WHAT CAUSES COMPLICATIONS IN GALACTOSEMIA?

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HARVARD MEDICAL SCHOOL
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No conflicts of interest to disclose

COMPLICATIONS

• What are the complications?
• Why are we worried about them?

COMPLICATIONS

• What are the complications?
  – Developmental language delay
  – Speech defect
  – Learning problems in school
  – Cognitive deficits
  – Decreased bone mineralization
  – Poor growth
  – Infertility in females (Premature Ovarian Insufficiency or POI)

COMPLICATIONS

• What are the neurological complications?
  – Tremor
  – Ataxia
  – Dystonia

COMPLICATIONS

• Why are we worried about them?
  – Because we cannot prevent them with a lactose restricted diet
  – They may occur even if the patient's galactose-1-phosphate levels have been between 1-4 mg% and galactitol between 100-400 umol / mmol creatinine for life
  – They may occur even if mother restricted lactose during pregnancy and diet therapy was begun at birth
LONG-TERM CHRONIC COMPLICATIONS WITH NO CLEAR CAUSE

- Anxiety
- Depression
- Rare history of males who have been biological fathers
- Introverted or shy personalities (seems to affect males greater than females)

What causes the complications?

- Leading Hypotheses:
  - Chronic elevation of galactose-1-phosphate in brain, ovarian and bone cells due to endogenous synthesis of galactose
  - Chronic deficiency of UDPgalactose in brain, ovarian and bone cells due to the inability to convert galactose-1-phosphate to UDPgalactose since that is what GALT does and it is absent in patients with classic galactosemia

Galactose Metabolism

When does this happen?

- Before birth when the fetus with galactosemia is growing in mother’s womb
- After birth but only when the infant is exposed to large amounts of lactose in the newborn period
- After birth for the lifetime of the patient with classic galactosemia
- Both before and after birth
Okay, but given the fact that patients still develop chronic complications even if they are started on a diet as soon as they are born, what is the most likely hypothesis?

Where’s the supporting data?


What evidence is there that galactose levels are elevated in the fetus with galactosemia?

- Galactose-1-phosphate is elevated in the tissues of the fetus and in the cord blood of the newborn
- Galactitol is elevated in the amniotic fluid of the pregnant woman
- And, the levels of galactose-1-phosphate in erythrocytes in cord blood and galactitol in amniotic fluid are still elevated even if the pregnant woman ingests NO lactose during pregnancy


Amniotic fluid Galactitol (umol/L)

G/N mothers on lactose 7.96, 9.21
G/N mothers on diet 6.35, 6.92
N/N mothers (n=30) 0.80+/-.015
What is the most likely explanation?

- The fetus with galactosemia synthesizes galactose during all or part of the pregnancy
- In other words, endogenous synthesis of galactose may be responsible for the elevated levels of galactose-1-phosphate and galactitol in many or all fetal tissues

Can this cause disease?

- Possibly
- Let’s go back to the last question

Okay, but given the fact that patients still develop chronic complications even if they are started on a diet as soon as they are born, what now is the most likely hypothesis?

Answer

- The most likely hypothesis is that chronic elevation before and/or after birth of galactose-1-phosphate in brain, ovarian and bone cells due to endogenous synthesis of galactose and/or chronic deficiency of UDPgalactose in brain, ovarian and bone cells cause the chronic complications seen in patients with classic galactosemia

Yea, but wait a minute, not so fast, Dr. Leslie’s knockout mouse has elevated levels of galactose-1-phosphate and galactitol and it does not become sick, what gives?

- Maybe the sensor for galactose-1-phosphate is missing in the mouse and the mouse fails to activate unwanted pathways
- Maybe the levels have not reached a “toxicity threshold” in the mouse

I still don’t buy it, Dr. Leslie’s knockout mouse has elevated levels of galactose-1-phosphate and galactitol and it just does not get sick, how can that be?

- Maybe there is an unknown genetic modifier element missing in certain murine species
- Maybe the GALT gene, mRNA or protein performs another function in man, but not in the mouse
- The answer is we just don’t know!
Why are the chronic long term complications so variable and does that change the hypothesis?

- We do not know
- Yes, to a degree

Let us take a look at this issue of variability!

THE BOSTON ADULT GALACTOSEMIA STUDY

DESCRIPTION

Prospective study with the same team of investigators and instruments and measurements

34 adults with galactosemia (17 females; 17 males, but one of the females turned out to have the benign Duarte variant galactosemia)

12 evaluations each, all in one weekend

DESCRIPTION OF SAMPLE

<table>
<thead>
<tr>
<th>AGE</th>
<th>Average: 32 years, (18-59 years)</th>
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</thead>
<tbody>
<tr>
<td>GENOTYPE</td>
<td>15 subjects: Q188R/Q188R</td>
</tr>
<tr>
<td></td>
<td>13 subjects Q188R/other</td>
</tr>
<tr>
<td></td>
<td>5 other/other</td>
</tr>
<tr>
<td></td>
<td>1 Q188R/N314D (Duarte Variant)</td>
</tr>
<tr>
<td>HEALTH</td>
<td>Normal blood pressure in all but 2 subjects, majority in good health</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>Average female height = 5 ft 5 in (US national average = 5 ft 4 in)</td>
</tr>
<tr>
<td></td>
<td>Average male height = 5 ft 9 in (US national average = 5 ft 9 in)</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>58% normal weight; 2 subjects underweight; 11 subjects overweight</td>
</tr>
<tr>
<td>BONE DENSITY</td>
<td>25% with bone mineral density below lower limit of normal (5 women/2 males)</td>
</tr>
</tbody>
</table>

METHODS

<table>
<thead>
<tr>
<th>HEALTH HISTORY</th>
<th>PHYSICAL EXAM</th>
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<tbody>
<tr>
<td>GALT ENZYME ANALYSIS</td>
<td>GALT GENE MUTATIONS</td>
</tr>
<tr>
<td>NEUROLOGICAL EXAM</td>
<td>ENDOCRINE AND FERTILITY (sperm for men)</td>
</tr>
<tr>
<td>REPRODUCTIVE HISTORY</td>
<td>BONE DENSITY (DEXA)</td>
</tr>
<tr>
<td>PSYCHOLOGICAL EVALUATION</td>
<td>SPEECH &amp; LANGUAGE EVALUATION</td>
</tr>
<tr>
<td>NUTRITION EVALUATION</td>
<td>LABORATORY STUDIES</td>
</tr>
<tr>
<td>EEG</td>
<td></td>
</tr>
</tbody>
</table>
Adult Galactosemia Study
(n=33 subjects with classic disease)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male = 17, Female = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>33 ± 12 (range 18-59)</td>
</tr>
<tr>
<td>Mean Education</td>
<td>Two years of college</td>
</tr>
<tr>
<td>Mean SES</td>
<td>Lower middle class</td>
</tr>
<tr>
<td>Tried to conceive</td>
<td>3 males (18%); 5 female (31%)</td>
</tr>
<tr>
<td>Has a child</td>
<td>2 males (12%); 1 female (6%)</td>
</tr>
</tbody>
</table>

INTELLIGENCE

<table>
<thead>
<tr>
<th>Mean IQ</th>
<th>88 ± 20 (55-122)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scores &lt; 85 (Borderline Range)</td>
<td>13 subjects (39%)</td>
</tr>
<tr>
<td>Scores &lt; 70 (Range of Intellectual Disability)</td>
<td>8 subjects (24%)</td>
</tr>
<tr>
<td>Verbal vs. Performance</td>
<td>No difference</td>
</tr>
</tbody>
</table>

PSYCHOLOGICAL OUTCOMES

| Adaptive Behavior Deficits | 7 (21%) |
| Executive Function Deficits | 5 (15%) |
| Depression (observed or reported) | 13 (39%) |
| Anxiety | 17 (52%) |

SPEECH & LANGUAGE

- Motor speech deficits: 25 subjects or 78%
- Dysarthria (articulation problems): 25%
- Apraxia of speech: 9%
- Reduced tongue strength: 73%
- Low phonation duration (breath support): 64%
- Reduced receptive vocabulary: 42%
- Hearing loss in 3 men (2 unilateral, 1 bilateral)

NEUROLOGICAL

- TREMOR 46%
  - Intention tremor in 8 subjects (24%)
  - Postural tremor in 5 subjects (15%)
  - Both kinds of tremor in 2 subjects (6%)
  - No subject exhibited a Parkinsonian tremor
- ATAXIA 15%
- DYSTONIA 6%

NEUROLOGICAL

- One subject experienced seizures; these first occurred during adulthood
- No subject had upper motor neuron disease (such as spastic paraparesis and spastic quadriplegia)
Physiological outcomes

<table>
<thead>
<tr>
<th></th>
<th>16 females (100%)</th>
<th>8 (24%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary ovarian insufficiency (POI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased bone mineral density (BMD)</td>
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GENOTYPE AND OUTCOME

- Subjects homozygous for the common Q188R mutation experienced a similar range of scores on IQ tests and measures of depression and anxiety as subjects with deletions or other mutations on one allele.
- Percentage of patients with tremor, ataxia or speech defects did not differ with genotype.

NEWBORN SCREENING AND OUTCOME (NOT ACCURATE!!!)

<table>
<thead>
<tr>
<th></th>
<th>Newborn Screened (6)</th>
<th>Not newborn screened (27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average IQ</td>
<td>91</td>
<td>88</td>
</tr>
<tr>
<td>Depression</td>
<td>0</td>
<td>11%</td>
</tr>
<tr>
<td>Anxiety</td>
<td>33%</td>
<td>52%</td>
</tr>
<tr>
<td>Tremor</td>
<td>0</td>
<td>52%</td>
</tr>
<tr>
<td>Ataxia</td>
<td>0</td>
<td>19%</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>20%</td>
<td>26%</td>
</tr>
<tr>
<td>Apraxia</td>
<td>20%</td>
<td>7%</td>
</tr>
</tbody>
</table>

FINDINGS

- Only one subject identified by newborn screening showed evidence of tremor/ataxia/dystonia
- Speech and intellectual functioning did not differ between those identified by newborn screening and those not identified by newborn screening.

Chemical Structure of Lactose

D - Lactose

D - Glucose

D - Galactose

D - Glucose
SUMMARY

- Individuals with galactosemia experience challenges in adulthood that may affect
  - Living independently
  - Social relationships
  - Becoming parents
- Included in this sample, however, were successful college students, teachers and many with successful marriages

CONCLUSIONS

- Galactosemia is not a progressive neurodegenerative disease
- Subjects appear to overcome handicaps over time
- The impact of newborn screening on the health and outcome of patients with galactosemia needs to be further examined

CONCLUSIONS

- POI is the one complication that is almost universal in women with galactosemia and appears completely resistant to diet therapy
- The structure and function of the male reproductive tract does not appear to be severely affected and does not easily explain why so few men have become fathers

SO, AS YOU CAN SEE, EXCEPT FOR POI IN FEMALES WITH CLASSIC GALACTOSEMIA, ALMOST ALL OF THE OTHER CHRONIC COMPLICATIONS ARE VARIABLE IN THEIR EXPRESIVITY

DOES THE GALT GENOTYPE PROVIDE AN EXPLANATION AS TO WHY CLASSIC PATIENTS DEVELOP COMPLICATIONS?

NO
Common SEVERE GALT Gene Mutations

- Q188R
- K285N
- L195P
- 5.2 kb deletion
- More than 230 GALT gene mutations have been detected

Have we made a mistake with our diet therapy?

Lee et al, Lancet, 362:446, 2003

- 38 year old female with Q188R/Q188R genotype, mild speech delay, hand tremor, verbal/performance IQ of 88/78 and ovarian failure
- No diet therapy after age 3: on 2690 mg galactose per day from dairy products!
- History of poor feeding and jaundice at 6 days of age while on lactose

Lee et al, Lancet, 362:446, 2003

- < 0.8% RBC GALT enzyme activity
- RBC galactose-1-phosphate 3.4 mg%
- Urine galactitol 68 umol/mmol creatinine

Panis et al, Molecular Genetics & Metabolism, 446, 2006

- 34 year old male with Q188R/Q188R genotype
- He is married and the father of one healthy daughter
- No diet therapy after age 3: on 9000 mg galactose per day from dairy products!
- History of liver disease and sepsis as a newborn infant while on lactose

Panis et al, Molecular Genetics & Metabolism, 446, 2006

- 1.5 % RBC GALT enzyme activity
- RBC galactose-1-phosphate 1.7 mg%
- Urine galactitol 83 umol/mmol creatinine
- Galactose breath test consistent with severe classic phenotype
What is responsible for this GALT-independent oxidation/metabolism of galactose? Is it the reason that patients over 3 years of age can tolerate dairy products?


Complete absence of GALT protein does not prevent oxidation of galactose to carbon dioxide.

IgG N-glycan profiles in diet relaxed Gal

Scriver et al., MMBID 2001; 1-2:453.
Postulated Mechanisms of Disease Pathogenesis in Galactosemia

- Phosphate trapping due to galactose-1-phosphate excess
- Galactose-1-phosphate inhibition of enzymes/transporters
- Galactitol (cytotoxic edema, NADPH deficiency)
- Galactonate toxicity
- Unidentified galactose metabolite
- Myo-inositol deficiency
- Reduced concentration of the GALT-product, UDP-galactose
- Reduced synthesis of galactose-containing glycoproteins, galactocerebrosides and glycosaminoglycans due to ER and/or Golgi lesions
- Moonlighting function of GALT protein
- Effect of GALT gene mutation on IL-11Rα gene

- Data support galactose-1-phosphate as the key pathogenic factor

- But is it sufficient to produce all of the complications seen in patients with classic galactosemia?

Clinical Research: An Evidence-Based Medicine Approach Is Mandatory

Establish an International Consortium for Galactosemia

- International Database
- Repository
- Patient care
- Education
- Multicenter Research Studies
International Consortium for Galactosemia

Patient Care
Education
Research

Initiate Multicenter Clinical Study of Dietary Galactose Tolerance in adolescents and adults, and assess the effect of Exogenous Galactose Exposure on Glycoconjugate formation and Endogenous galactose synthesis

Research goal #1

• Establish an international database on the prevalence of complications in thousands of patients with known GALT genotypes and correlate outcomes with modifier gene variations determined via whole genome sequencing

Human iPS cells (2007)

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Reprogramming of human somatic cells to pluripotency with defined factors

Inhyun Park

Research goal #2

• Use neurons derived from iPS cells of patients with classic galactosemia to study epigenetic effects
THANK YOU!

• We greatly thank the Galactosemia Foundation for supporting our work.