Congenital diaphragmatic hernia: a genetic-environmental mismatch

Retinoic acid in human CDH; is it really relevant

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**CONGENITAL ANOMALIES AT BIRTH**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Environment</th>
<th>Maternal metabolism</th>
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</thead>
<tbody>
<tr>
<td>NO</td>
<td></td>
<td></td>
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<tr>
<td>YES</td>
<td></td>
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</tbody>
</table>

**THE "SOLUTION" OF CONGENITAL ANOMALIES DEPENDS ON**

- Increased knowledge of risk factors (nutrition?; environmental exposure)
- Integrating knowledge of cell biological regulatory mechanisms and human DNA/gene data
- Structured interdisciplinary follow-up into adolescence and "targeted" genetic counseling (second generation)
**Strategy**

1. CHD+ patients
2. Abnormal karyotype? yes
   - Refine breakpoints
   - Candidate region → Candidate genes
3. Abnormal deletions / duplications
   - Search for smaller deletions / duplications
4. No
   - Mutation-analysis
   - Larger group of CDH patients

**Techniques available**

- Standard G-banding
- Fluorescent In Situ Hybridization (FISH)
- Array-based Comparative Genome Hybridization (Array-CGH): BAC, oligo, …

**CDH and deletion 15q**

- Literature:
  - Numerical anomalies: trisomy 13, 18 & 21
  - Structural anomalies: almost all chromosomes (except chr 19)

- Rotterdam cohort:
  - Numerical anomalies
  - Structural anomalies: as described in literature

- Deletion 15q: 3 patients

**CDH and chromosome 15q anomalies**

- Deletion 15q: 3 patients
- Ring chromosome 15
CDH and chromosome 15q anomalies

- 7 patients with CDH and deletion 15q (∗)
- 3 patients with deletion 15q, but without CDH (#)

15q CDH critical region

3D viewer: chromosome 15q

3D View CDH critical region

Other patients with deCOUP-TF2 and CDH?

- Collaboration Baylor College of Medicine (B. Lee & D. Scott)
- Array-CGH: whole-genome coverage, resolution 300 kb!
- Patient: left-sided Bochdalek-CDH, severe cardiac defect, † 7 hours

- 46,XX.del(15)(q21)(q37;q26) ??
- COUP-TF2 deleted !!
Additional prenatal cases

- Baby girl: left-sided CDH, IUGR, limb abnormalities, dysmorphic features, t. 1h

25 cases of deletion 15q and CDH

> 450 reported chromosomal anomalies

Common pathway CDH candidate genes?
Role for COUP-TF2 in etiology CDH?

- Transcription factor
- Involved in retinoic acid metabolism (sequesters RXR)
- Interacts with FOG2
- Essential for limb- and skeletal muscle development
- COUP-TF2 \( \rightarrow \) E9 (arrest of cardiac development)
- COUP-TF2 \( \rightarrow \) 75% \( \uparrow \) neonatally
  (Perreira, Tsai et al., 1999)

Yes: COUP-TF2 mouse model of CDH

- Tissue specific ablation
- Ablation in foregut mesoderm (incl. posthepatic mesenchymal plate) \( \rightarrow \) left-sided CDH
- Few patients....
- Mutation analysis COUP-TF2
- 50 patients normal
- Mechanism?

Where did this approach bring us ???

>450 chromosomal aberrations

Candidate genes ??

Mutation analysis

- COUPTFII (Tsai et al. KO mouse model)
- >50 CDH pl for 15q gene (COUPTFII)
- Total of research to date: >500 pl for 15q, 50 pl for COUPTF II, GATA4, FOG2, ROBO3/4...
- STR_MAP (Donahoe-Barron) & LRP2 (PDAC) - recessive mutation

Only sporadic small (bp) changes!

The congenital diaphragmatic hernia network

Candidate genes involved in CDH related phenotypes in mice or humans are yellow

Donahoe PK. 2009 Birth Defects Res
Conclusions

- Chromosome 15q26 significant region in CDH
- Contains several "retinoic acid genes"
- Several 15q genes might play an important role in CDH
- COUP-TFIi important candidate gene
- Most important pathway: Retinoic Acid Signaling Pathway
- Also influence Neural Crest Cell development
- Collaborative studies are needed to collect sufficient number of patients
- Functional studies are needed: human RA levels
- human diaphragm tissue

Plasma and cellular forms of retinoid and major reactions in retinoid metabolism.

<table>
<thead>
<tr>
<th>Retinoid</th>
<th>Cell Type</th>
<th>Metabolism</th>
<th>Retinoid Metabolism</th>
<th>Metabolites</th>
<th>Products</th>
<th>Target Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicron</td>
<td>RBP</td>
<td>LDL</td>
<td>Albumin</td>
<td>Retinoic acid</td>
<td>S-carotene</td>
<td>RXR</td>
</tr>
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</table>


History

- 40's: Vitamin A
- 60's: Surgical models
- 80's: Nitrofen
- 90's: Genetics
- 2000: RA pathway?
Distribution of retinoid receptors in human lungs

Steroid hormone receptor expression in normal and Nitrofen induced hypoplastic lungs at 15 days of gestation

Suggestive evidence for vitamin A and related genes in the etiology of CDH

Compound mutants for retinoic acid receptor (RAR) beta and RARA alpha1 reveal developmental functions for multiple RAR beta isoforms
Luo J et al. 1996 Mechanisms of Development 55;33-44

Prenatal retinoic acid upregulates pulmonary gene expression of COUP-TFII, FOG2 and GATA4 in pulmonary hypoplasia of Nitrofen induced CDH.

Vitamin A deficiency (VAD), teratogenic, and surgical models of congenital diaphragmatic hernia (CDH)

Suggestive evidence for vitamin A and related genes in the etiology of CDH

Children CDH: low retinol and RBP
Mothers CDH: high retinol and RBP

Literature

<table>
<thead>
<tr>
<th></th>
<th>Cord Retinol (ng/L)</th>
<th>Cord RBP (mg/L)</th>
<th>Maternal Retinol (ng/L)</th>
<th>Maternal RBP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>293 +/- 2 (n=7)</td>
<td>27 +/- 2 (n=5)</td>
<td>288 +/- 36 (n=7)</td>
<td>28 +/- 5 (n=7)</td>
</tr>
<tr>
<td>CDH</td>
<td>179 +/- 40 (n=7)</td>
<td>11 +/- 2 (n=5)</td>
<td>434 +/- 46 (n=7)</td>
<td>51 +/- 3 (n=5)</td>
</tr>
</tbody>
</table>

Major et al. 1998
Retinol in pregnancy

Cikot et al., Br J Nutr. 2001

Retinoic acid in human CDH; is it really relevant
A proof of principle study

Retinoid study

- Original initiative by J Greer, Edmonton
- Primary objective:
  Determine vitamin A status in pregnant women at diagnosis and in mothers and children at delivery
- Cohort study
- Control group
  - Same centre, presentation (year, month)
  - Parity, maternal age, gestational age, ethnicity, fertilization
- \( n = +/- 50 \)

Primary objective:
Determine vitamin A status in pregnant women at diagnosis and in mothers and children at delivery

Cohort study
Control group
- Same centre, presentation (year, month)
- Parity, maternal age, gestational age, ethnicity, fertilization
- \( n = +/- 50 \)
the Rotterdam protocol: HERNIA-study

- Blood mother Retinol, RBP, β-ctn
- Amniotic fluid Retinol, RBP, β-ctn
- Cord blood Retinol, RBP, β-ctn
- Blood child DNA
- Blood father DNA

- Food frequency questionnaire
- Questionnaire mother + child
- Questionnaire father

T = 0
- Diagnosis CDH
- Week 8

T = 1
- Delivery
- Week 20

T = 2
- Week 38

T = 3
- 15 months postpartum
- Blood mother

15 months
- no pregnancy effects left
- usually no breastfeeding
- Season comparable to period of conception
- Assumption: nutrition stays the same

HERNIA – study

T = 0
- Week 8
- Diagnosis CDH
- 33 cases
- 58 controls

T = 1
- Delivery
- Blood mother
- 43 cases
- 37 controls

T = 2
- Blood mother

T = 3
- 15 months postpartum
- Blood mother
- 10 cases
- 29 controls

Lung and diaphragm development: common pathways?

Lung development

NCC development

Diaphragm development
In Conclusion:

- Animal data suggest disturbances in retinoic acid metabolism as an etiological factor in CDH.
- The supplementation of retinoic acid diminishes the incidence of CDH in animal models.
- A number of candidate genes in humans have been identified and are known to be involved in retinoic acid metabolism.
- Only one study with small numbers suggested a pivotal role of disturbed vitamin A metabolism in the etiology of CDH.
- Patient recruitment of “the proof of principle study” is finalized, and results will be available within 6 months.

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University of Alberta, Canada:
J. Greer

Additional prenatal cases 1

Candidate genes:
Additional prenatal cases

- Baby girl: left-sided CDH, IUGR, DORV, dysmorphic features, † 7h
- Prenatal karyotype: 46,XX
- Postnatal array-CGH: 46,XX,der(15)(q26.2)(q37.3)
  
FISH on cultured amniocytes:

MLPA: