WHAT CAUSES COMPLICATIONS IN GALACTOSEMIA?

DIVISION OF GENETICS
BOSTON CHILDREN'S HOSPITAL
HARVARD MEDICAL SCHOOL

Gerard T Berry, MD

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

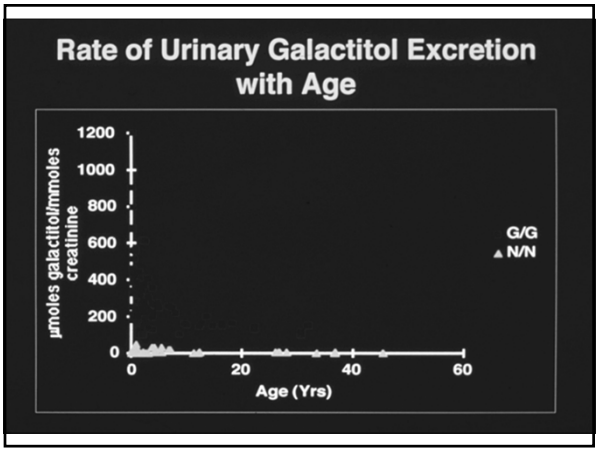
LONG-TERM CHRONIC COMPLICATIONS WITH NO CLEAR CAUSE

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

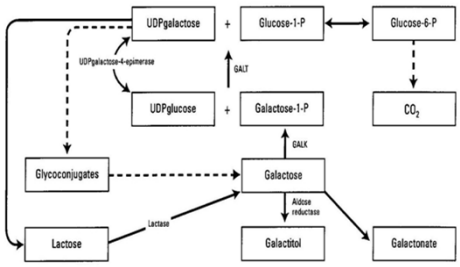
What causes the complications?

- 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

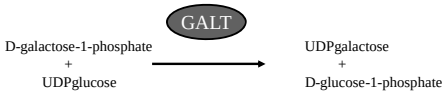


Galactose Metabolism



Galactosemia

Deficiency: galactose-1-phosphate-uridyltransferase(GALT)



Gene: GALT on chromosome 9p13

Frequency: 1/35,000 to 1/60,000 (1/16,476 in Ireland)

Inheritance: autosomal recessive

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

Long-term Prognosis in Galactosaemia: Results of a Survey of 350 Cases

D. D. WAGGONER[1], N. R. M. BUIST[2]* and G. N. DONNELL[3]
[1]*Crippled Children's Division, Oregon Health Sciences University, USA*
[2]*Departments of Pediatrics and Medical Genetics, Oregon Health Sciences University, USA*
[3]*Southern California School of Medicine and Children's Hospital of Los Angeles, USA*

Summary: An international survey of the long term results of treating galactosaemia has shown poor results. These do not seem to be related to any of the relevant variables studied, for example delayed diagnosis or poor dietary compliance.

Table 10 Sibling cases: older sibling diagnosed because of clinical symptoms or newborn screening, younger sibling treated within first two days of life

	Proband ($n = 28$) *Result* (n)	*Sibling* ($n = 31$) *Result* (n)	p
Age			
Mean (range)	14 y (3–33 y)	10 y (0–31 y)	0.1
Neonatal history			
Symptomatic	100%	3%	0.0001
Age diet begun			
Mean (range)	63 d (7–400 d)	1 d (1–2 d)	
Milk restriction during pregnancy	0% (22)	76% (25)	0.0001
DQ/IQ (Mean ± SD)			
IQ 3–5 y	87 ± 8 (9)	96 ± 20 (13)	0.2
IQ 6–9 y	83 ± 23 (11)	93 ± 21 (12)	0.3
IQ 10–16 y	69 ± 19 (9)	84 ± 15 (9)	0.1
Speech			
Abnormal	64% (25)	62% (26)	0.9
Ovarian function (F ≥ 14 y)			
Abnormal	75% (8)	71% (7)	0.7

from Waggoner D.D. et al 1990

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?

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

Where's the supporting data?

- Donnell et al. In: Galactosemia: New Frontiers in Research. Donnell G, De la Cruz F, Koch R, Levy HL, eds., NIH Publication 93-3438, 1993.
- Jakobs C, Kleijer WJ, Bakker HD, van Gennip AH, Przyrembel H, Niermeijer MF. Dietary restriction of maternal lactose intake does not prevent accumulation of **galactitol** in the amniotic fluid of fetuses affected with galactosaemia. Prenat Diagn. 1988 8:641-5.
- Irons M, Levy HL, Pueschel S, Castree K. Accumulation of galactose-1-phosphate in the galactosemic fetus despite maternal milk avoidance. J Pediatr. 1985 107:261-3.

"Dietary restriction of maternal lactose intake does not prevent accumulation of galactitol in the amniotic fluid of fetuses affected with galactosaemia", C.Jakobs, W.J. Kleijer, H.D. Bakker, A. H. Van Gennip, H. Przyrembel and M. F. Niermeijer, Prenatal Diagnosis, 8: 641, 1988.

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+/-0.15

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

J Inherit Metab Dis
DOI 10.1007/s10545-011-9372-y

ORIGINAL ARTICLE

The adult galactosemic phenotype

Susan E. Waisbren · Nancy L. Potter · Catherine M. Gordon · Robert C. Green · Patricia Greenstein · Cynthia S. Gubbels · Estela Rubio-Gozalbo · Donald Schomer · Corrine Welt · Vera Anastasoaie · Kali D'Anna · Jennifer Gentile · Chao-Yu Guo · Leah Hecht · Roberta Jackson · Bernadette M. Jansma · Yijun Li · Va Lip · David T. Miller · Michael Murray · Leslie Power · Nicolle Quinn · Frances Rohr · Yiping Shen · Amy Skinder-Meredith · Inge Timmers · Rachel Tunick · Ann Wessel · Bai-Lin Wu · Harvey Levy · Louis Elsas · Gerard T. Berry

J Inherit Metab Dis. 2012 Mar;35(2):279-86. Epub 2011 Jul 21.

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

METHODS

HEALTH HISTORY	PHYSICAL EXAM
GALT ENZYME ANALYSIS	GALT GENE MUTATIONS
NEUROLOGICAL EXAM	ENDOCRINE AND FERTILITY (sperm for men)
REPRODUCTIVE HISTORY	BONE DENSITY(DEXA)
PSYCHOLOGICAL EVALUATION	SPEECH & LANGUAGE EVALUATION
NUTRITION EVALUATION	LABORATORY STUDIES
EEG	

DESCRIPTION OF SAMPLE

AGE	Average: 32 years, (18-59 years)
GENOTYPE	15 subjects: Q188R/Q188R 13 subjects Q188R/other 5 other/other 1 Q188R/N314D (Duarte Variant)
HEALTH	Normal blood pressure in all but 2 subjects, majority in good health
HEIGHT	Average female height = 5 ft 5 in (US national average = 5 ft 4 in); Average male height = 5 ft 9 in (US national average =5 ft 9 in)
WEIGHT	58% normal weight; 2 subjects underweight; 11 subjects overweight
BONE DENSITY	20% with bone mineral density below lower limit of normal (5 women/2 males)

Adult Galactosemia Study (n=33 subjects with classic disease)

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

INTELLIGENCE

Mean IQ	88 ± 20 (55-122)
Scores < 85 (Borderline Range)	13 subjects (39%)
Scores < 70 (Range of Intellectual Disability)	8 subjects (24%)
Verbal vs. Performance	No difference

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

Primary ovarian insufficiency (POI)	16 females (100%)
Decreased bone mineral density (BMD)	8 (24%)

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

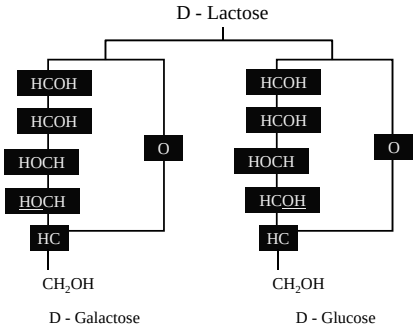
NEWBORN SCREENING AND OUTCOME
(NOT ACCURATE!!!)

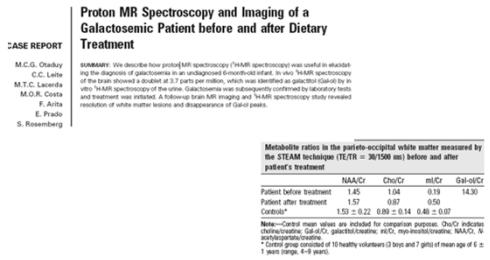
	Newborn Screened (6)	Not newborn screened (27)
Average IQ	91	88
Depression	0	11%
Anxiety	33%	52%
Tremor	0	52%
Ataxia	0	19%
Dysarthria	20%	26%
Apraxia	20%	7%

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




CASE REPORT

Proton MR Spectroscopy and Imaging of a Galactosemic Patient before and after Dietary Treatment

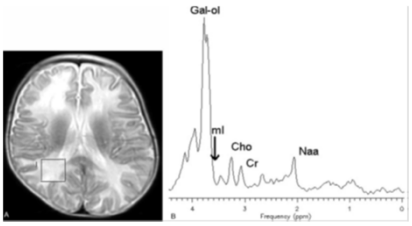
M.C.G. Otaduy
C.C. Leite
M.T.C. Lacerda
M.O.R. Costa
F. Arita
E. Prado
S. Rosemberg

SUMMARY: We describe how proton[MR spectroscopy (¹H-MR spectroscopy) was useful in elucidating the diagnosis of galactosemia in an undiagnosed 6-month-old infant. In vivo ¹H-MR spectroscopy of the brain showed a doublet at 3.7 parts per million, which was identified as galactitol.Galctol by in vitro ¹H-MR spectroscopy of the urine. Galactosemia was subsequently confirmed by laboratory tests and treatment was initiated. A follow-up brain MR imaging and ¹H-MR spectroscopy study revealed resolution of white matter lesions and disappearance of Gal-ol peaks.

Metabolite ratios in the parieto-occipital white matter measured by the STEAM technique (TE/TR = 30/1500 ms) before and after patient's treatment

	NAA/Cr	Cho/Cr	mI/Cr	Gal-ol/Cr
Patient before treatment	1.45	1.04	0.19	14.30
Patient after treatment	1.57	0.87	0.50	
Controls*	1.53 ± 0.22	0.86 ± 0.14	0.46 ± 0.07	

Note:—Control mean values are included for comparison purposes. Cho/Cr indicates choline/creatine, Gal-ol/Cr, galactitol/creatine, mI/Cr, myo-inositol/creatine, NAA/Cr, N acetylaspartate/creatine.
* Control group consisted of 10 healthy volunteers (3 boys and 7 girls) of mean age of 6 ± 1 years (range, 4–9 years).



Axial localizer T2-weighted image showing the MR spectroscopy voxel location (*A*). STEAM (TE/TR, 30/1500 milliseconds) (*B*) in vivo 1H-MR spectroscopy spectrum of the patient before treatment.

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

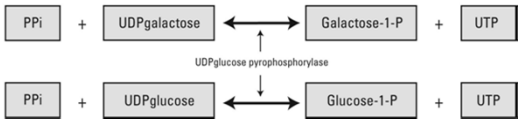
- 1.5 % RBC GALT enzyme activity
- RBC galactose-1-phosphate 1.7 mg%
- Urine galactitol 83 umol/mmol creatinine
- Galactose breath test consistent with severe classic phenotype

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?

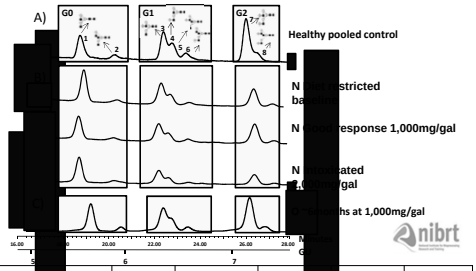
"Evidence for Alternate Galactose Oxidation in a Patient with Deletion of the Galactose-1-Phosphate Uridyltransferase Gene", Gerard T. Berry, Nancy Leslie, Robert A. Reynolds, Claire T. Yager and Stanton Segal, Molec. Genet. Met., 72: 316, 2001.

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

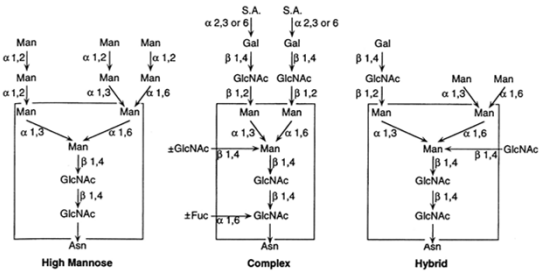


IgG N-glycan profiles in diet relaxed Gal

Coss et al., (2012) MGM 105 (2012) 212-220

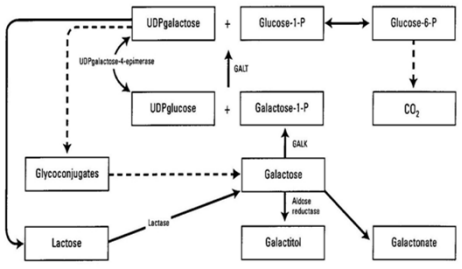


	Ga… <3…	…gal	Healthy controls	N baseline <300mg/gal	N 1,000mg/gal	N …2,000mg/gal	O ~6months at 1,000mg/gal
G0	mean **0.81**	95% CI 0.69-0.92	**0.5**	0.95	0.84	**1.28**	**0.78**
(G0/G1)/G2	**0.03**	0.02-0.03	**0.01**	0.04	0.03	**0.06**	**0.02**



Scriver et al., *MMBID* 2001; 1-2:453.

Galactose Metabolism

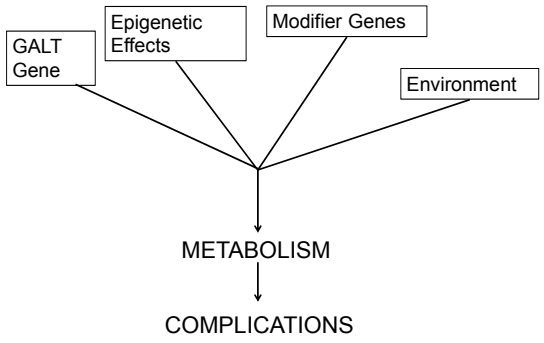


Postulated Mechanisms of Disease Pathogenesis in Galactosemia

- 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

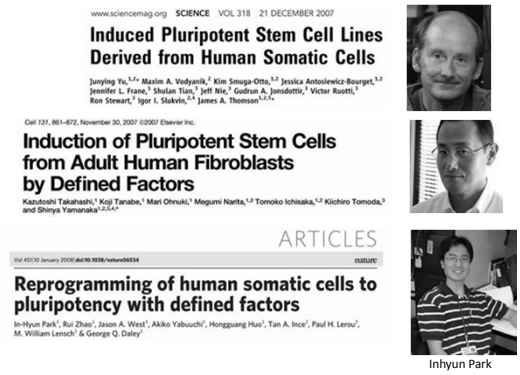
Patient Care
Education
Research

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

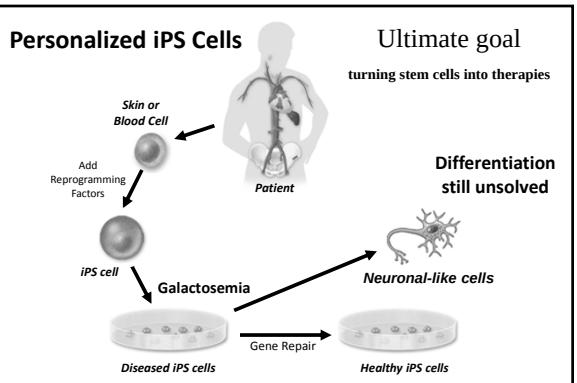
Research goal #1

- 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)



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.