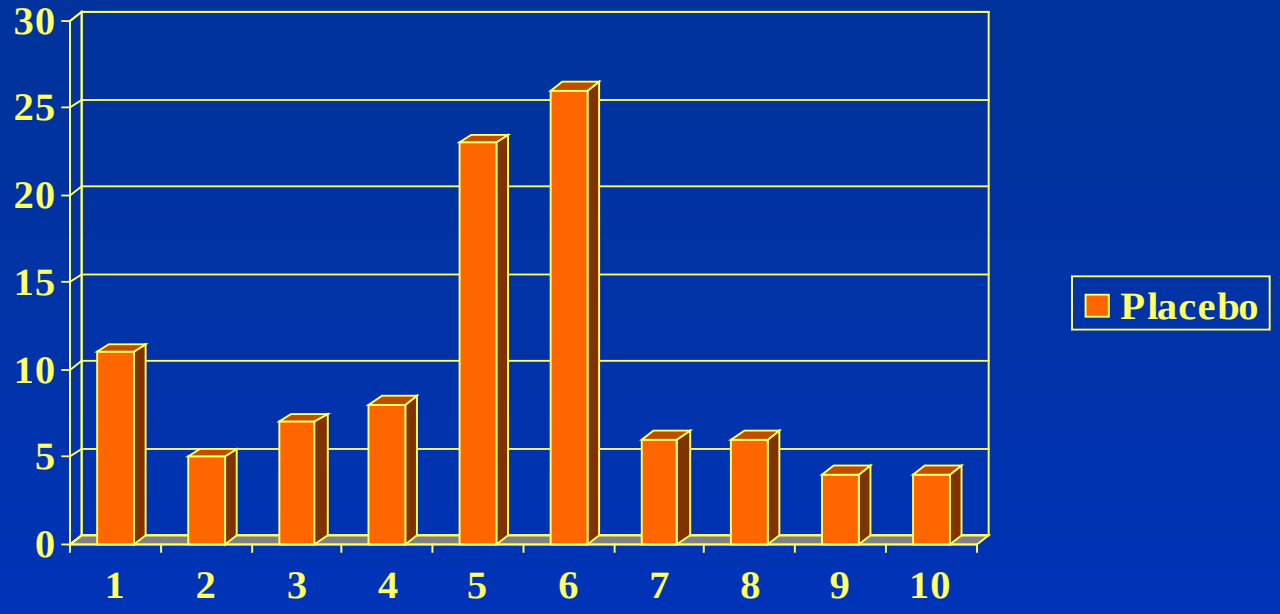
- Six children had MRS studies
- 3 on placebo: all have a myoinositol peak and one had a creatine peak
- 3 on medications: 2 had a myoinositol peak, 1 of them had in addition a creatine peak, 1 child had a normal study

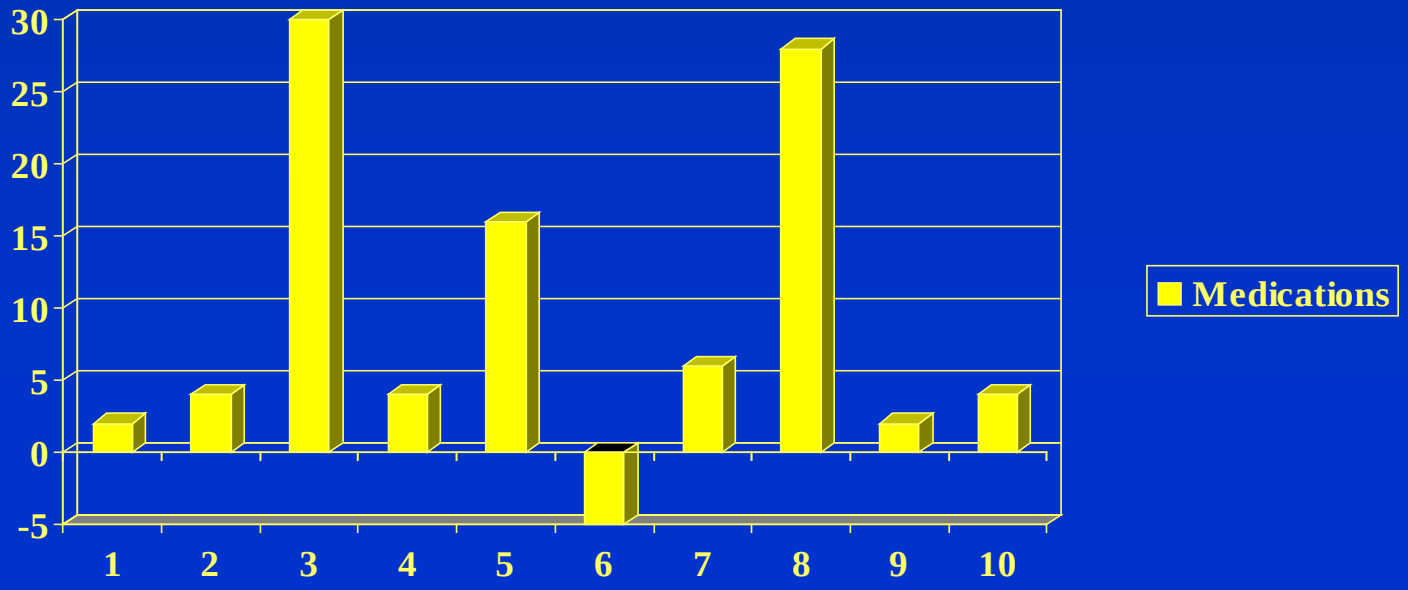


Betaine Levels

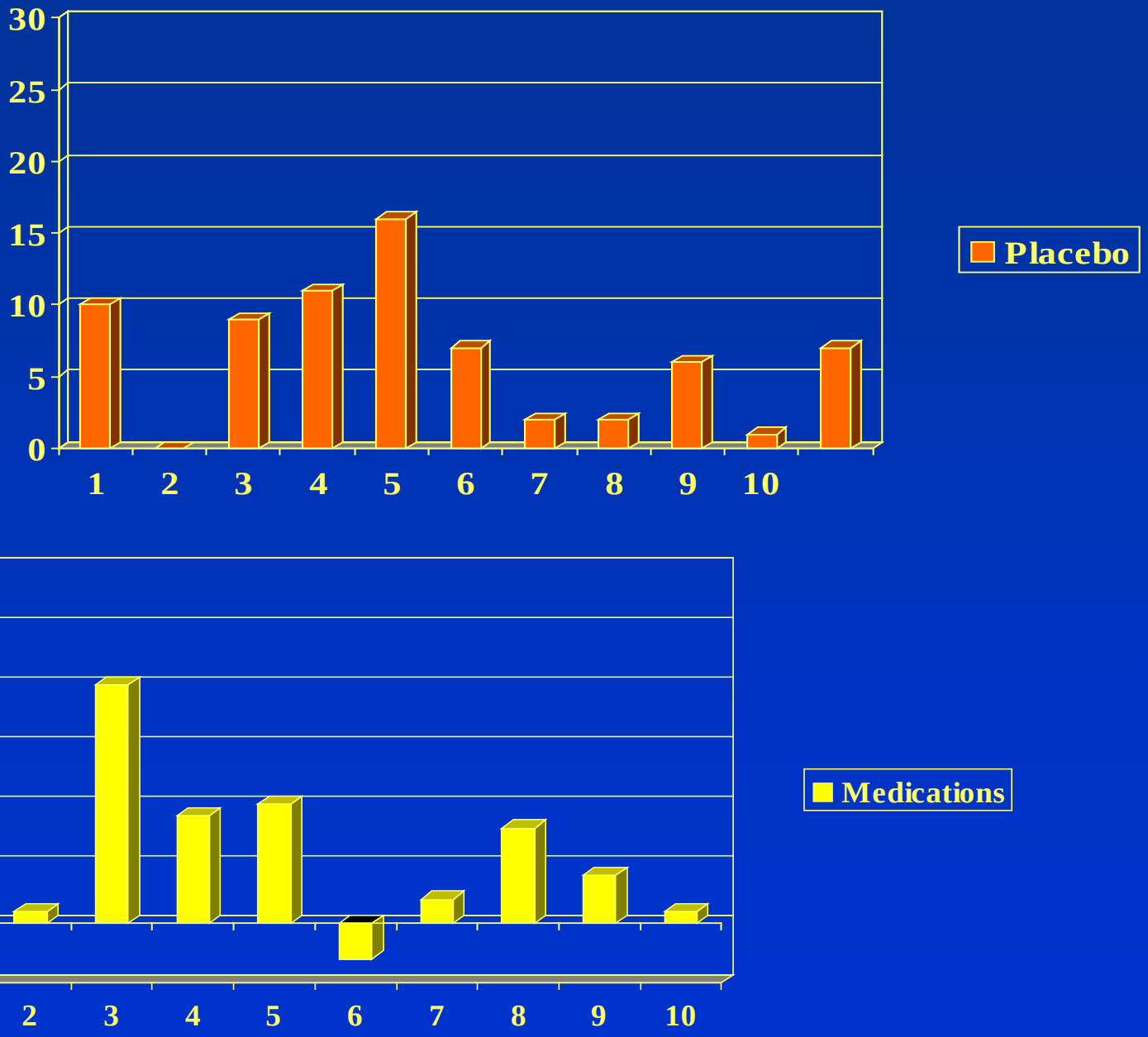


Bayley Mental Scores - MDI

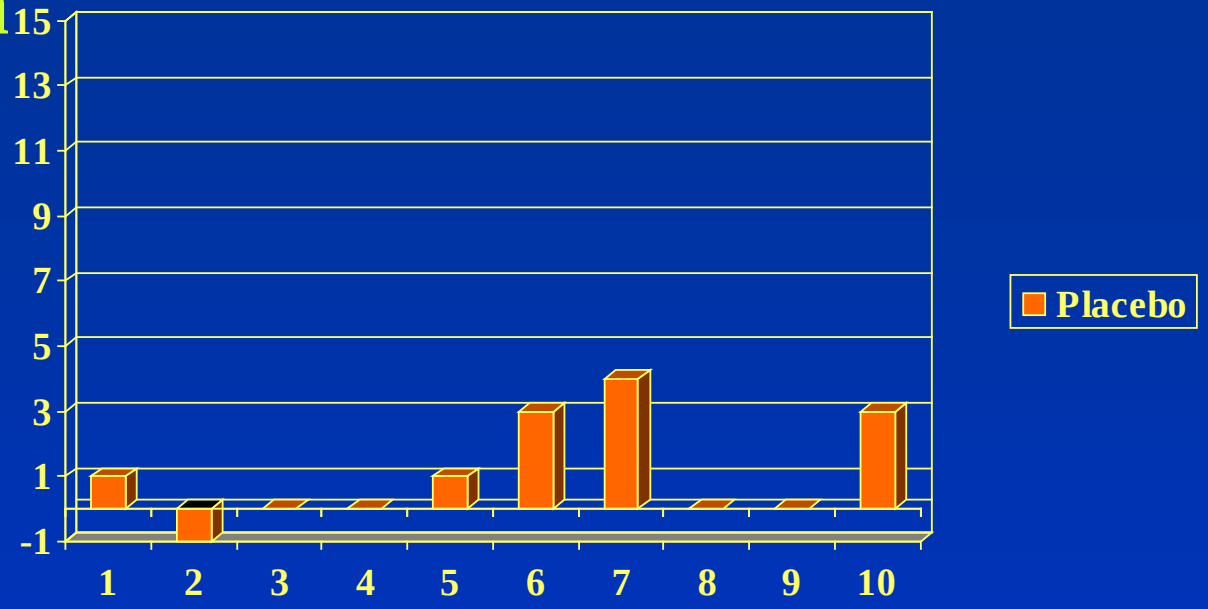


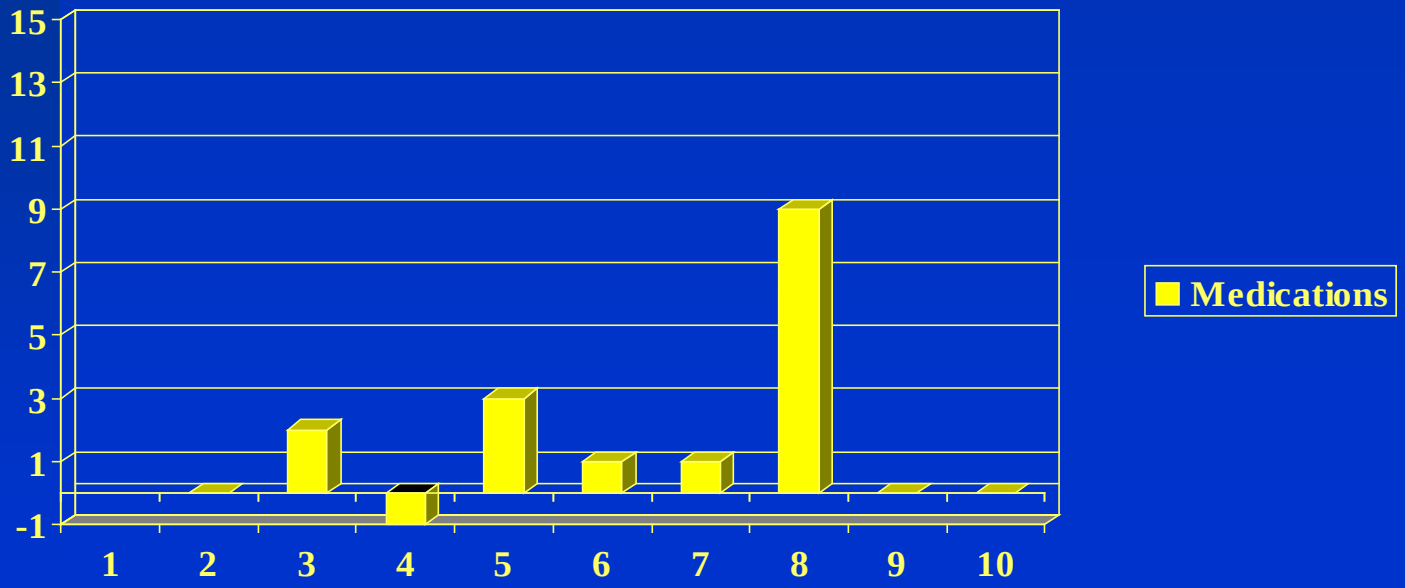


Bayley Mental Scores – PDI (motor)

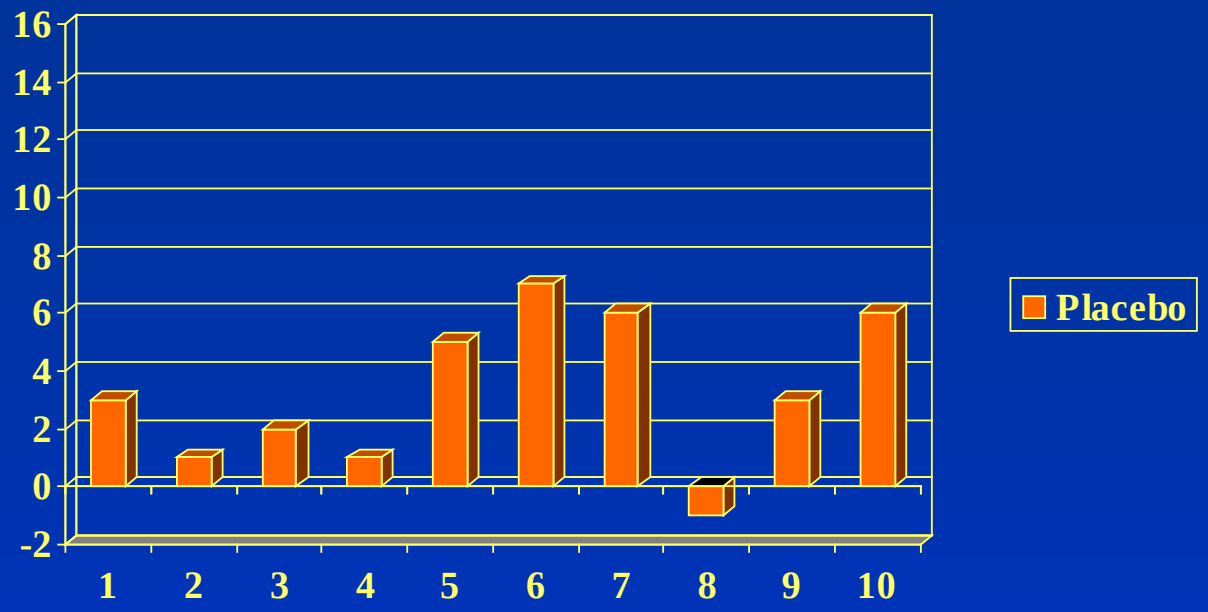


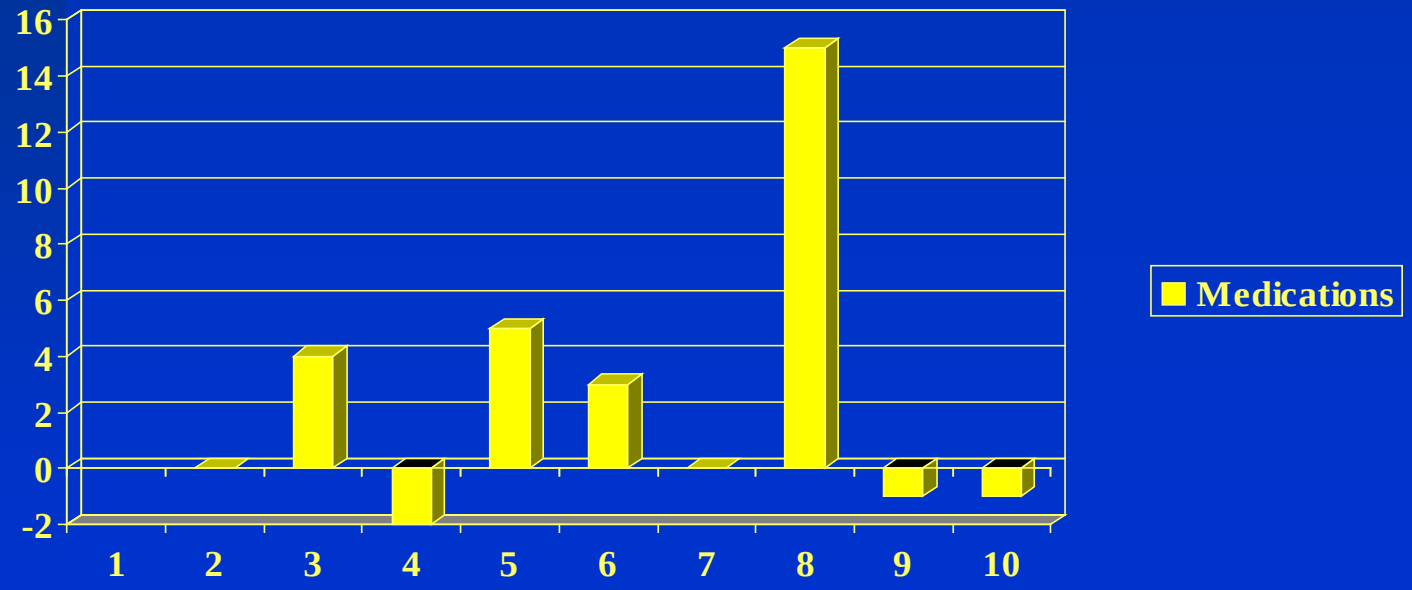
Pre-school language scale – Expressive communication



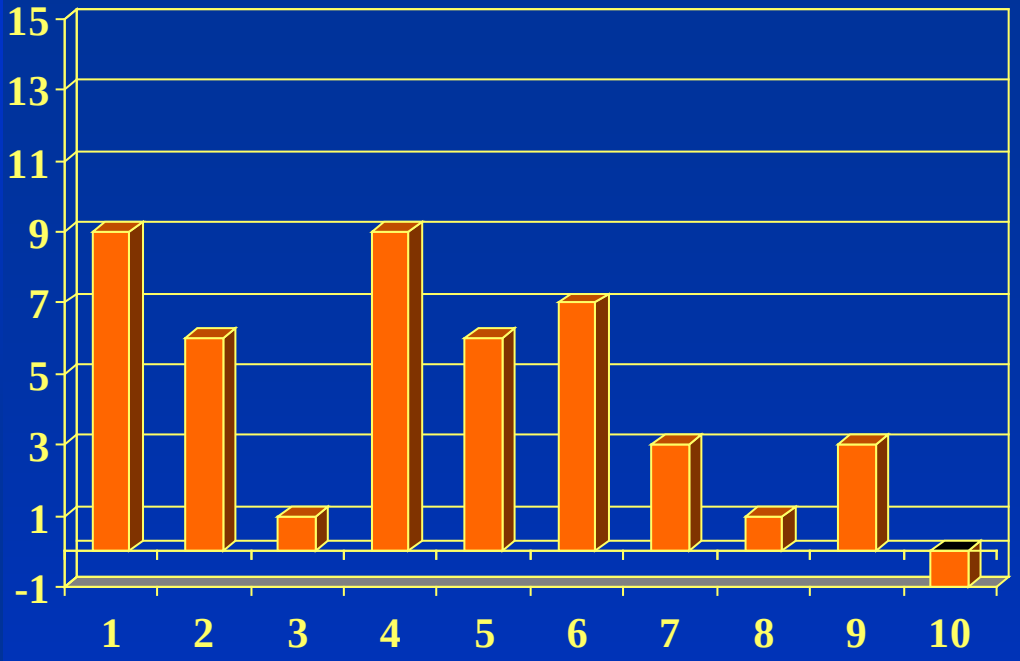


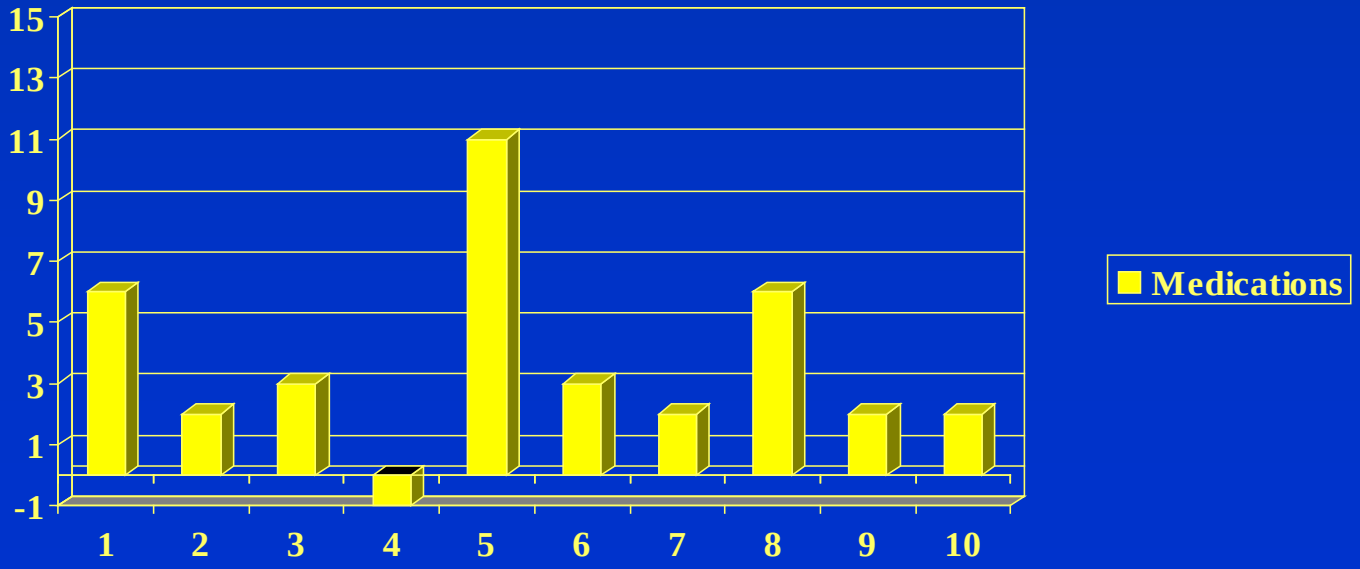
Pre-school language scale – Total language score



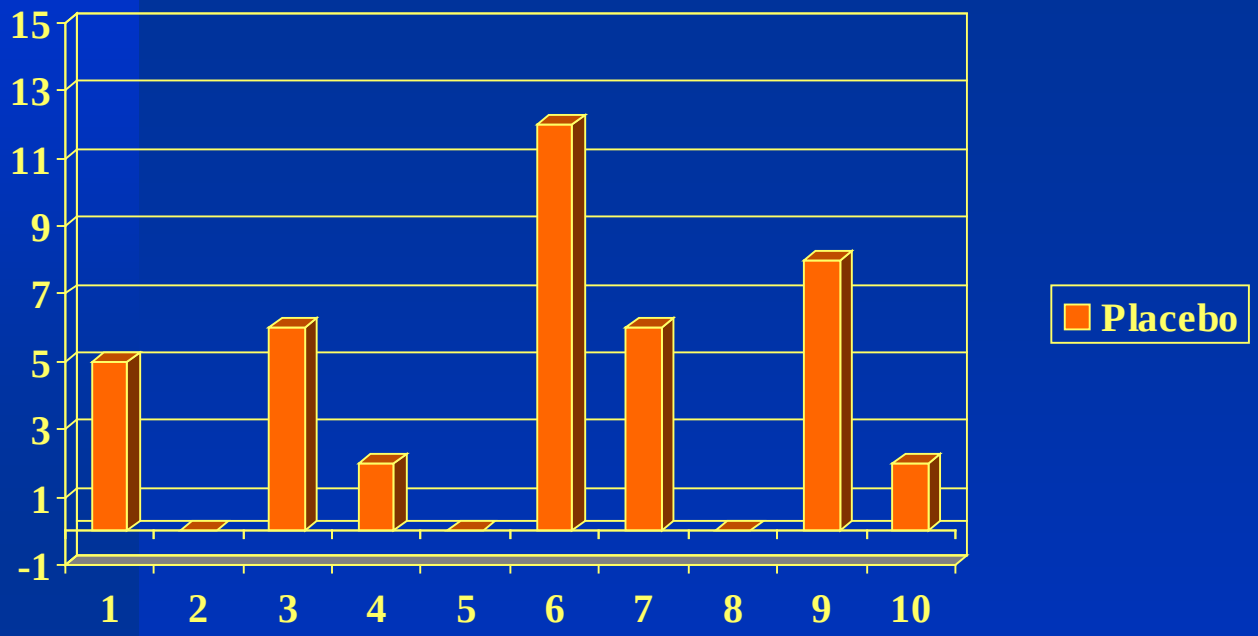


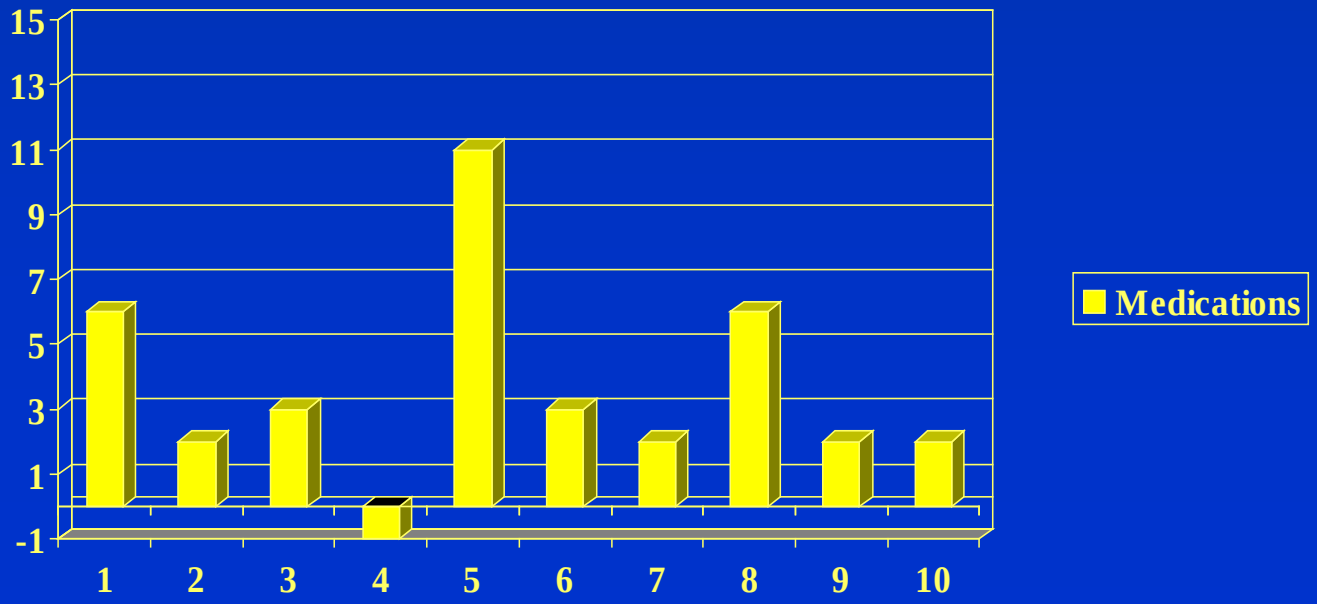
Vineland Raw Scores: Communication



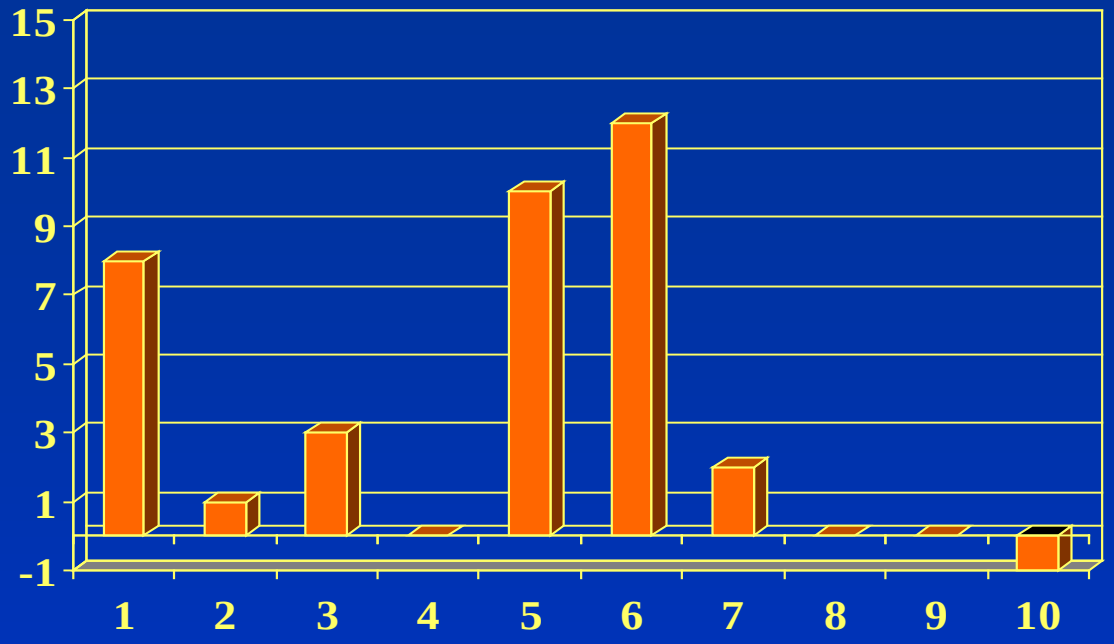


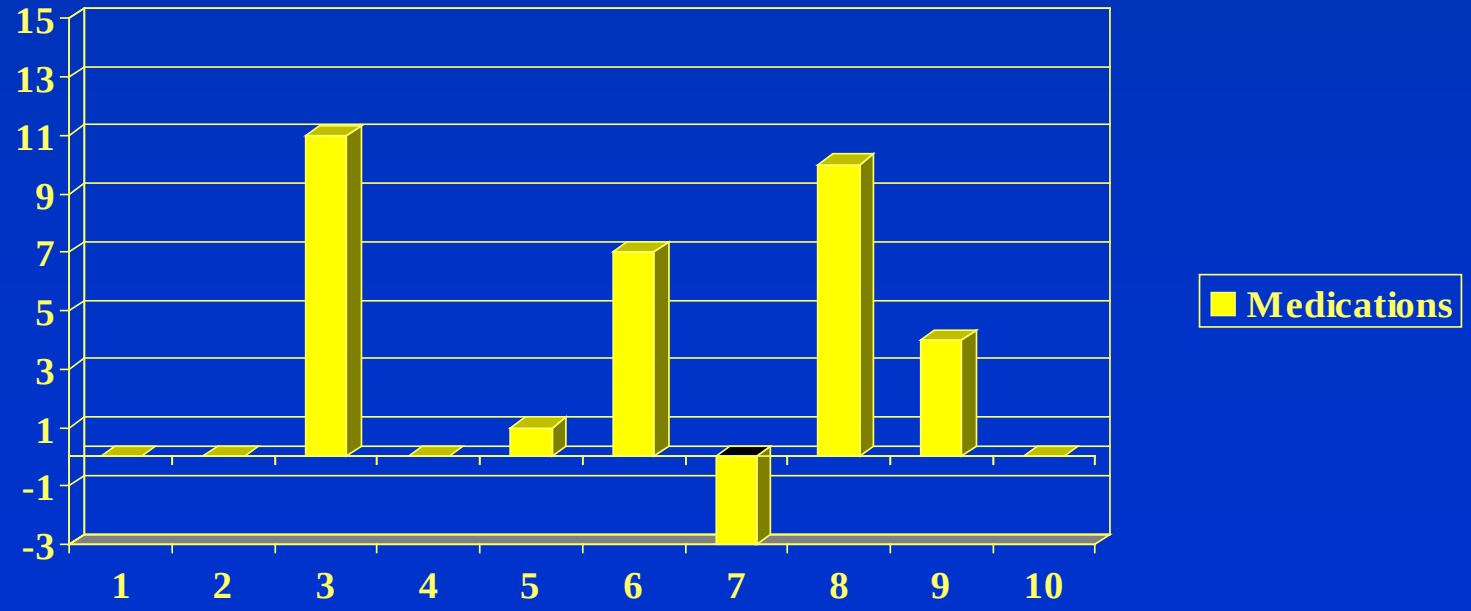
Vineland Raw Scores: Daily living skills



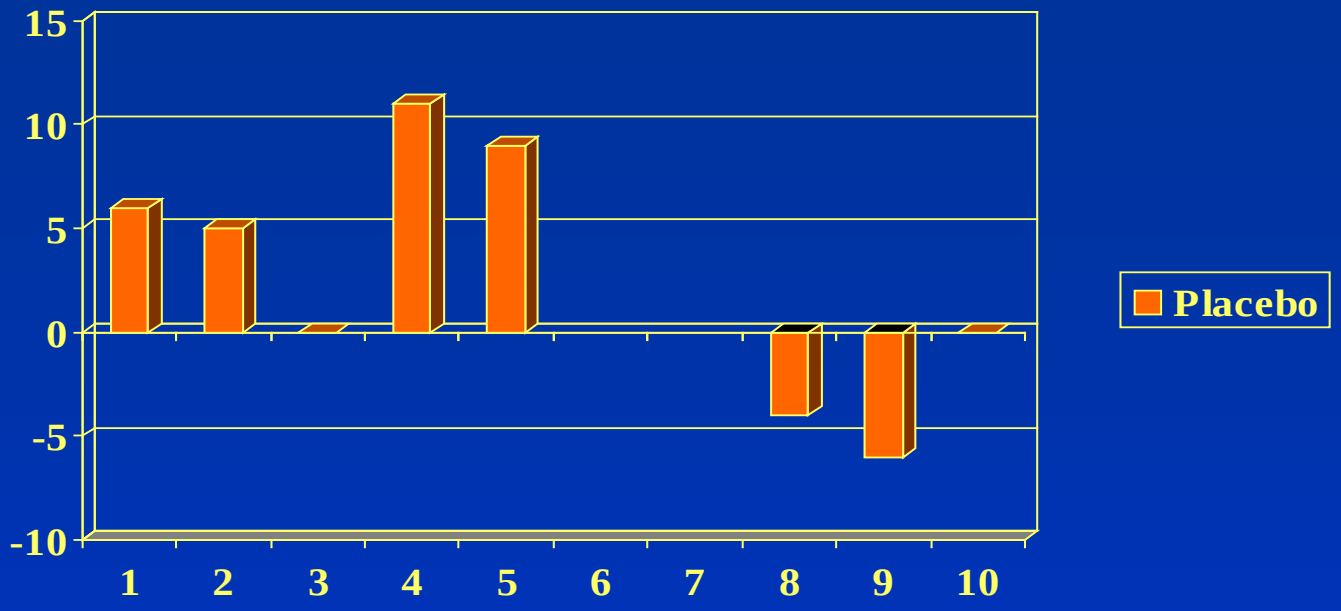


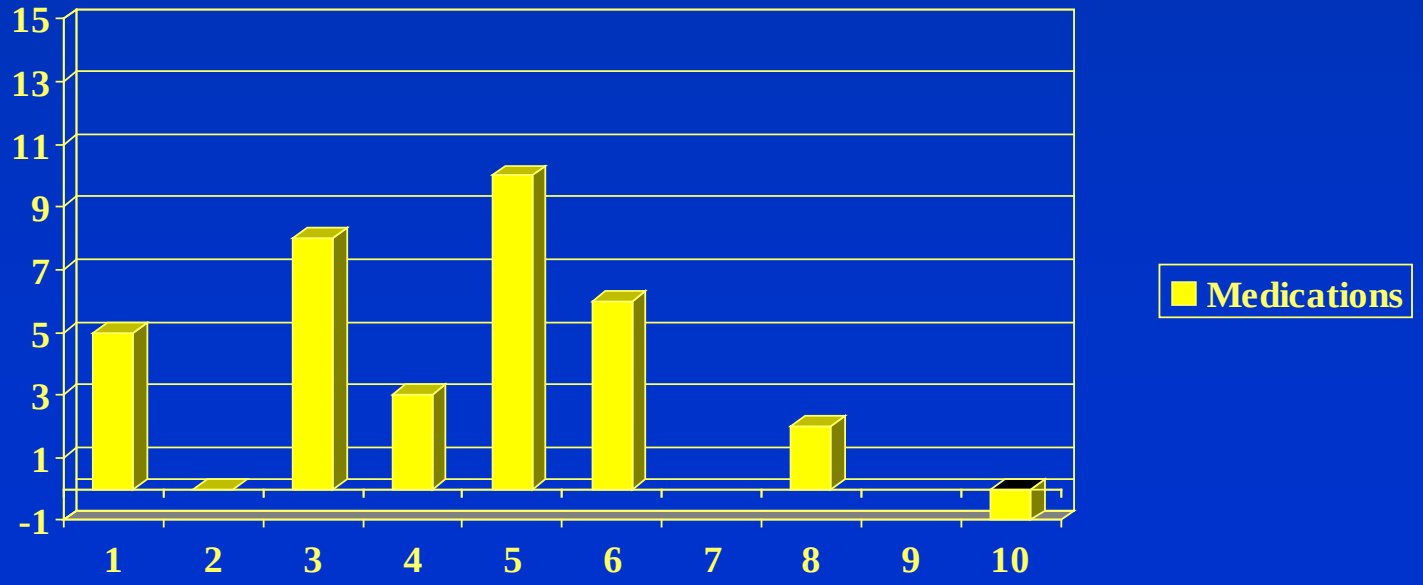
Vineland Raw Scores: Socialization





Vineland Raw Scores: Motor skills

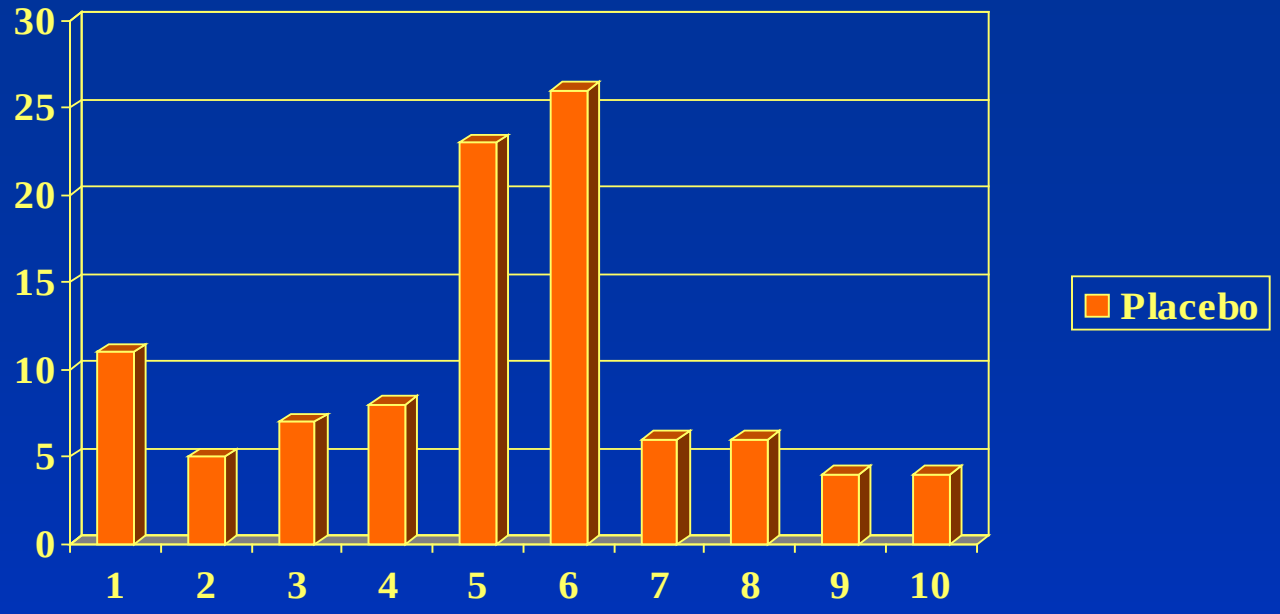


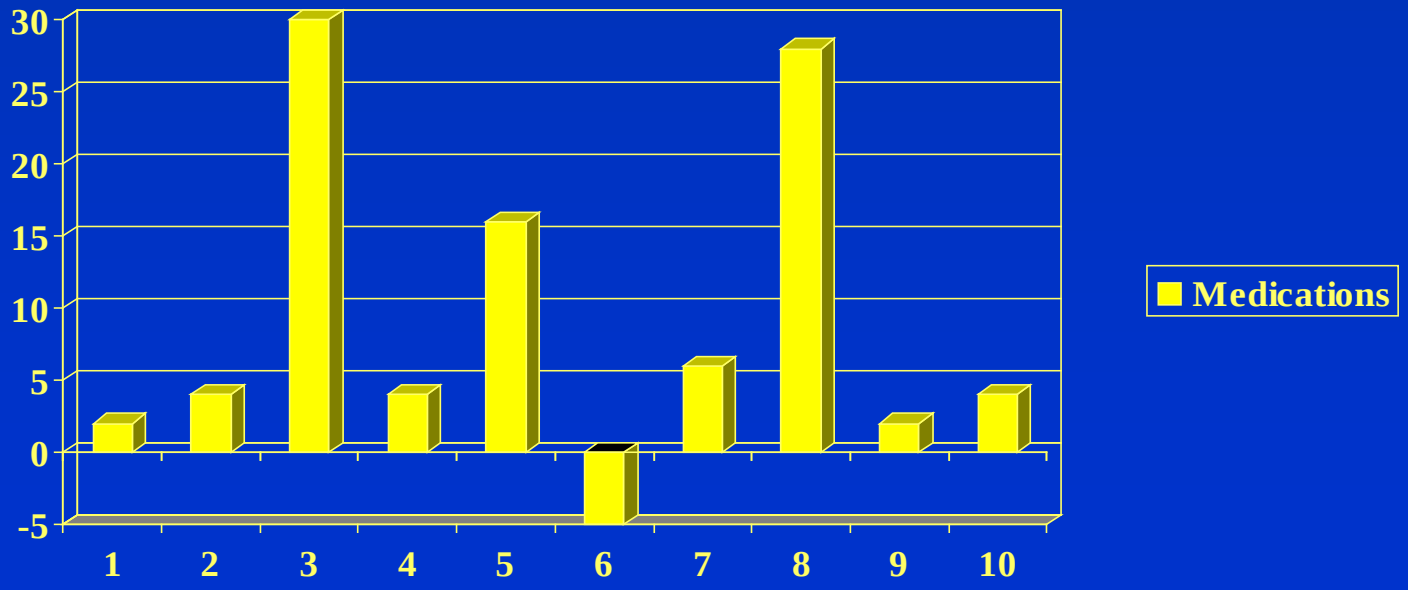


Results

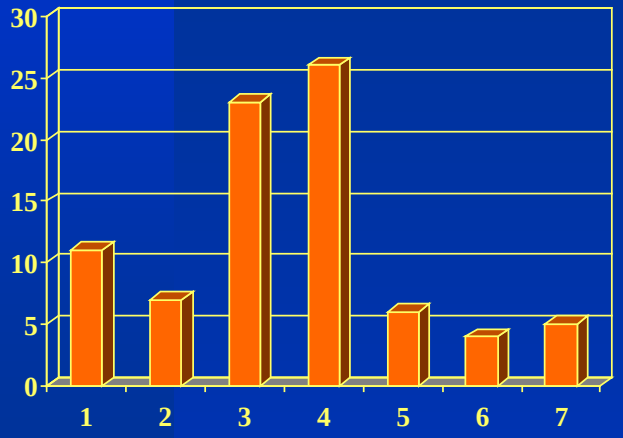
- During the developmental evaluations, some children were recognized to meet diagnostic criteria for autism (ADI and ADOS)
- Autism is defined as “difficulties with communication and reciprocal social interaction skills, accompanied by repetitive/stereotypic behaviors. Delays in nonverbal communication are most relevant in children with AS”
- The diagnosis of autism is made having taken into account children developmental delay

Bayley Mental Scores - MDI

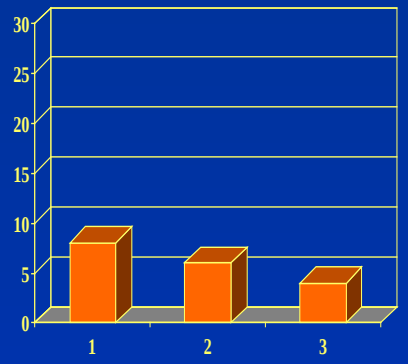




Bayley Mental Scores - MDI



Placebo Non-Autistic



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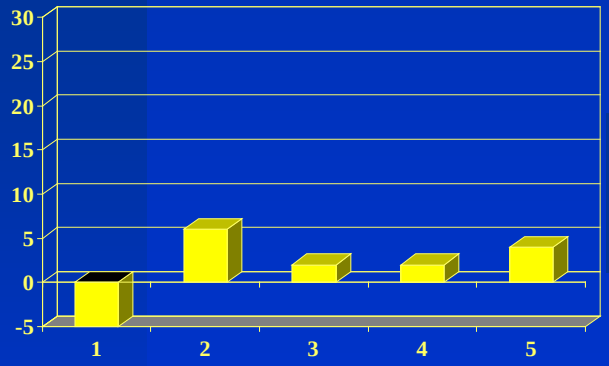
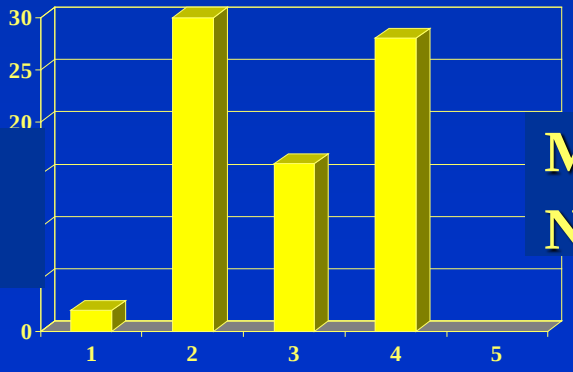


Medications Autistic



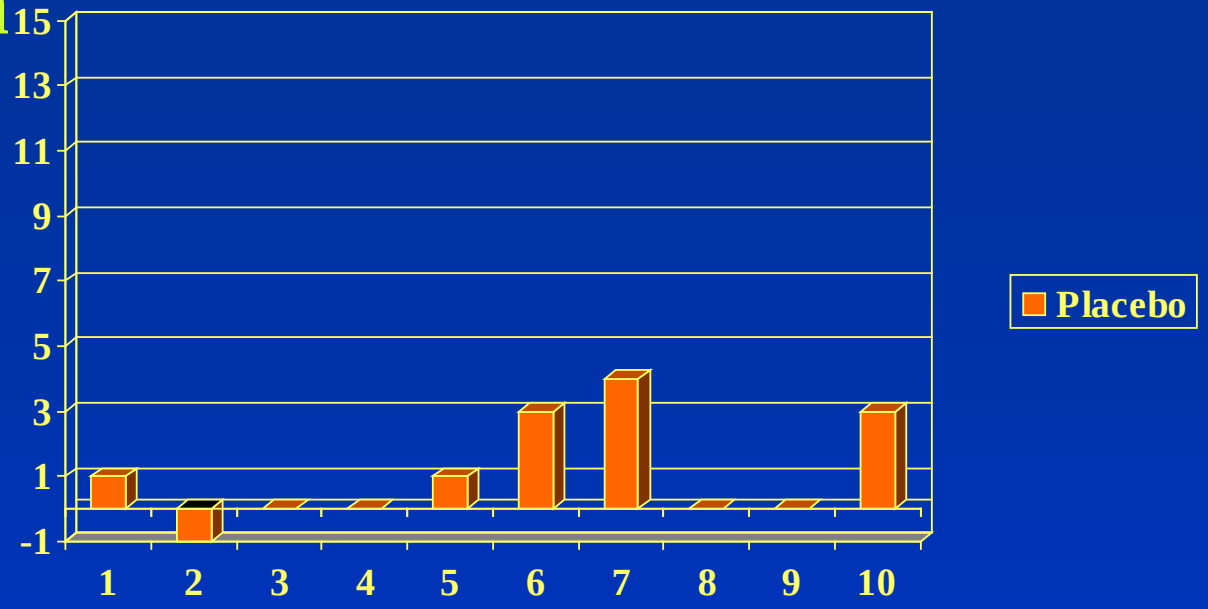
Medications Non-Autistic

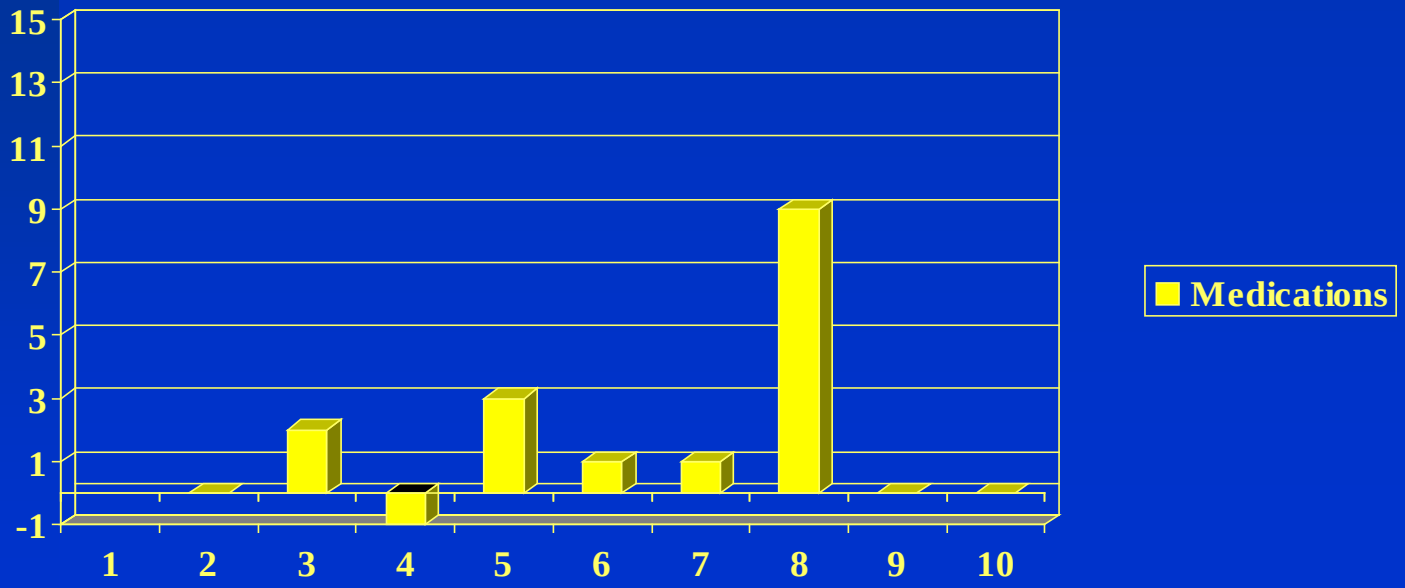
Mean for improvement after correction for autism in medication group

Autistic group: 9.796

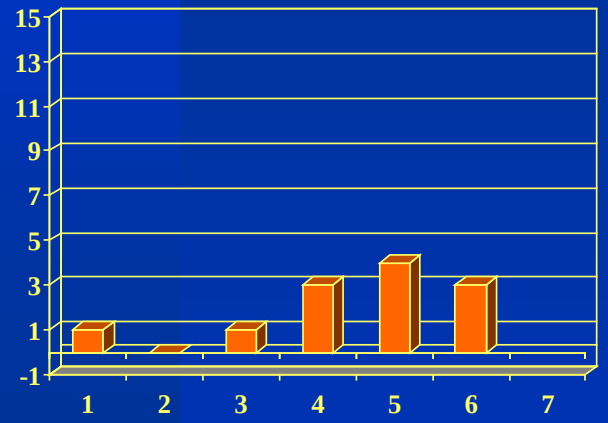
Non-autistic: 11.533

Pre-school language scale – Expressive communication





Pre-school language scale – Expressive communication



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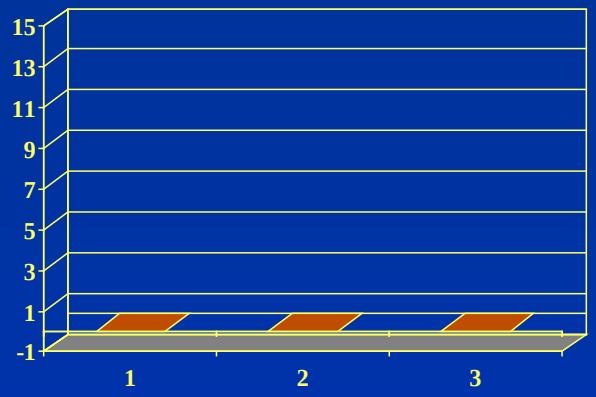

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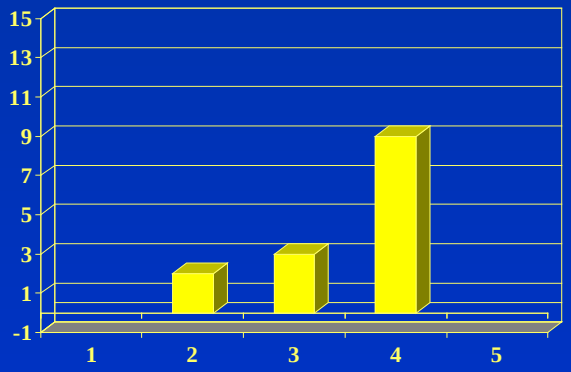
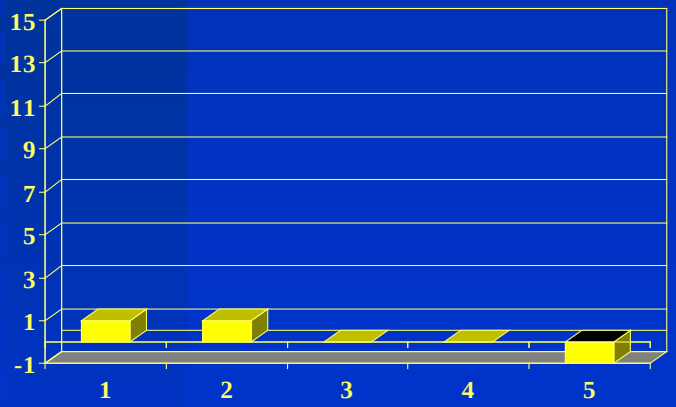


Medications Non-Autistic



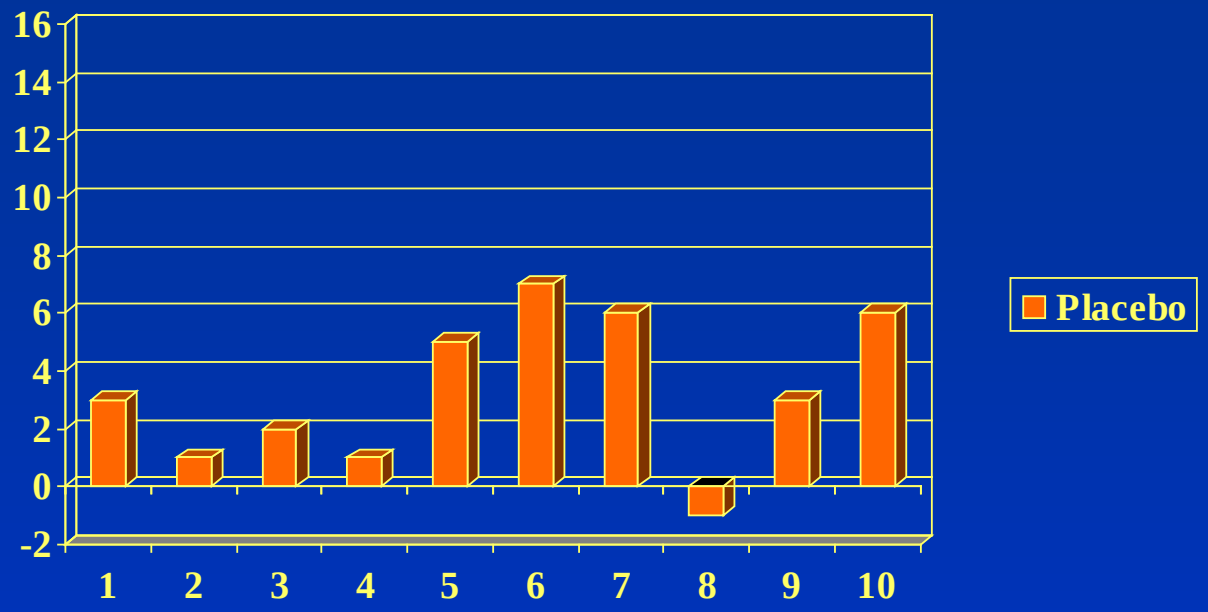
Medications Autistic

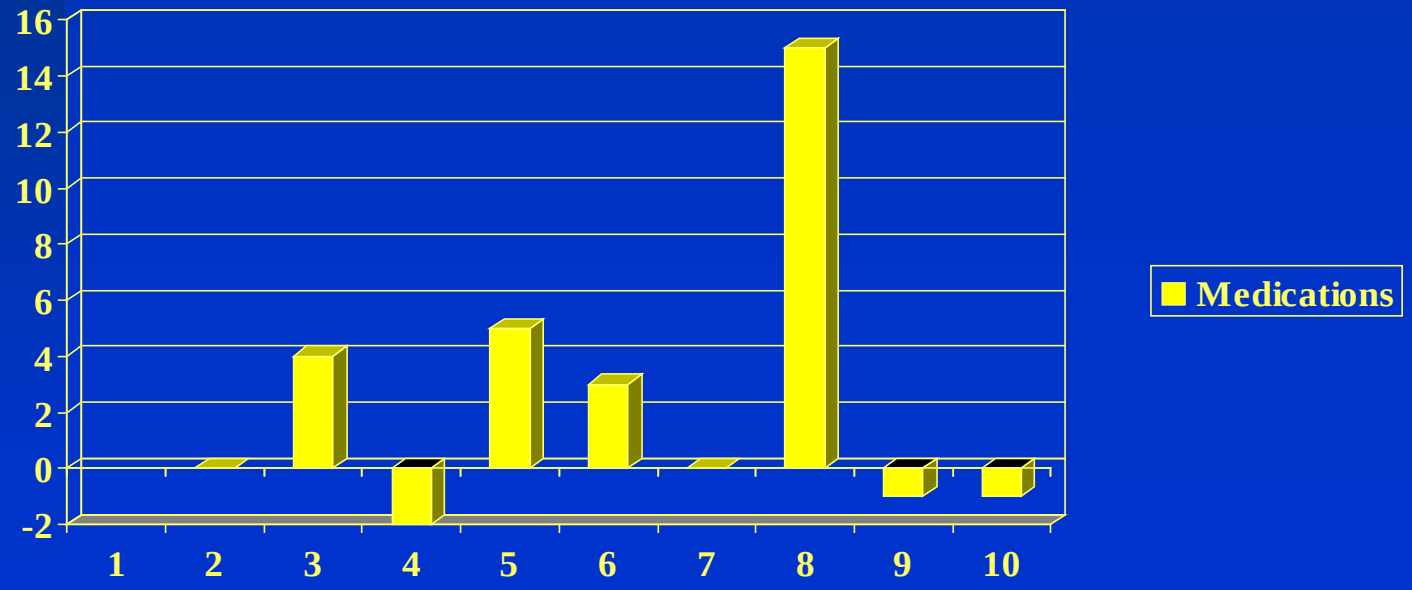
Mean for improvement after correction for autism in medication group

Autistic group: 0.971

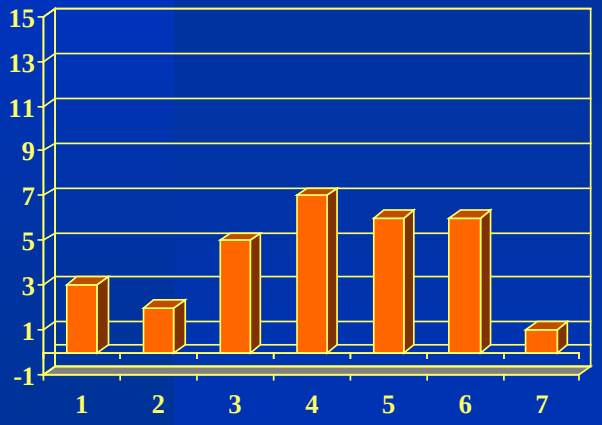
Non-autistic: 2.565

Pre-school language scale – Total language score





Pre-school language scale – Total language score



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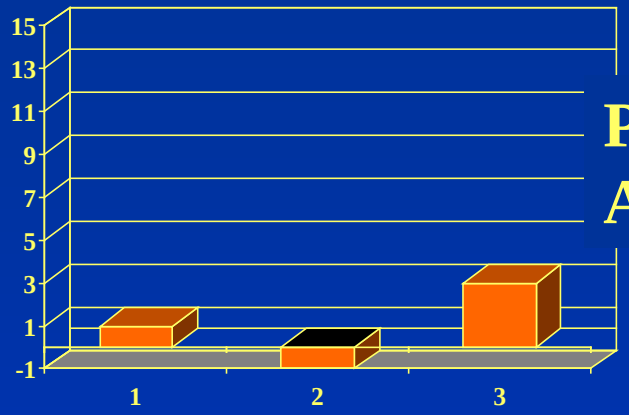

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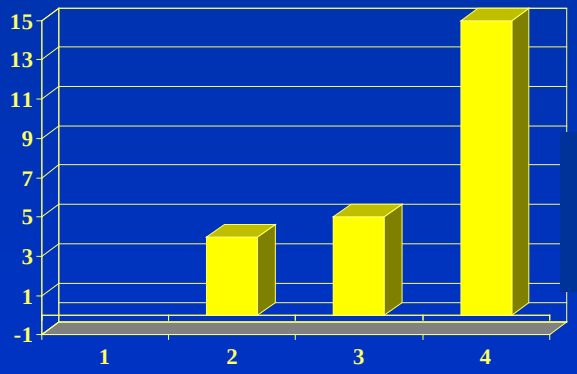
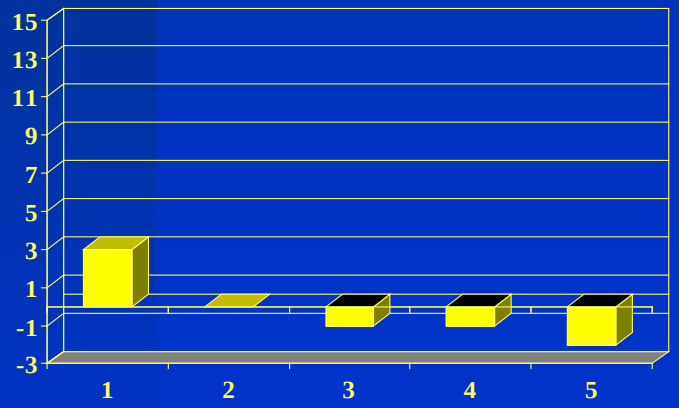


Medications Non-Autistic



Medications Autistic

Mean for improvement after correction for autism in medication group

Autistic group: 2.941

Non-autistic: 4.141

Risks and Side Effects of Folate and Betaine

- Increase seizures (folic acid)
- Betaine: hair loss, body odor

Side Effects Reported on our study patients

- Seizures increment in 7 children out of 20:
  - 4 were on placebo and
  - 3 were on drugs
- Nail abnormalities on 1 child (on drugs)
- Abnormal urine odor or body odor in 2 children (on drugs)

Conclusions

- There was a trend for improvement in a group of children on medications in particular with reference to communication and expressive skills.
- The non-autistic group had the most benefit.

Conclusions

- The number of patients in each sub-group is very small and the evidence for any benefit is not conclusive.
- Increasing the number of patients studied as in San Diego or Boston may or may not prove a statistical significant benefit.

What to do? To treat or not to treat

- More numbers are needed to establish the trend shown by preliminary results
- An independent group should review the data and make recommendations for the medical and the Angelman syndrome communities

Other issues:

Cost of medications per year for each child

- Betaine <30 kg $1,278 per year
- Betaine <30 kg $2,555 per year
- Folic acid $1,432 per year (however 1.000 x 1 mg tablets are $45)

Acknowledgments

- **Houston Team**

  Marie Turcich
  Sarika Peters , Ph.D.
  Jan Goddard-Finegold, M.D.
  Dan Glaze, M.D.
  William O'Brien, Ph.D.
  Art Beaudet, M.D.
  Lina Shinawi
  O'Brian Smith, Ph.D.
- **Angelman Syndrome Foundation**
- **March of Dimes Foundation**
- **General Clinical Research Center (TCH)**
- **Angelman Syndrome families**
- **San Diego**

Ringraziamenti

- Signore Ivano Pillon
- Organizzazione Sindrome di Angelman (OrSA)

Grazie per la vostra ospitalità