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Folic Acid and NTDs

Smithells 1983:

- 973 mothers of NTD infants
- Multivitamin vs no supplement
- NTDs in 0.7% multivitamin vs 4.7% no supplement

Trials of Folic Acid Supplementation

Medical Research Council Vitamin Study, 1991:

- Randomized, double blind
- 33 European centers -- 1817 NTD mothers
- Four treatment groups; FA alone or with vitamins, vitamins only, no supplement

Lancet 1991

Trials of Folic Acid Supplementation

MRC Study, 1991:

	With FA	Without FA
NTDs	5/593, 1.0%	21/602, 3.5%

RR with folic acid supplement = 0.28

Lancet 1991

Trials of Folic Acid Supplementation

Czeizel et al, 1992

- Randomized, prospective trial
- 4753 low risk women
- 12 vitamins + 0.8 mg FA vs trace elements

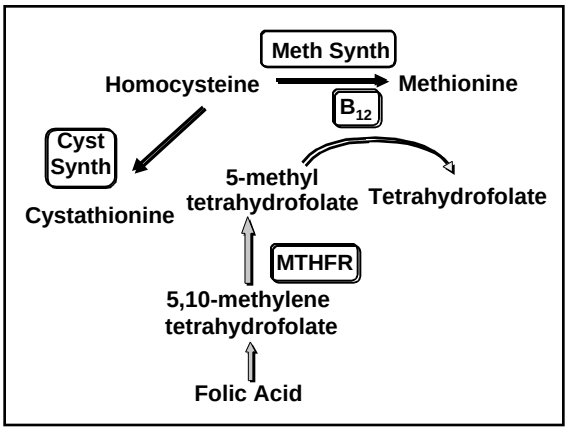
NEJM 1992

Trials of Folic Acid Supplementation

Czeizel et al, 1992:

	With FA	Without FA
NTDs	0/2104 (0%)	6/2052 (0.3%)

NEJM 1992



Folic Acid and NTDs

Van der Put et al, 1995:

- **NTD children and their parents**
- **Homozygous MTHFR 677 C →T in:**
  16% mothers
  10% fathers vs 5% of controls
  13% patients

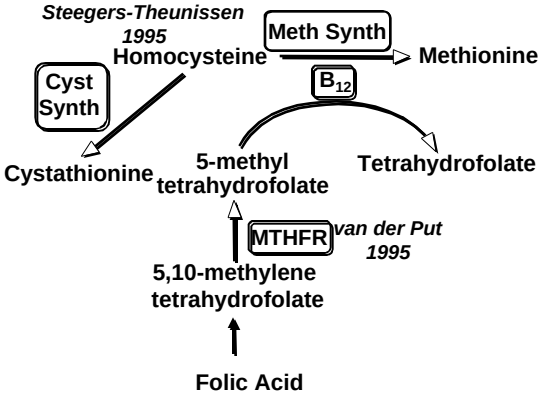
Lancet 1995

MTHFR and NTDs

Steegers - Theunissen et al, 1995

- **27 NTD mothers vs 31 controls**
- **AF homocysteine ↑ in NTDs, but MS homocysteine normal**

The AF homocysteine is fetal in origin



Public Health Recommendations

- **Public Health Service, 1992:**
  All women: 0.4 mg FA daily
- **ACOG Committee Opinion #120, 1993:**
  If previous NTD child: 4.0 mg FA daily
- **Teratology Society, 1997; Institute of Medicine, 1998**
  All women: 0.4 mg FA daily

Effectiveness of Folic Acid Recommendations

MMWR Survey of FA Knowledge:

	Heard Of FA	FA Prevents BDs	FA Before Preg?	Take FA Daily
1995	52%	5%	2%	28%
1998	68%	13%	7%	32%

Effectiveness of FA Recommendations

FDA, March 1996:
- **Issued regs requiring fortification of grain starting January 1998; 0.1 mg /day**

March of Dimes
- **Now investing $10 million for 3 year national FA campaign**

Mechanisms of Folic Acid Effects

Methyl Donor:
- **Hcys + Methyl = Methionine;**
- **Methionine → adenosylmethionine**
- **S-adenosylmethionine is major intracellular methyl donor**

Mechanisms of Folic Acid Effects

DNA & RNA Replication:
- **Methyl groups required for meiosis / mitosis**
- **Hypomethylation → chromosome rearrangements, aneuploidy**

Mechanisms of Folic Acid Effects

James et al, 1999:
- **50 DS mothers vs 50 age-matched controls**
- **Homozygosity for MTHFR 677 → T = 2.8X ↑ risk DS**

Mechanisms of Folic Acid Effects

Gene Regulation
- **CpG dinucleotides located in gene regulatory regions:**
- **Methylation of the cytosine prevents transcription, turning gene "off"**

Folic Acid and Malignancy

Folate and S-AM deficiency leads to:
- **↓ methylation → inappropriate activation of proto oncogenes**
- **↓ DNA synthesis and repair Chromosome breakage**

Duthie, Br Med Bulletin, 1999

Mechanisms of Folic Acid Effects

Global hypomethylation found in human neoplasms:

- **Cervical Carcinoma**
- **Colorectal Cancers**
- **Hematologic Malignancies**

Folic Acid and Malignancy

Netherlands Cohort Study on Diet and Cancer (n = 58, 279):

- **Folate levels are inversely related to incidence of small cell, squamous cell, and adenocarcinomas of the lung.**

Voorrips et al, Cancer Epid 2000

Folic Acid and Malignancy

Kato et al, 1999

- **Case-control study.**
- **105 cases colorectal cancer and 523 controls.**
- **The highest folate levels were associated with lowest cancer risk (OR = 0.52).**

Br J Cancer, 1999

Folic Acid and Malignancy

Zhang et al, 1999:

- **Prospective cohort study of 88,818 women, including 3,483 cases of breast cancer.**
- **In alcohol users, folic acid level associated with breast cancer risk.**

JAMA, 1999

Folic Acid and Malignancy

In Breast cancer patients who used ≥ 15 g alcohol/day:

Cancer risk highest if low folate:

- **If FA intake < 300 μg/d, RR = 1.32**
- **If MV + FA, RR = 0.74**

Zhang et al, JAMA, 1999

Folic Acid and Malignancy

Colorectal cancer is associated with ↑ alcohol and ↓ folate:

- **Microbes turn alcohol to acetaldehyde**
- **Acetaldehyde breaks down folate**
- **Low folate is carcinogenic**

Homann et al, Int J of Cancer,1999

Mechanisms of Folic Acid Effects

Transmethylation Reactions

- **Methyl groups required for synthesis of AA, proteins, lipids (myelin), etc.**
- **↓ Methyl group availability → slowed tissue growth**

Folic Acid and Other Defects

Shaw et al, 1995; Botto et al:

- **Population-based case-control studies of pre-pregnancy diet**
- **FA ↓ risk of oro-facial clefts by 25-50%**

Lancet 1995

Folic Acid and Other Defects

Czeizel et al, 1993:

- **Reanalyzed original FA trial data**
- **FA associated with:**

↓ urinary tract defects

↓ heart defects

Br Med J, 1993

Folic Acid and Other Defects

Wenstrom et al, 2001:

- **Tested AF from 26 cardiac defect fetuses and 116 controls**
- **58% of cardiac cases had MTHFR 677 C → T ± ↑ homocysteine**

AJOG, 2001

AF Homocysteine and MTHFR Analysis

	Cases (n = 26)	Controls (n = 116)	P	OR
Either mut or Hcys > 90%	50% (13/26)	20% (23/116)	0.003	4.0 (1.6 - 9.9)
Both mut and Hcys > 90%	12% (3/23)	0 (0/116)	0.006	34.7 (1.7 - 694.3)

Folic Acid and Other Defects

Mechanism?

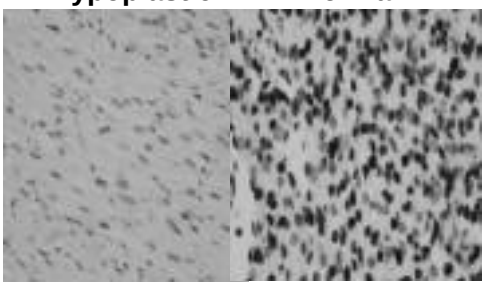
- **Most if not all defects associated with MTHFR 677 C → T arise by:**
  - **Slowed growth, leading to**
  - **Abnormal timing of events**

Cardiac Defects Associated with MTHFR 677 C→T and/or High AF Homocysteine

- Hypoplastic Left Ventricle
- Coarctation of the Aorta
- Pulmonary Stenosis
- Pulmonary Atresia
- Atrial Septal Defect
- Truncus Arteriosis
- Complete AV Canal

Left Ventricle, Third Trimester

Hypoplastic Normal



% Methylated Nuclei and % Moderate-Strong in HLH

	Controls (n = 28)	Cases (n = 34)
% methylated	97%	74%*
% mod-strong	84%	47%*

* P < .0001

Discussion

- Migrating neural crest cells form aortico-pulmonary septum, aortic and pulmonary valves
- Are these cells particularly sensitive to ↓ methyl groups?

Folic Acid Deficiency

Jacob et al, 1998:

- 8 healthy elderly women placed on FA-deficient diet
- Genome-wide DNA hypomethylation in lymphocytes after 5 weeks

Journal of Nutrition, 1998

Folic Acid and Heart Disease

Boushy et al, 1995:

- Meta analysis of 27 publications

  ↑ homocysteine is independent risk factor for arteriosclerotic heart disease

JAMA, 1995

Folic Acid and Heart Disease

Risk of CAD ↑ by 60% in men and 80% in women for every 5 μmol ↑ in homocysteine.

Boushy et al, JAMA, 1995

Folic Acid and Heart Disease

Kang et al, 1988; 1993:

- **Individuals with CAD have 3X ↑ incidence of MTHFR 677 C → T**

Metabolism, 1988
Circulation, 1993

Folic Acid and Heart Disease

Kluijtmans et al, 1996:

- **60 Dutch men with premature vascular dz vs healthy controls.**
- **15% homozygous for MTHFR C→T vs 5% of controls.**

Am J Hum Gen, 1996

Folic Acid and Heart Disease

Kang et al, 1988; 1993:

Kluijtmans et al,1996: MTHFR 677 C → T significantly more common in individuals with:

- **Coronary Artery Disease**
- **Severe Coronary Artery Stenosis**
- **Myocardial Infarction**
- **Cerebrovascular Disease**
- **Peripheral Vascular Disease**

Metabolism, 1988; Circulation, 1993; Am J Hum Gen, 1996

Folic Acid and Heart Disease

Rozen et al, 1995:

- **MTHFR 677 C → T associated with heart disease <u>only if folic acid level < 50% of normal</u>**

Am J Hum Genet, 1995

Folic Acid and Psychiatric Disease

Folate deficiency is associated with:

- **Schizophrenia**
- **Obsessive-Compulsive Disease**
- **Major Depression**

Heran et al, 1999

Folic Acid and Psychiatric Disease

Folate deficiency lowers levels of:

- **S-adenosylmethionine**
- **5-hydroxytryptamine**

Both antidepressants

Folic Acid and Malignancy

Godfrey et al, 1990:

- **Double blind, placebo control trial**
- **40 patients with acute schizophrenia or major depression and folate deficiency took:**
  - **-- psychotropics alone, or**
  - **-- psychotropics plus folic acid**

Lancet 1990

Folic Acid and Psychiatric Disease

Outcome Scores Showed:

- **Folate significantly improved clinical and social recovery.**

Godfrey et al, Lancet 1990

Mental Effects of Folate Deficiency

Copen et al, 1986:

- **83 patients with affective disorder (53 unipolar, 23 bipolar, 7 schizoaffective)**
- **Double-blind comparison of:**
  - **Lithium plus Folate vs**
  - **Lithium plus Placebo**

Br J Psychiat, 1982; 141:87-9

Mental Effects of Folate Deficiency

Copen et al, 1986:

- **Patients with highest plasma folate had significant reduction in affective morbidity.**

Br J Psychiat, 1982; 141:87-9

Mental Effects of Folate Deficiency

Experimentally-Induced Folate Deficiency:

- **Sleeplessness**
- **Forgetfulness**
- **Irritability**
- **Organic Brain Syndrome**
- **Affective Disorders**

Herbert et al, Trans Assoc Amer Phys, 1962

Mental Effects of Folate Deficiency

Anticonvulsant-Associated Folate Deficiency:

- **Depression**
- **Behavioral disturbances**
- **Psychosis**

Young & Ghadjrian
Prog Neuro Psycho-Pharm & Biol Psych, 1989

Folic Acid and Aging

Senescence caused in part by:

- **Defective somatic cell methylation**
- **Accumulated genetic instability**
- **Oxidative damage to DNA**

All related to folate availability

Mental Effects of Folate Deficiency

Clark et al, 1986:

Case-control study of Alzheimer's Disease (AD)

164 patients with AD
(88 clinical dx plus 76 hist dx)
108 normal controls
All ≤ age 55

Arch Neurol 1998

Mental Effects of Folate Deficiency
Odds Ratio of Confirmed AD

	Analyte Levels		
	Top Third	Middle Third	Lowest Third
Hcys	4.5 (2.2-9.2)	1.0 (0.4-2.3)	1.0 (0.4-2.7)
Folate	1.0 (0.3-3.1)	0.4 (0.1-1.5)	3.3 (1.8-6.3)

Clarke et al, Arch Neurol 1998

Folate Deficiency

- **Deficient methylation of uracil to thiamine**
- **Incorporation of ↑ uracil into DNA**
- **Repair of uracil in DNA requires transient single-strand breaks**
- **↑ risk of double-strand breaks**
- **↑ risk of micronucleus formation**

Folic Acid and Aging

Fenech et al, 1998:

- **Cross-sectional study of: 49 males, 57 females**
- **DNA damage (micronucleated cells and DNA methylation) assessed before and after folate/B_{12} therapy* vs placebo**

•*3.5 x the recommended dietary intake

Carcinogenesis, 1998

Folic Acid and Aging

- 25% of MNC frequency explained by age
- Micronucleated cell frequency ↑ with ↑ homocysteine ↓ B_{12}
- Folate / B_{12} decreased MNC by 25% in those with initially high values

Fenech et al, Carcinogenesis, 1998

The MTHFR 677C →T Allele

Munoz-Moran et al, 1998:

- Tested 695 people < 40 years old in southern Spain
- TT genotype (homozygous 677C →T) increased from 13% before 1982 to 26% after 1982
- 35% took FA in 1982, 55% in 1986

Lancet Vol. 352, October 1998

Summary

Folic Acid reduces risk of:

- First incidence birth defects
- Recurrent birth defects
- Coronary artery disease
- Vascular disease
- Certain psychiatric disorders

Summary

Folic Acid Influences:

- Certain cancers
- Alzheimer's Disease
- Aging