Dr. Arthur Beaudet

Hosted By:
U.S. Department of Health and Human Services
National Institutes of Health
EPIGENETICS

• The study of changes in gene function that are stable and heritable (or potentially heritable as in terminally differentiated neurons) and do not entail a change in DNA sequence.
EPIGENETICS AS THE FONT OF DNA SEQUENCE (LETTERS)

Epi-genetics on top of genetics
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 expression from the maternal and paternal alleles of a diploid locus.

Well described in plants and mammals, but not in egg-laying vertebrates.
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.
- Mule vs hinney.
Phenotype

Environment

Genotype

Stochastic events

Epigenotype

Phenotype
Phenotype

Health or disease

Epigenotype

Genotype

Stochastic events

Environment

Genetic defects/susceptibility

Epigenetic/ imprinting defects

Phenotype

Health or disease
PRADER-WILLI SYNDROME

- Infantile hypotonia & feeding problems
- Hyperphagia & obesity
- Moderate MR
- Gonadal hypoplasia
- Short stature
ANGELMAN SYNDROME

Severe learning def.
Absent speech
Happy disposition
Seizures
Ataxia / tremor
Microcephaly
Prominent mandible

Behavior = anti-autism and like autism

Learning = like autism
<table>
<thead>
<tr>
<th>Prader-Willi syndrome</th>
<th>Angelman syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td>Genetic</td>
</tr>
<tr>
<td>Epigenetic</td>
<td>Epigenetic</td>
</tr>
<tr>
<td>Paternal deficiency</td>
<td>Maternal deficiency</td>
</tr>
<tr>
<td>15q11-q13</td>
<td>15q11-q13</td>
</tr>
<tr>
<td>70%</td>
<td>70%</td>
</tr>
<tr>
<td>30%</td>
<td>Rare</td>
</tr>
<tr>
<td>or</td>
<td>or</td>
</tr>
<tr>
<td>or</td>
<td>or</td>
</tr>
</tbody>
</table>
DISEASE DEFINITIONS

- **Genetic disease** – an aberration in nucleotide sequence causing a disease phenotype
- **Epigenetic disease** – an aberration in epigenotype (stable / heritable change in gene expression) causing a disease phenotype in the absence of nucleotide aberration
- **Both** – through altered gene expression
GENOMIC IMPRINTING IN 15q11-q13

- Nonimprinted
- PWS
- AS

CH₃
UBE3A ENCODES E6-AP

- E6-AP discovered as a protein that interacts with papilloma E6 to promote degradation of p53
- E6-AP is a ubiquitin-protein ligase; gene symbol *UBE3A*
- Maternal deficiency is the cause of AS
- Imprinted with tissue-specific silencing of the paternal copy in brain
RELATIONSHIP OF SNRPN, UBE3A AND IMPRINTING CENTER

Invariant
Mat = meth
Pat = unmeth

Plasticity
Brain = unmeth/meth
Other = meth

PO-DMR  UBE3A Antisense  TS-DMR

UBE3A sense

SNRPN

AS-IC  PWS-IC

CpG

1 or 2 of 11 autism brains abnormal vs 0/60 control brains

Yong-hui Jiang
Imprinting

Mom → Eggs → Child → Sperm → Dad

Erase and reset

Maintain
Mom

Sperm

Erase and reset

Angelman Child

Dad

ICSI

Eggs

Maintain

Imprinting defect
Imprinting defect

Mom

Autism? Child

Erase and reset

Dad

Eggs

Sperm

Maintain
An epigenetic defect can give the same phenotype as a genetic defect

<table>
<thead>
<tr>
<th>Angelman</th>
<th>Deletion</th>
<th>UPD</th>
<th>Imprint</th>
<th>UBE3A</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td>Epi-genetic</td>
<td>Mixed</td>
<td>Genetic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity of types of defects causing one phenotype

*↓*
MTHFR deficiency in a patient with typical AS but no identifiable defect.

Arn et al., 1998
PMID 9605586

Does MTHFR deficiency silence (maternal) UBE3A?
**Normal**

- **Brain**: CAGT
- **Deletion**: CAGT
- **UPD**: CAGT
- **Imprint def.**: CAGT

**Angelman brain**

- **Brain**: CAGT
- **Deletion**: CAT
- **UPD**: CAGT
- **Imprint def.**: ????

- **Non-CNS**: CAGT
- **UBE3A mut.**: CAGT
- **MTHFR -/-?**: CAGT
- **Other**: ????
AUTISM (NARROW) AND AUTISM SPECTRUM DISORDER (BROAD)

• A neurological or brain disorder that profoundly affects a person’s ability to communicate, form relationships with others and respond appropriately to the environment.

• Look perfectly normal.

• Abnormal behaviors such as hand flapping.
GENETIC AND OTHER FACTORS IN AUTISM?

- Incidence 10-15 in 10,000 (16-24 in 10,000 males; 4 to 6 in 10,000 females)
- Male predominance (4:1 ratio)
  - Unknown
- High concordance MZ twins but low in DZ twins
  - 10-20 loci?
- Association with higher mat. education
  - Unknown
AUTISM, 15q11-q13, AND GENOMIC IMPRINTING

- Maternal but not paternal dup15q11-q13 cause autism; often maternally inherited
- Inv dup 15q causing autism are always of maternal origin
- Evidence for genomic imprinting & parent of origin effect
- Nurmi et al., linkage disequilibrium with D15S122 at 5’-UBE3A
- Shao et al., LOD 4.71 at GABRB3 using ordered-subset analysis (OSA); 5’ of UBE3A; maternal sharing
Figure 1. Distribution of birth dates of regional center eligible persons with autism

Without evidence for an artificial increase in autism cases, we conclude that some, if not all, of the observed increase represents a true increase in cases of autism in California, and the number of cases presenting to the Regional Center system is not an overestimation of the number of children with autism in California.

http://www.dds.cahwnet.gov/autism/mindreport.cfm
• “Therefore, from available evidence it can be concluded that recent rates for both ASD (autism spectrum disorder) and autism disorder are 3 to 4 times higher than 30 years ago.”
• Unless comparisons also control rigorously for changing case definitions, interpretation of differences in prevalence rates over time and across surveys will be virtually impossible.

• Moreover, there is strong evidence that differences in methods for case finding can account for a huge proportion of the variability of prevalence estimates between surveys.

• Claims about an epidemic of autism and its putative causes have the most weak empirical support.
<table>
<thead>
<tr>
<th>Disorder</th>
<th>MZ</th>
<th>DZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Down syndrome</td>
<td>100%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Achondroplasia and Rett de novo</td>
<td>100%</td>
<td>nil</td>
</tr>
<tr>
<td>Autism narrow</td>
<td>~60%</td>
<td>nil</td>
</tr>
<tr>
<td>Autism broad</td>
<td>~90%</td>
<td>~10%</td>
</tr>
<tr>
<td>De novo gametic or preMZ imprinting defect</td>
<td>100%</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>
THE USUAL GENETIC HYPOTHESIS FOR AUTISM

- “These results are most compatible with a model specifying a large number of loci (perhaps $\geq 15$) and are less compatible with models specifying $\leq 10$ loci.”
ALTERNATIVE HYPOTHESIS

• Autism is an oligogenic disorder (perhaps even one major locus with modifiers) caused in most cases (e.g., singleton families) by de novo genetic or epigenetic defects arising in germ cells or the early embryo (prior to MZ twinning).

• Over-expression of *UBE3A* may be the unifying pathophysiology; the major gene?
EVIDENCE

- Mat. but not pat dupes 15q cause autism
- Increased sharing of parental alleles in affected sib pairs
- Tissue specific DNA methylation in 15q
- Abnormal DNA methylation in 1-2 of 11 autism brains
- Hypomorphic allele for \textit{MTHFR} may be protective
Homocysteine

5MTHF

BETAINE

(m-B12)

meth synth MTR/MTRR

Dimethylglycine

Choline

5,10-MTHF

THF

Pyrimidine biosynthesis

Purine biosynthesis

MTHFR

MTHFD1

DHFR

FOLIC ACID

METHYL TRANSFERASES

MTHFR

MTHFD1

DHFR

SAM

DNA

RNA

S-adenosyl-methionine

Protein

Lipids

SAH
High folic acid  Low folic acid

methylation

Wolff et al., FASEB J 1998; 12:949-957
MTHFR AND FOLATE

- Use transmission disequilibrium test (TDT) to avoid matched control problems and allow use of parent child trios
- Is the hypomorphic V allele of MTHFR protective for autism?
A. Germ cells

- High Methylation
- Low Methylation
- PGCs
- E13

B. Embryo

- Mature oocytes
- Fertilization
- DNA replication
- Blastocyst
- EM
- EX
- High Methylation
- Low Methylation
- Developmental time
Table 3

Grandparental Origin of the Chromosome Carrying the Imprinting Defect

<table>
<thead>
<tr>
<th>Origin</th>
<th>AS</th>
<th></th>
<th>PWS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC Mutation</td>
<td>No IC Mutation</td>
<td>IC Mutation</td>
<td>No IC Mutation</td>
</tr>
<tr>
<td>Maternal:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grandfather</td>
<td>5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grandmother</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Paternal:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grandfather</td>
<td>0</td>
<td>0</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Grandmother</td>
<td>0</td>
<td>0</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
• Autistic children more often inherit paternal 15q11-q13 from their grandmother than from their grandfather because the need to switch imprint increases risk of an imprinting defect.
• Unpublished model for autism presented
Normal

Brain
CAGT
CAGT
CAGT

Non-CNS
CAGT
CAGT

Autism brain

Interstitial dup
CAGT
CAGT
CAGT

Inv dup
CAGT
CAGT
CAGT

Pat imprint def?
CAGT
CAGT

Mat hypermorph?
CAGT
CAGT

PWS UPD
CAGT
CAGT
ICSI AND IMPRINTING

- 19 Hits in PubMed
- 1995 – Theoretical concerns
- 1998 – Yanagimachi: success in mouse even with round spermatids and secondary spermatocytes
- 1998 – Steirteghem: no imprinting defects found in first 165 cases
- 2000 - Steirteghem: normal DNA methylation at 15q11-q13 normal in 95 children
ICSI AND IMPRINTING

- 2002 – Horsthemke: two cases of Angelman “sporadic” imprinting defect
- 2003 – 6 of 149 Beckwith-Weidemann (3 ICSI & 3 IVF) vs expected 1.7; $P \sim 0.01$; similarity to large offspring syndrome.
- 2003 – Another case of AS and ICSI
- About 50 % of ART uses ICSI at present.
<table>
<thead>
<tr>
<th>RECOGNITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWS/AS/Autism</td>
</tr>
<tr>
<td>• Trilochan Sahoo</td>
</tr>
<tr>
<td>• Yong-hui Jiang</td>
</tr>
<tr>
<td>• Jan Bressler</td>
</tr>
<tr>
<td>• Dani Bercovich</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Igne Buyse</td>
</tr>
<tr>
<td>• David Stockton</td>
</tr>
<tr>
<td>• Ben Roa</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Greenwood SC (SCAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Roger Stevenson</td>
</tr>
<tr>
<td>• Ron Michaelis</td>
</tr>
<tr>
<td>• Dick Schroer</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AGRE NIMH/Stanford</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistical analysis</td>
</tr>
<tr>
<td>• Richard Speilman</td>
</tr>
</tbody>
</table>