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

W H O

Gene polymorphisms and Folate metabolism as maternal risk factors for Down syndrome child

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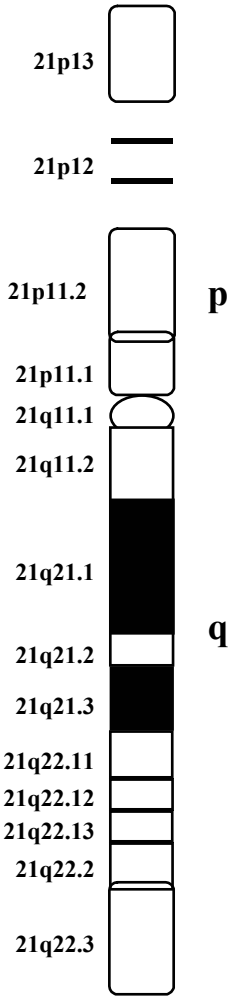
HC 21

- 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 pregnacy
- Economic cost and burden



Chromosome 21

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??

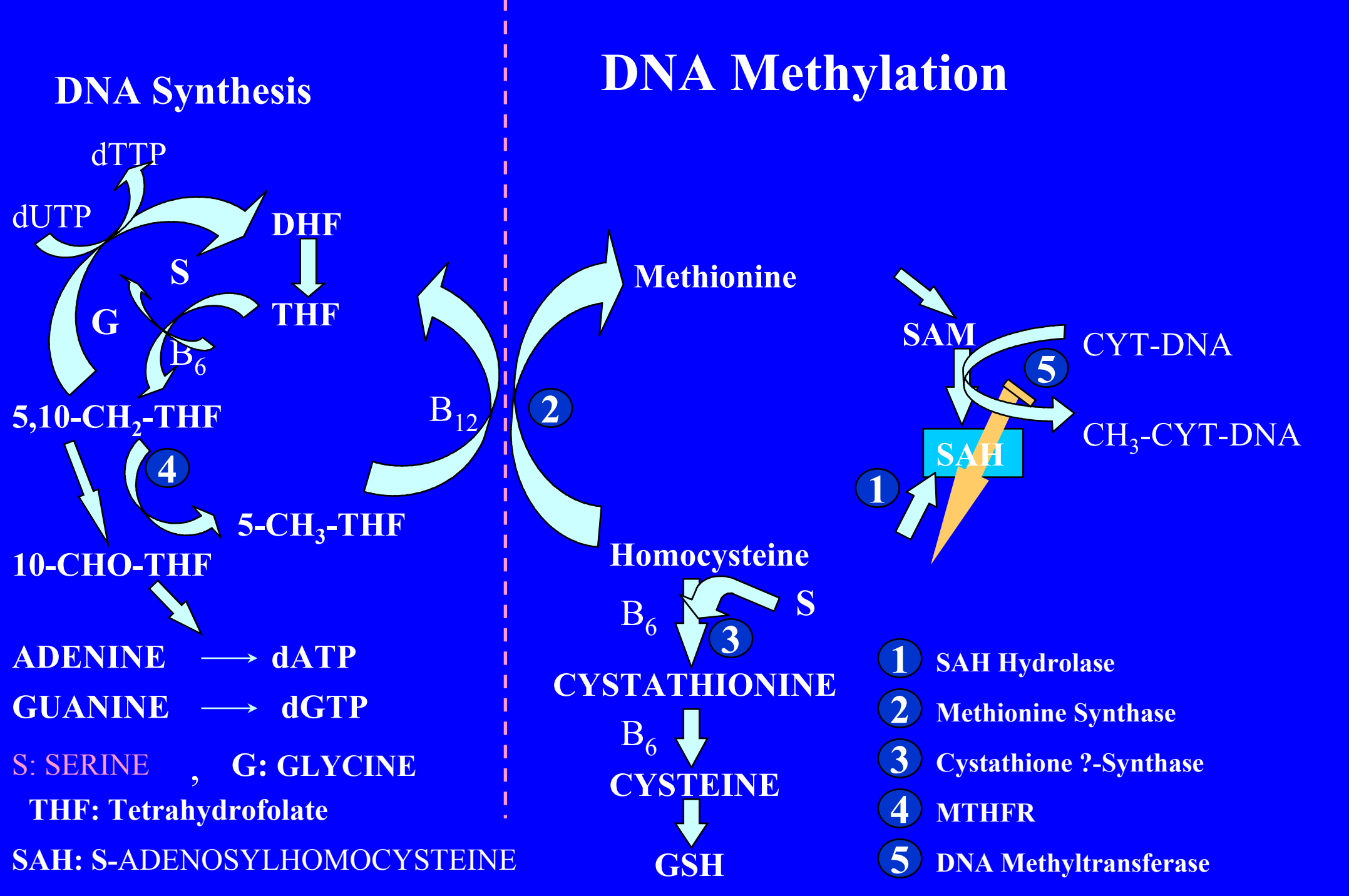
DNA Methylation and Synthesis

- **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
  Balaghi & Wagner (1993) Biochem Biophy Res comm 193:1184-90

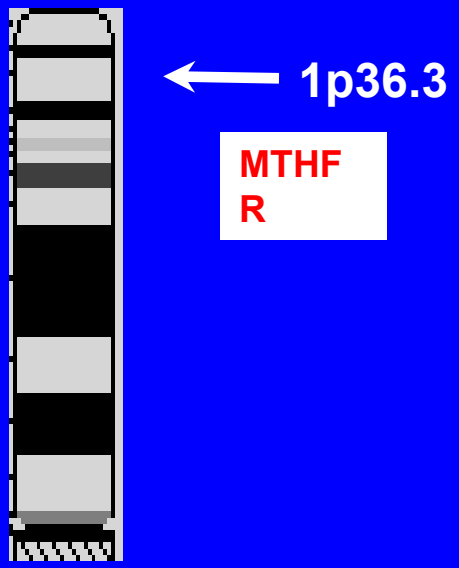
- **MTHFR :Regulation of cellular methylation**

  Frosst et al (1995): Nat Genet, 10:111-3

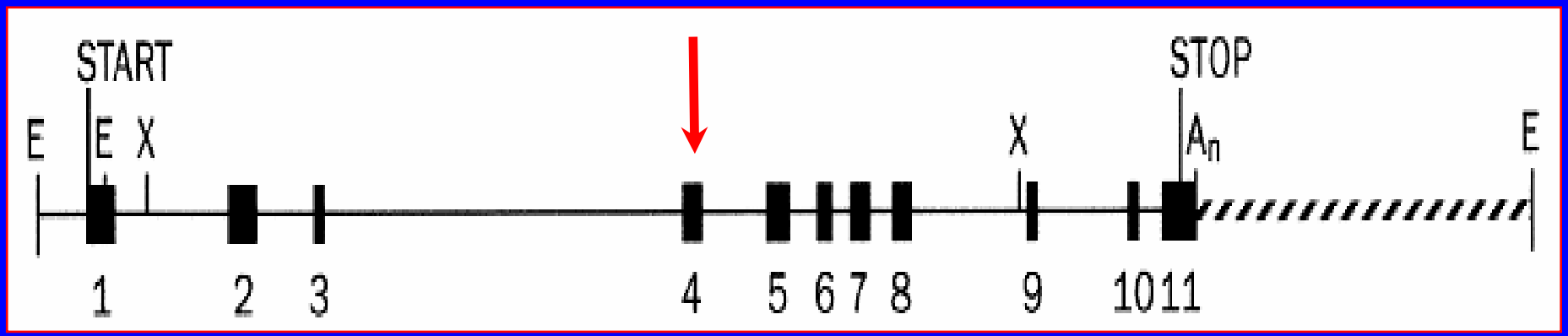

DNA Synthesis
dTTP
dUTP
DHF
S
THF
G
B6
5,10-CH2-THF
B12
2
Methionine
4
5-CH3-THF
10-CHO-THF
ADENINE ⟶ dATP
GUANINE ⟶ dGTP
S: SERINE , G: GLYCINE
THF: Tetrahydrofolate
SAH: S-ADENOSYLHOMOCYSTEINE
Homocysteine
B6
S
3
CYSTATHIONINE
B6
CYSTEINE
GSH
DNA Methylation
SAM
CYT-DNA
5
CH3-CYT-DNA
SAH
1
1 SAH Hydrolase
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 fpr DNA, RNA and phospholipid
- MTRR: 5p15.3: functional state

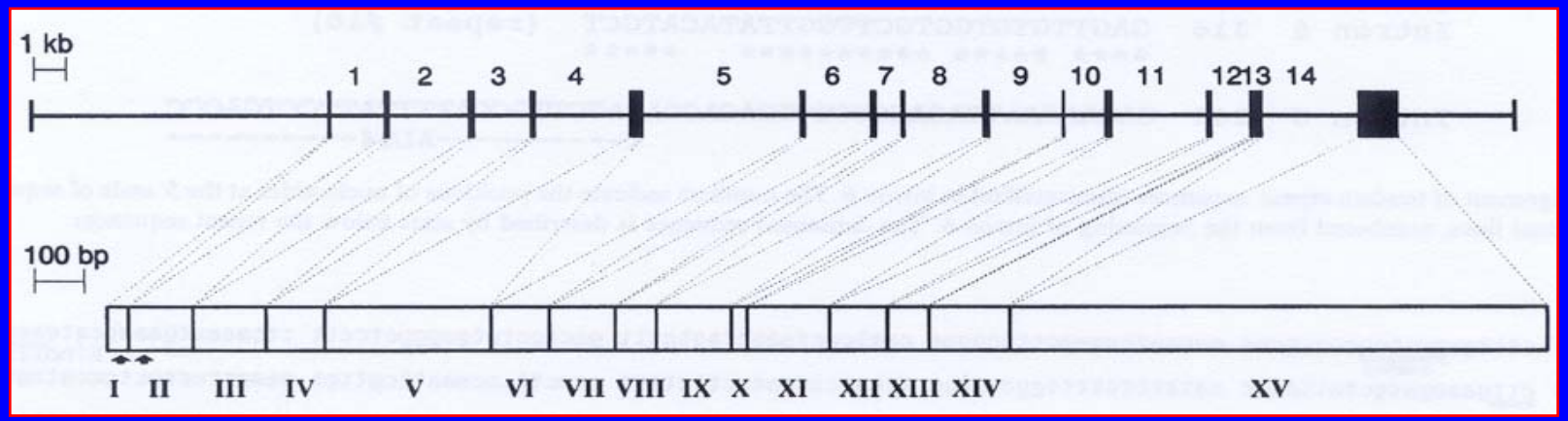


MTHFR Gene (polymorphism location)



- **Exon 4:**
- **677 C-T**
- **Alanine to valine**

MTRR Gene (polymorphism location)



Normal

			22					
A	K	A	I	A	E	E	M	C
GCA	AAG	GCC	ATC	GCA	GAA	GAA	ATG	TGT
			↓					
GCA	AAG	GCC	GTC	GCA	GAA	GAA	ATG	TGT
A	K	A	M	A	E	E	M	C

Polymorphism

Abnormal Folate metabolism and mutation in the MTHFR gene may be a maternal risk factor for Down syndrome

- James SJ et al. (1999) Am J Clin Nutr. 70: 495-501
- Hobbs CA et al (2000) Am J Hum Genet. 67: 623-630
- Gazali et al(2001) Am J Med Genet. 103: 128-132

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

Genotype	No(%) of case Mothers n=157	No (%) of control Mothers (140)	Odds Ratio	95% CI	P value
CC	51(32)	67(48)	1.0		
CT	84(54)	59(42)	1.87	1.14-3.06	.02
TT	22(14)	14(10)	2.06	.96-4.43	.09
CT 0r TT	106(68)	73(52)	1.91	1.19-3.05	.01

Am J Hum. Genet 67: 623, 2000

Maternal MTRR genotype in DS and control mothers (Am J Hum Genet, 67:623, 2000)

Genotype	No of control (139)	No of case (145)	Odd ratio	95% CI
AA	39 (28)	26 (18)	1.0	
AG	68 (49)	64 (44)	1.41	.77-2.56
GG	32 (23)	55 (38)	2.57	1.33-4.99
AG/GG	119 (82)	100 (72)	1.78	1.02-3.13

Maternal MTHFR study in Indian mothers

Subjects	CC Genotype	CT Genotype	TT Genotype
Control(13)	6/13 (46%)	6/13 (46%)	1/13 (7.6%)
Case(17)	11/17 (64.7%)	6/17(35%)	--

Plasma Hcy and Methionine levels

(James et al 1999: Am J Clin Nutrit)

	Control mothers		DS mothers	
	CC(n=9)	CT+TT(n=32)	CC(n=9)	CT+TT(n=32)
Homocysteine(umol/L)	7.9	8.3	10.9	12
SD	0.4	0.4	0.5	0.3
Plasma Methionine	36.7	33.6¦	23.2	28.3
SD	2.4	3.8	2.1	1.4

Preliminary Findings* unpublished data (Sheth and James et al)

	HOMOCYSTEINE (µm/L)	METHIONINE (um/L)	GSH (um/L)	CYS-GLU (um/L)	CYSTEINE (um/L)
DS MOM (Ind)*	8.3?2.79 (18)	21.528 ?6.052	4.686 ?0.996	28.898 ?5.272	251.61 ?73.730
CONTROL* MOM (Ind)	6.499 ? 1.837 (11)	25.089 ? 5.163	5.130 ? 1.314	26.069 ?7.039	248.600 ?32.420
USA DS MOM	12.01 ? 4.073	25.424?7.346	7.567 ±2.381	43.563 ±7.398	200.11 ? 25.194
Control MOM	7.568 ? 1.767	35.666±11.404	6.104 ?1.572	39.254 ±5.328	213.86 ±24.831

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 independant of gene polymorphism

** Maternal risk factors for meiotic nondisjunction

FUTURE

- 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.

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