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Neonatal Liver Failure
Lessons Learned

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Neonatal Liver Failure
Results from the PALF study

NLF is a prominent player in PALF

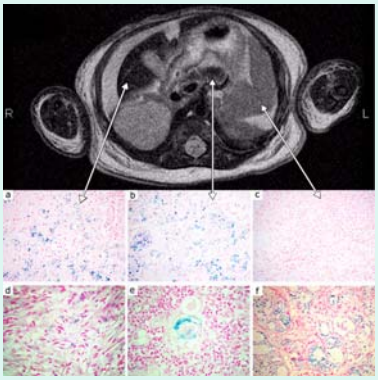
- 148/841 registrants were < 90 days of age
- Meaning 4% of the age spectrum contributed 17% of PALF patients

Etiology	Percent of NLF
Metabolic disease	18.9
Viral	16.2
Neonatal hemochromatosis	13.5
Other	12.8
Indeterminate	37.8

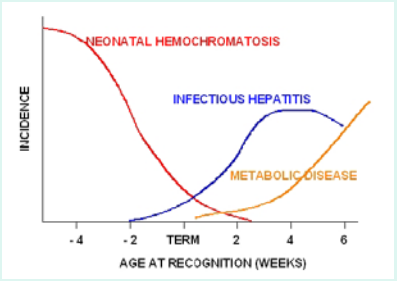
Neonatal Hemochromatosis

- Single diagnosis with highest prevalence in NLF
- Diagnosis requires demonstration of extrahepatic siderosis in association with severe neonatal liver disease
- NH is actually a phenotype, not a disease
- Strong evidence that the NH phenotype results from FETAL LIVER DISEASE

The NH Phenotype



Epidemiology of NH



Contrasting clinical features of NH and viral infection in cases of neonatal liver failure

	GALD-NH	Perinatal viral infection
Premature birth	Most (70-90%)	Usual population incidence
Oligohydramnios	Most (70-90%)	Exceedingly rare
Intrauterine growth restriction	Most (70-90%)	Rare
Ascites	Common (40-60%)	Exceedingly rare - never
Anasarca or hydrops	Uncommon (10-20%)	Exceedingly rare - never
Patent ductus venosus	Most (70-90%)	Exceedingly rare - never
Hepatomegaly	Uncommon (10-20%)	Common
Hard liver	Always when palpable	Exceedingly rare - never
Splenomegaly	Uncommon (10-20%)	Common though often mild

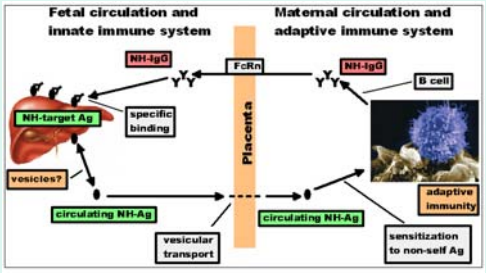
Lessons learned
From clinical observation

- NLF is a big part of PALF
- Not all NLF is NALF
- Birth is an ambiguous dividing line in the continuum of fetal-neonatal liver disease
- Some of NLF is the extension of fetal liver disease – NH is the prototype for this paradigm

Theorem: Gestational Alloimmune Liver Disease is the cause of NH

- NH is congenital and familial but not inheritable.
  - The apparent recurrence rate of lethal disease after the index case is as high as 92%
  - Many women have had several normal babies prior to the index case
  - Several instances of women having affected offspring with different fathers
  - No sisters of affected women ever reported to have a baby with NH
  - Offspring of women who survived NH are unaffected
- In the universe of known causes of fetal liver injury only **gestational alloimmune disease** can explain the phenomenon of NH

Proposed mechanism of NH involving maternal alloimmunity



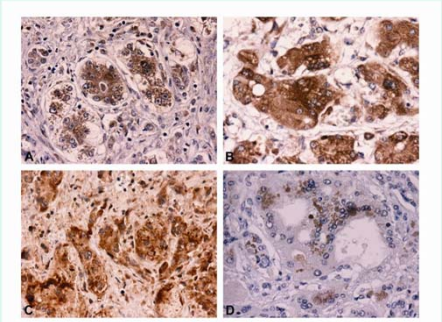
NH alloantibodies produce fetal liver injury via engagement of the fetus' innate immune system

- IgG binds to cell surface antigens
- Fetal complement is fixed leading to classical pathway activation of the terminal complement cascade
- Hepatocyte plasma membrane injury and cell death result from MAC-attack

Assessing Complement-Mediated Injury in Human Tissues

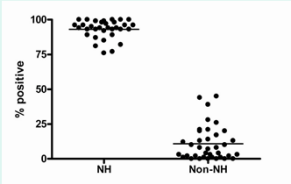
- In assembly of MAC a stable complex is formed comprising the complement elements C5b through 9
- The C5b-9 complex is a neoantigen that has been isolated and antibodies raised against it
- Specific anti-C5b-9 antibodies do not bind to any of the complement elements, only to the neoantigen
- Finding C5b-9 complex on or in a cell provides indisputable evidence that MAC has been assembled on the cell surface

Hepatocyte MAC in Cases of NH



Defining Gestational Alloimmune Liver Disease

Extensive hepatocyte MAC is the defining feature of GALD.



If the liver injury is due to GALD and there is extrahepatic siderosis, it is GALD-NH.

Atypical GALD scenarios

- Fetal death
- Unexplained death in the newborn
- Acute liver failure

GALD as a cause of fetal acute liver failure

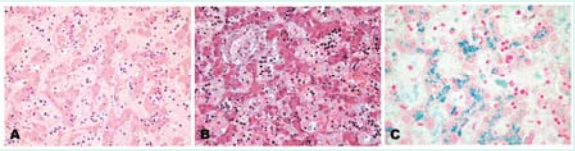
- Fetal liver failure has never been described or parameters for diagnosis defined
- IUFD must define “failure” as other measures of liver function are unavailable
- Conventional diagnosis of NH may not apply as siderosis is a secondary and perhaps late event

GALD-Associated Abrupt Fetal Demise

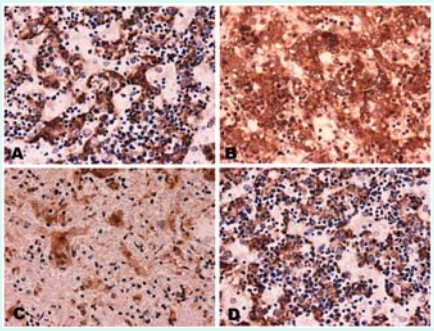
Eight cases with no fetal distress or other evidence of chronic or subacute liver disease

Case #	Gest age (weeks)	Birth Status	Sibling with NH	Extrahepatic Siderosis
1	22	live birth	yes	no
2	22	stillbirth	no	no
3	34	stillbirth	no	yes
4	30	live birth	yes	yes
5	22	stillbirth	no	yes
6	20	stillbirth	no	yes
7	20	stillbirth	no	no
8	21	stillbirth	no	yes

Confluent Hepatic Necrosis in a Fetus



C5b-9 in Hepatocytes Shows These Cases to be GALD



Stillbirth: Intrauterine fetal demise

- Late IUFD defined as loss in second half of pregnancy
- Affects ~1 in 150 pregnancies in the US
- Cause generally not determined
  - Chromosomal defects in ~10%
  - Hypercoagulable states may cause ~7%
- Approximately 20% of pregnancies with IUFD will recur

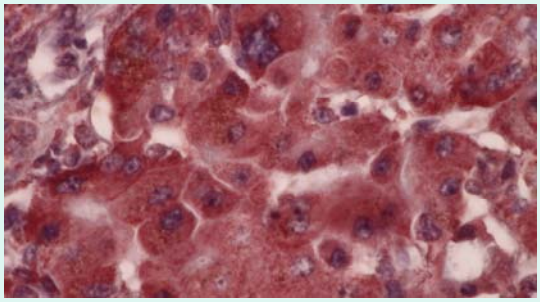
What about GALD as a cause of IUFD?

- Women with a baby affected by NH have a rate of late IUFD far in excess of normal – **one in seven pregnancies ended in fetal loss between 16 weeks and term**
- Re-examination of stillbirth autopsies has resulted in numerous GALD and/or NH diagnoses
- Case control study needed to determine if GALD is a common cause of IUFD
- If GALD causes 10% of IUFD, it would be 10-times more prevalent than biliary atresia as a cause of fetal-infant morbidity/mortality

GALD in obscure neonatal death

- 16-year autopsy study; death by 90 days of age
- 7 cases identified with “obscure” cause of death
  - Final pathological diagnoses: anasarca or hydrops in 4 and hemorrhagic diathesis in 3
  - Liver pathology reported as post-mortem change
- Siderosis negative in 6
- C5b-9 stain 4+ positive in all cases

Case of full-term AGA newborn with ALF on DOL 4 and death DOL 8.



C5b-9 immunohistochemistry of liver in a case of GALD-NH

Lessons learned

Exploring mechanisms of fetal liver injury

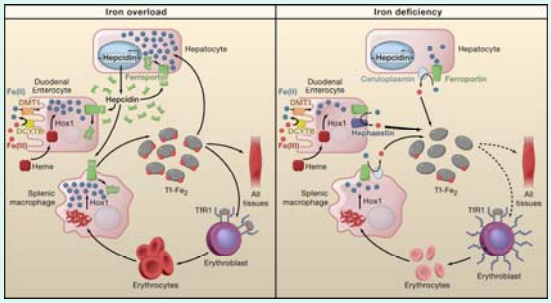
Gestational alloimmune liver disease

- It happens
- First known “pure” antibody mediated liver disease
- Cause of acute liver failure in fetuses and newborns
- Cause of typical NH with “congenital cirrhosis”
- Mechanism has led to prevention and improved medical therapy
- Mechanism opens new vistas for further improvement in detection and therapy

Where does the iron come from?

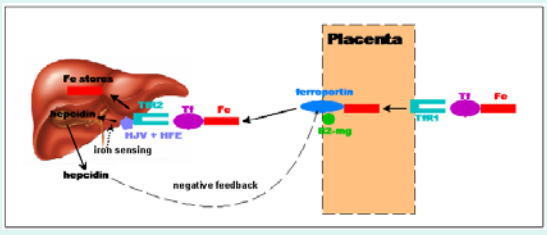
- From the mother of course
- How is regulation of maternal-fetal iron flux disturbed?
- Why the specific tissue distribution of siderosis?

Regulation of Systemic Iron Homeostasis



Cell 142, July 9, 2010

Regulation of fetal iron homeostasis



The fetal liver regulates the flux of iron from the relatively immense maternal pool of iron via the iron-sensitive secretion of hepcidin resulting in negative feedback on ferroportin-permissive iron extrusion from the placenta

HAMP expression in GALD-NH versus normal newborn and fetal liver

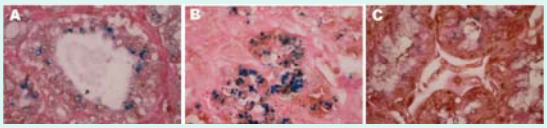
Normal newborn iron transporter expression

	TfR2	DMT1	Zip14	Ferroportin
Hepatocytes	+++	+	+++	+++
Pancreatic acinar cells	-	-	+++	+
Thyroid follicle epithelia	-	-	+++	-
Hassall's corpuscles	-	+	+++	-
Myocardium	-	-	++	++
Adrenal cortex	+	-	+++	++
Renal tubular epithelium	+	-	++	++
Respiratory epithelium	++	-	+++	+++
Small intestinal epithelium	++	+	+ - +++	+++
Spleen pulp	+++	-	+	+++

Relationship between siderosis and iron transporter expression in GALD-NH

	Siderosis	Zip14	Ferroportin
Pancreatic acinar cells	++	+++	+
Thyroid follicle epithelia	++	++	-
Hassall's corpuscles	++	++	+
Myocardium	+	++	++
Adrenal cortex	+	++	+
Renal tubular epithelium	+	+	+
Submucosal salivary glands	++	+++	-

Colocalization of ZIP14 expression and siderosis in GALD-NH



A = thyroid
B = exocrine pancreas
C = minor salivary gland

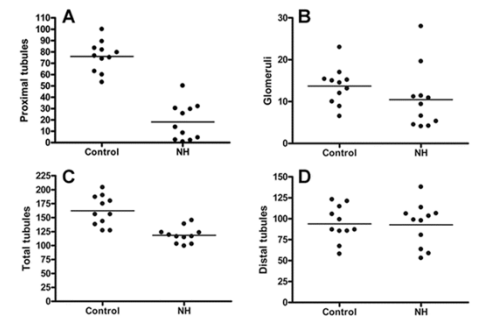
Mechanism of iron overload and tissue siderosis in NH

- Severe fetal liver disease no matter the cause may result in low HAMP expression
- Hepcidin deficiency leads to unrestricted placental iron flux and iron overload
- Iron overload with or without low transferrin leads to excess NTBI
- Cells capable of taking up NTBI via ZIP14 and incapable of eliminating iron via ferroportin develop siderosis

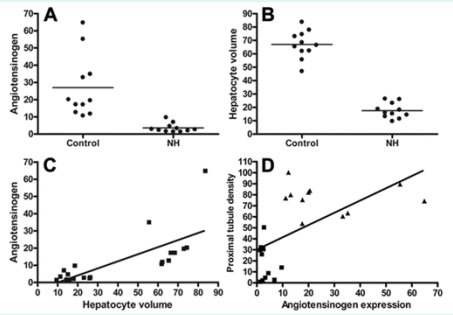
Renal proximal tubular dysplasia in NH

- Must be more than coincidence
- Hypothesis: renal tubular dysplasia is a consequence of fetal liver injury as is NH
- Development of proximal tubules is dependent upon angiotensinogen
- Angiotensinogen is produced entirely by the liver
- Can we show that GALD produces angiotensinogen deficiency and thus impairs proximal tubule development?

Quantitative morphometry of kidney shows that proximal tubule density is specifically reduced in GALD-NH relative to controls



Liver angiotensinogen expression is markedly reduced in NH and correlates with proximal tubule density



Mechanism renal proximal tubular dysplasia in GALD-NH

- Low angiotensinogen expression correlates with severity of fetal liver injury
- Angiotensinogen deficiency impairs development of proximal tubules
- Severe impairment of developmental process may lead to critically reduced density of proximal tubules
- The condition called renal proximal tubular dysplasia is just the tip of the iceberg

Lessons learned

Exploring mechanisms of epiphenomena

- Iron overload is a symptom of fetal liver disease
  - HAMP/hepcidin deficiency leads to iron overload
  - Classification of NH among the hereditary hemochromatosis disorders is incorrect
  - Iron probably plays no role in tissue injury in NH
- Fetal liver injury affects renal development
  - Angiotensinogen deficiency is the cause
  - Renal proximal tubular dysplasia is the extreme

Remaining questions

Many careers worth

Those we are currently exploring

- What mechanisms lead to fibrosis without inflammation
- What is the fetal liver antigen
- What it is the mechanism of maternal sensitization and what determines its frequency with which it occurs

Acknowledgements

- Sue Kelly – clinical research coordinator
- Padmini Malladi – research scientist
- Xiaomin Pan – senior technician
- Silvana Bonilla, Josh Prozialeck and Aki Asai – fellows
- The families of children with NH and physicians everywhere caring for them