Nutrition is a fundamental pillar of human life, health and development across the entire life span. From the earliest stages of fetal development, at birth, through infancy, childhood, adolescence and on into adulthood and old age, proper food and good nutrition are essential for survival, physical growth, mental development, performance and productivity, health and well being.
Gene polymorphisms and Folate metabolism as maternal risk factors for Down syndrome child

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• First description T21: Lejeune et al 1959
• Cloning: Lieman et al 1982
• Complex genetic disorder with many metabolic disorder
• Most common cause of Human MR
• Birth frequency 1:700/1,800
• 1/150 conceptions have T21
• 80% lost during pregnancy
• Economic cost and burden
How does it occur?

- Failure of chromosome 21 to segregate
- Attributed to the presence of three copies of chromosome 21
- Maternal origin in 95% cases (Antonarakis, 1991)
- Maternal age is the only well established risk factor for Down syndrome completion of sequence and cSNP map: (Hattori et al, 2000 and Samuel et al 2001)
Therefore what do we need?

• Gene involved in the phenotype
• Molecular pathophysiology
• Its prevention and management?
Prevention of DS Birth

A  Chorionic villus sampling
B  Triple/Double marker study
C  Amniocentesis

**Perinatal diagnosis**

- MTHFR & MTRR study before conception??
- Folate /Hcy status
- Dietary modifications??
DNA Methylation and Synthesis

• Folic acid is essential for the de novo synthesis of nucleotide precursors for normal DNA synthesis & methylation

  Christman et al. (1993) Carcinogenesis, 14:551-7

• MTHFR: Regulation of cellular methylation
DNA Synthesis

- dTTP
- dUTP
- DHF
- 5,10-CH₂-THF
- 5,10-CHO-THF
- ADENINE → dATP
- GUANINE → dGTP
- THF: Tetrahydrofolate
- S: SERINE, G: GLYCINE

DNA Methylation

- Methionine
- SAM → CH₃-CYT-DNA
- SAH: S-ADENOSYLHOMOCYSTEINE
- MTHFR
- DNA Methyltransferase

Key Reactions:

1. SAH Hydrolase
2. Methionine Synthase
3. Cystathione ?-Synthase
4. MTHFR
5. DNA Methyltransferase
What is MTHFR

- MTHFR: 1p36.3
- catalyze-synthesis of 5-MTHF the methyl donor (B12 dependant) for remethylation of homocysteine to methionine
- Mth: precursor for SAM, a donor for DNA, RNA and phospholipid
- MTRR: 5p15.3: functional state
MTHFR Gene (polymorphism location)

- Exon 4:
- 677 C-T
- Alanine to valine
MTRR Gene (polymorphism location)

Normal

A  K  A  I  A  E  E  M  C
GCA  AAG  GCC  ATC  GCA  GAA  GAA  ATG  TGT

Polymorphism

A  K  A  M  A  E  E  M  C
Abnormal Folate metabolism and mutation in the MTHFR gene may be a maternal risk factor for Down syndrome

Study Population

• Blood samples from young women (below 40 yrs) with karyotypically confirmed trisomy 21 baby
• Age matched controls
• Dietary habits/vitamin supplementation
Sample Collection

- Blood from cases (n=157) as well as age matched controls (n=150). (Hobbs et al 2000)

Genotype Analysis

- Genomic DNA by standard extraction method
- PCR followed by restriction enzyme digestion for MTHFR/MTRR gene polymorphism
- Thiols by HPLC
# Association between Maternal MTHFR genotypes and Down Syndrome

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No(%) of case Mothers n=157</th>
<th>No (%) of control Mothers (140)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>51(32)</td>
<td>67(48)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>84(54)</td>
<td>59(42)</td>
<td>1.87</td>
<td>1.14-3.06</td>
<td>.02</td>
</tr>
<tr>
<td>TT</td>
<td>22(14)</td>
<td>14(10)</td>
<td>2.06</td>
<td>.96-4.43</td>
<td>.09</td>
</tr>
<tr>
<td>CT or TT</td>
<td>106(68)</td>
<td>73(52)</td>
<td>1.91</td>
<td>1.19-3.05</td>
<td>.01</td>
</tr>
</tbody>
</table>

## Maternal MTRR genotype in DS and control mothers

*Am J Hum Genet, 67:623, 2000*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No of control (139)</th>
<th>No of case (145)</th>
<th>Odd ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>39 (28)</td>
<td>26 (18)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>68 (49)</td>
<td>64 (44)</td>
<td>1.41</td>
<td>.77-2.56</td>
</tr>
<tr>
<td>GG</td>
<td>32 (23)</td>
<td>55 (38)</td>
<td>2.57</td>
<td>1.33-4.99</td>
</tr>
<tr>
<td>AG/GG</td>
<td>119 (82)</td>
<td>100 (72)</td>
<td>1.78</td>
<td>1.02-3.13</td>
</tr>
</tbody>
</table>
Maternal MTHFR study in Indian mothers

<table>
<thead>
<tr>
<th>Subjects</th>
<th>CC Genotype</th>
<th>CT Genotype</th>
<th>TT Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(13)</td>
<td>6/13 (46%)</td>
<td>6/13 (46%)</td>
<td>1/13 (7.6%)</td>
</tr>
<tr>
<td>Case(17)</td>
<td>11/17 (64.7%)</td>
<td>6/17(35%)</td>
<td>--</td>
</tr>
</tbody>
</table>
**Plasma Hcy and Methionine levels**

(James et al 1999: Am J Clin Nutrit)

<table>
<thead>
<tr>
<th></th>
<th>Control mothers</th>
<th></th>
<th>DS mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC (n=9)</td>
<td>CT+TT (n=32)</td>
<td>CC (n=9)</td>
</tr>
<tr>
<td>Homocysteine (umol/L)</td>
<td>7.9</td>
<td>8.3</td>
<td>10.9</td>
</tr>
<tr>
<td>SD</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Plasma Methionine</td>
<td>36.7</td>
<td>33.6(^i)</td>
<td>23.2</td>
</tr>
<tr>
<td>SD</td>
<td>2.4</td>
<td>3.8</td>
<td>2.1</td>
</tr>
</tbody>
</table>
### Preliminary Findings

*unpublished data (Sheth and James et al)*

<table>
<thead>
<tr>
<th></th>
<th>HOMOCYSTEINE (um/L)</th>
<th>METHIONINE (um/L)</th>
<th>GSH (um/L)</th>
<th>CYS-GLU (um/L)</th>
<th>CYSTEINE (um/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DS MOM (Ind)</strong></td>
<td>8.3±2.79 (18)</td>
<td>21.528±6.052</td>
<td>4.686 ±0.996</td>
<td>28.898 ±5.272</td>
<td>251.61±73.730</td>
</tr>
<tr>
<td><em><em>CONTROL</em> MOM (Ind)</em>*</td>
<td>6.499±1.837 (11)</td>
<td>25.089±5.163</td>
<td>5.130±1.314</td>
<td>26.069±7.039</td>
<td>248.600±32.420</td>
</tr>
<tr>
<td>USA DS MOM</td>
<td>12.01±4.073</td>
<td>25.424±7.346</td>
<td>7.567±2.381</td>
<td>43.563±7.398</td>
<td>200.11±25.194</td>
</tr>
</tbody>
</table>
Observations

- Mild Elevated Homocysteine levels: Independant risk factor
- MTHFR polymorphism: 1.9 fold? risk of DS
- MTRR polymorphism: 1.78 fold? risk of DS
- Low MTHFR polymorphism in Indian mothers
Increased Risk of Meiotic Non-disjunction and Down Syndrome Is Associated With

- Folate deficiency (genetic and/or nutritional deficiencies affecting negatively on folate metabolism)
- DNA hypomethylation
- Other autosomal trisomies: No association (Hassold et al: Am J Hum Genet: 2001)
Conclusion

* Mutation in MTHFR(677C-T) and/or MTRR (66 A-G) leads to impaired folate metabolism

* ?Hcy and ?Mth in mothers of DS child independent of gene polymorphism

** Maternal risk factors for meiotic nondisjunction
• Polymorphism in other candidate genes in the folate pathway needs to be studied

• Interactions with other micronutrients: Folate/methyl metabolism

• Greater opportunity to improve public health and prevention of DS child
Health and sustainable human development are equity issues. In our globalized 21st century, equity must begin at the bottom, hand in hand with healthy nutrition.

Gro Harlem Brundtland
Director General, WHO
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