Gene Polymorphism and Human Diseases

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Genome

The totality of genetic information belonging to a cell or an organism; in particular, the DNA that carries this information (2n=46 chromosomes, 3.2×10^9 bp).

Gene: Region of DNA that is transcribed as a single unit and carries information for a discrete hereditary characteristic, usually corresponding to

(1) **a single protein** (or set of related proteins generated by variant post-transcriptional processing), or

(2) **a single RNA** (or set of closely related RNAs).

Single Nucleotide Polymorphism(SNP)

- SNP: A single nucleotide polymorphism is a site in the genome that has a different DNA base in >1% of a population.
- Individual A …… G G A G C C G A T……..
- Individual B …… G G A G T C G A T……..
- **single nucleotide polymorphism (SNP)** A site in the DNA that has a different base in at least 1 percent of a population.
- **SNPs, also called** "snips"

https://en.wikipedia.org/wiki/Single-nucleotide_polymorphism

SNP and Human Genome

- **2005/2007:** The International HapMap(Haplotype Map) Consortium reports increasingly detailed single nucleotide polymorphism (SNP) maps for the human genome. The 2007 map has >3.1 million SNPs.

- High-density single nucleotide polymorphism (SNP) maps were developed by the International HapMap (haplotype mapping) Consortium to help identify DNA variants contributing to common multifactorial diseases.

Folate

Folate is the natural (complex) form found in foods such as dark-green leafy vegetables, broccoli, asparagus, lentils, beans, peanuts, strawberries, orange juice, liver.

Folate in foods can be lost through processing and cooking, reducing the amount of available folate.

A diet rich in folate is important, however the average daily intake of folate from foods is about **200 micrograms.**

Efficacy of folate absorption is estimated at **50%.** So, of the 200 micrograms that are eaten, only about **100** micrograms are actually used by the body.

Folic Acid (Vitamin B_9 or pteroyl-L-monoglutamic acid)

Folic acid is the **synthetic (simple) form** of folate.

Used in nutritional supplements and food fortification.

Only form that can be transported across membranes.

Most oxidized and stable form of folate.

Altered folate metabolism and congenital defects

- Several evidences have emerged that mothers with congenital defects children may have altered folate or methionine metabolism, which suggests the folate- methionine cycle may play a key role in the etiology of birth defects.

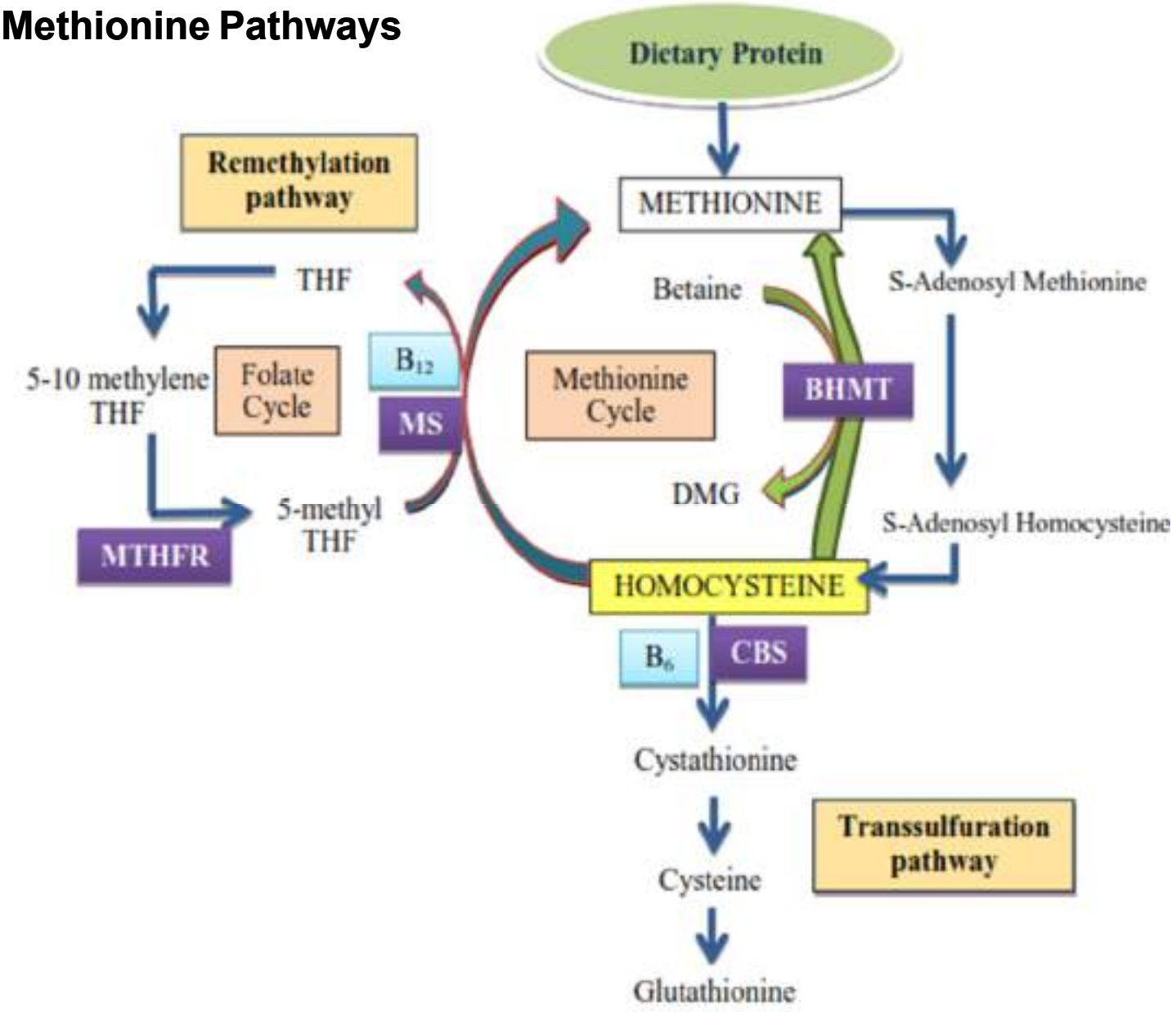
- Humans cannot synthesize folic acid, and folate is essential for-
- **Folate is essential for-**

  -synthesis of nucleotide precursor for DNA synthesis

  -methylation reaction i.e. DNA, RNA, histone, lipid

  -Chromosome segregation

Folate and Methionine Pathways



1.Methylene tetrahydrofolate reductase (MTHFR); 2. Methionine synthase (MTR) ; 3.Methionine synthase reductase (MTRR) ; 4. SAH hydrolase (SH) ; 5. methyltransferases (MT); 6. Cystathionine b synthase(CBS) ; 7.Dihydrofolate reductase (DHFR) ; 8.Betaine -homocysteine(S-methyltransferase(BHMT) ; Serin hydroxy methytlransferase(SHMT) ; Methylene tetrahydrofaalte dehydrogenase(MTHFD) ; Thymidylate synthase (TYMS) ; RFC(FOLH1)

Methylenetetrahydrofolatereductase (MTHFR)

- Methylenetetrahydrofolate reductase (MTHFR) is one of the most critical enzymes involved in folate metabolism.
- It irreversibly catalyzes the conversion of 5,10-methylenetetra hydrofolate to 5-methyltetra hydrofolate (5-THF).
- 5-THF donates methyl group for the conversion of homocysteine to methionine, which is further converted into S-adenosylmethionine (SAM).
- SAM is the main methyl group donor for all cellular methylation reactions.
- Human MTHFR enzyme is a 77-kilodalton protein.

MTHFR gene and C677T polymorphism

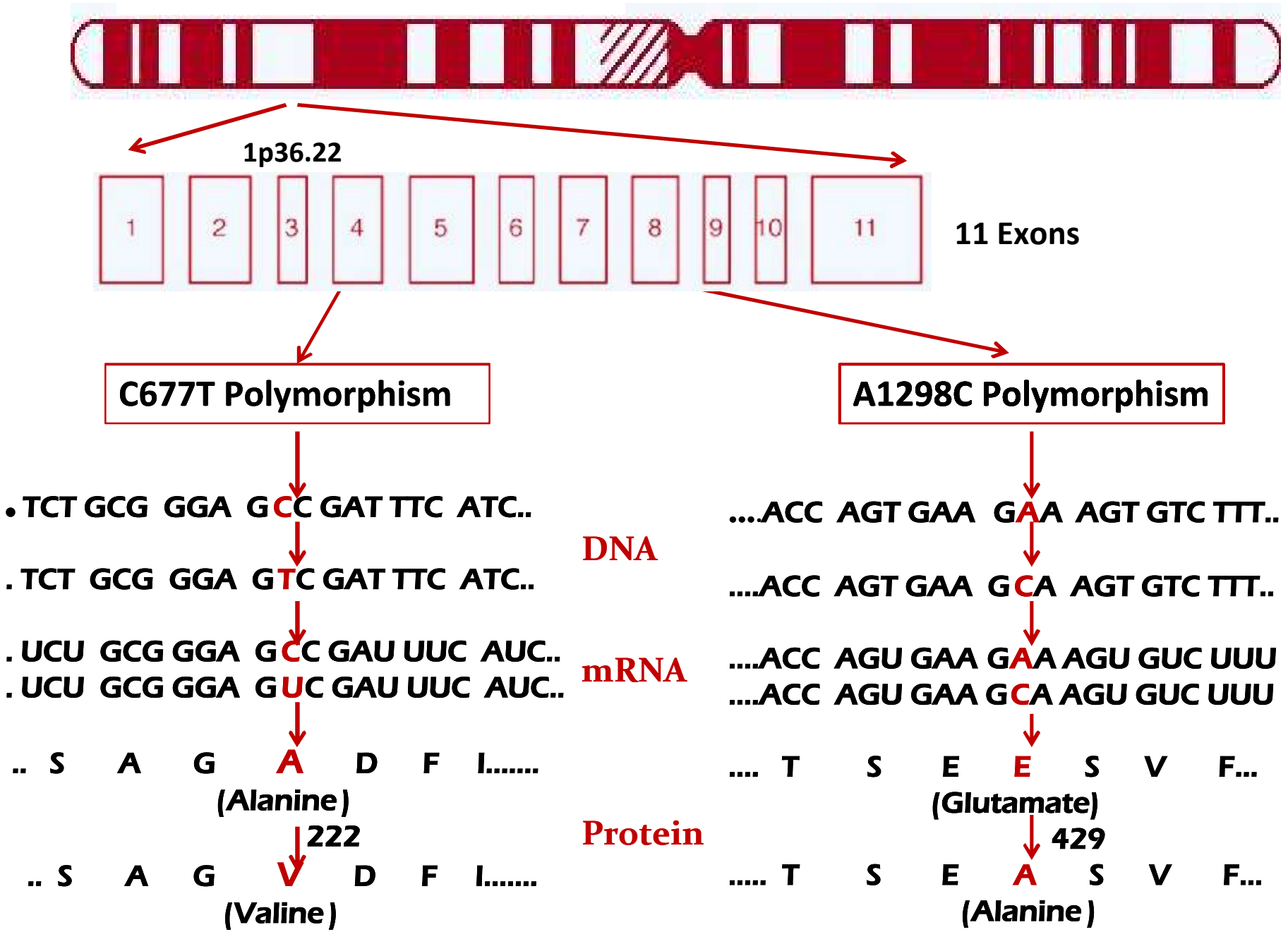
MTHFR gene

- **Cytogenetic Location: 1p36.22,short (p) arm**
- ~20 kb long gene.
- 11 Exons
- Clinically most important SNPs are-

  -C677T (Frosst et al., 1995) (Exon 4)

  -A1298C (Weisberg et al., 1998)

- **C677T mutation (ala222val)** is within the **catalytic domain** of the enzyme, and in hetero/homozygous conditions the enzyme activity declines by about **35% and 70%** respectively.
- MTHFR functions in dimeric form and Flvin adenosine dinucleotide (FAD)

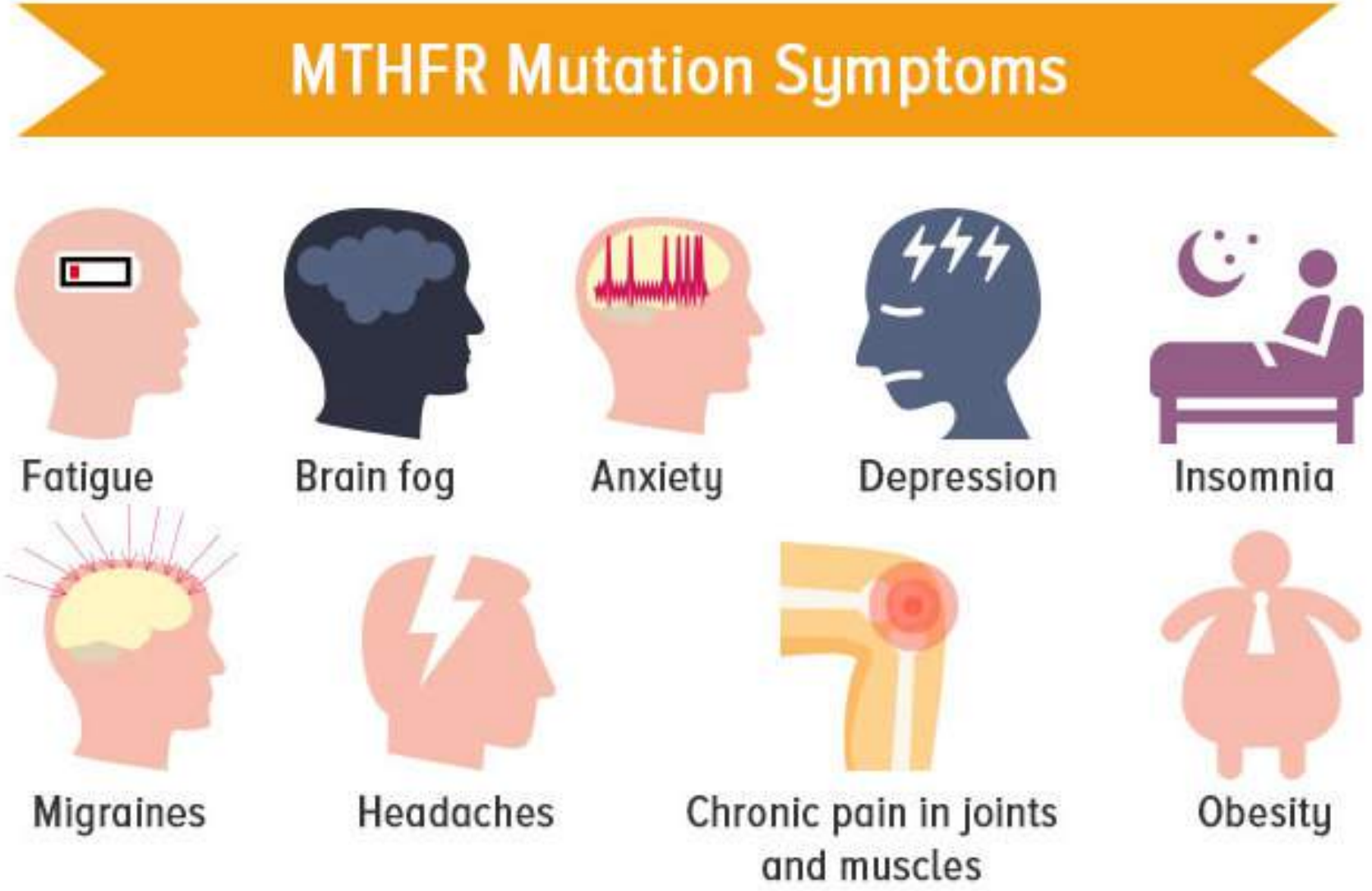

1p36.22
1
2
3
4
5
6
7
8
9
10
11
11 Exons
C677T Polymorphism
A1298C Polymorphism
• TCT GCG GGA GCC GAT TTC ATC..
. TCT GCG GGA GTC GAT TTC ATC..
. UCU GCG GGA GCC GAU UUC AUC..
. UCU GCG GGA GUC GAU UUC AUC..
.. S A G A D F I.....
(Alanine)
222
.. S A G V D F I.....
(Valine)
DNA
mRNA
Protein
....ACC AGT GAA GAA AGT GTC TTT..
....ACC AGT GAA GCA AGT GTC TTT..
....ACC AGU GAA GAA AGU GUC UUU
....ACC AGU GAA GCA AGU GUC UUU
.... T S E E S V F...
(Glutamate)
429
..... T S E A S V F...
(Alanine)

Variant MTHFR reduces the conversion of 5, 10-methylene THF to 5-methyl THF, and elevates plasma homocysteine concentration.

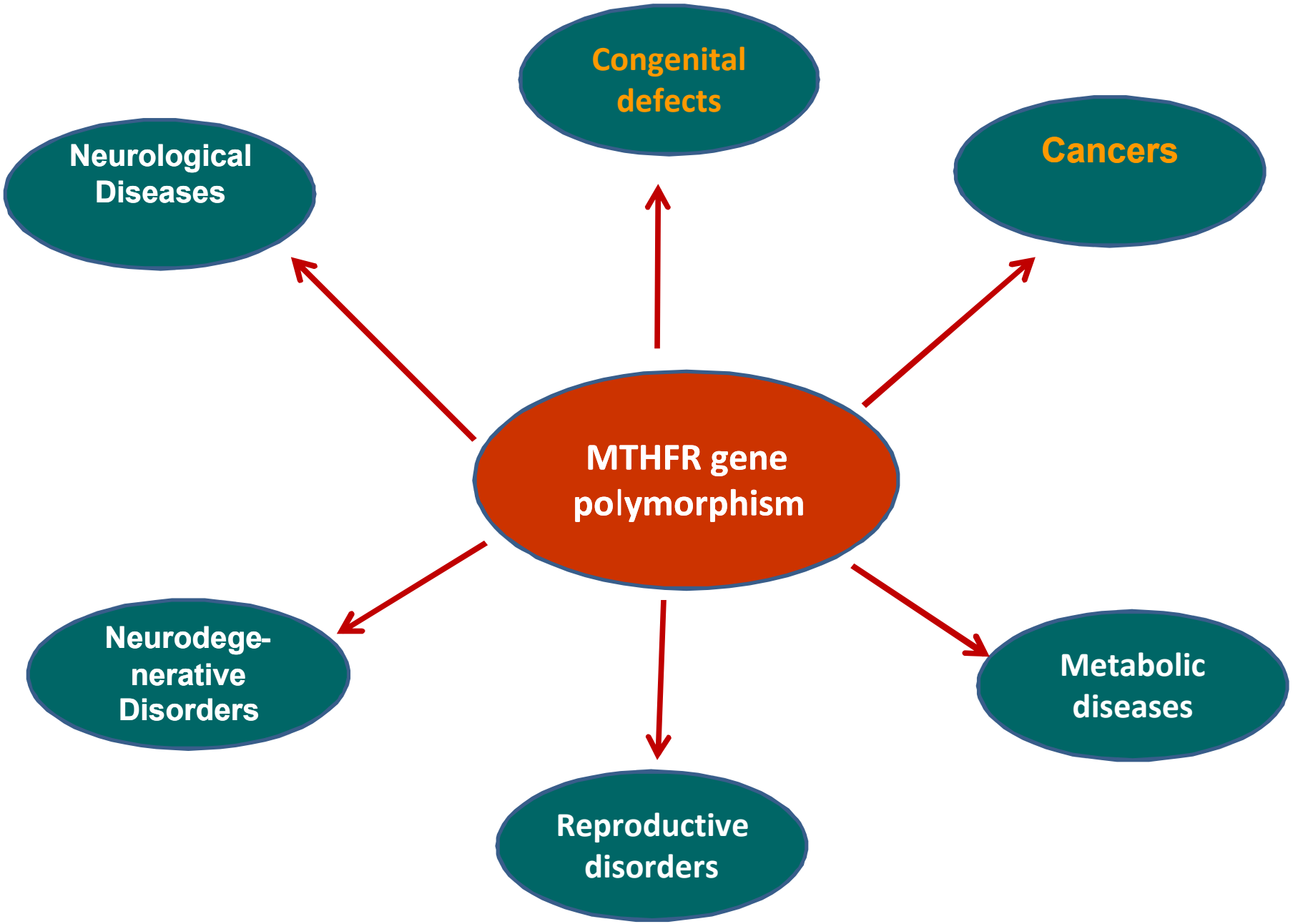
The reduction in enzyme activity associated with the C677T MTHFR polymorphism raises the dietary requirement for folic acid to maintain normal remethylation of homocysteine to methionine.

Allele Frequency

Population	T allele Freq.	Study
European	0.20 to 0.55	Van der Put et al.,1997
American	0.11 to 0.35	Schneider et al.,1998
African	0.063 to 0.094	Pepe et al.,1998
Asian	0.04 to 0.38	Spirinidova et al., 2004



https://blog.paleohacks.com/mthfr-mutation/


Congenital defects
Neurological Diseases
Cancers
MTHFR gene polymorphism
Neurodege-nerative Disorders
Metabolic diseases
Reproductive disorders

Congenital defects

Epidemiological studies showed that the prevalence of different birth defects vary in different populations in the world, pointing to variations in genetic, genomic, environmental, lifestyle, and other factors among them.

The prevalence rate of congenital malformation in India is 19.4 per 1000 birth.

Commonest malformation includes

neural tube defects (NTD),

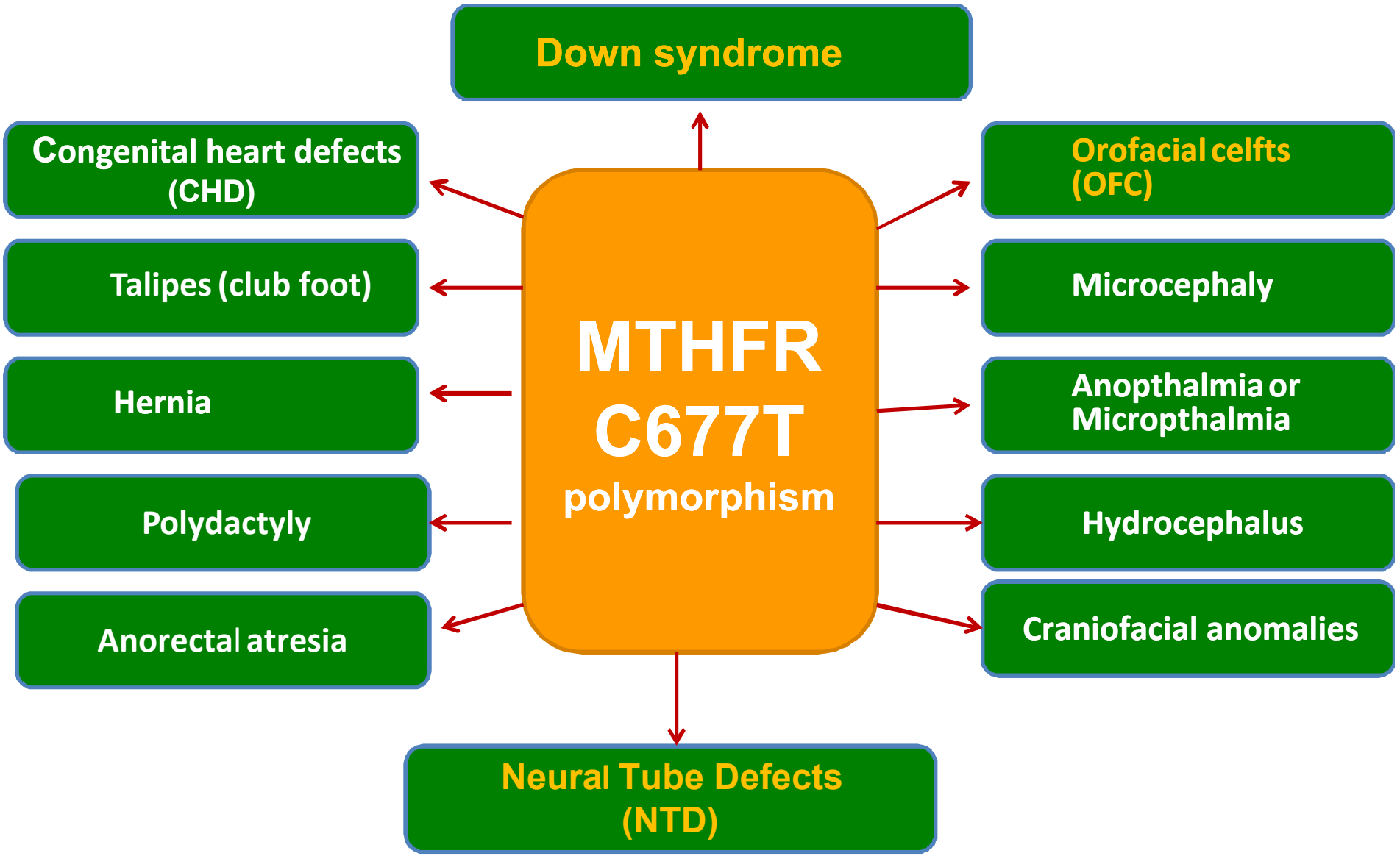
orofacial celfts (OFC)

talipes (club foot),

polydactyly,

Verma et al.,2000

MTHFR C677T polymorphism and congenital defects



Burden of Genetic Diseases at Birth in India

Disorder	*Incidence*	*Number per year*
Congenital malformations	1 : 49	495,096
Down syndrome	1 : 1139	21,412
Beta- thalassemia	1 : 2700	9,000
Sickle cell disease	–	5,200
Metabolic disorders	1 : 2497	9,760

Verma IC (2000) Burden of genetic disorders in India*IndianJPediatr* 67: 893–898.

Common Malformations and Estimated Number Born Per Year in India

	Rate per 10,000	Estimated births
Neural Tube Defects	36.3	88,532
Talipes	14.5	35,364
Polydactyly	11.6	28,291
Hydrocephalus alone	9.5	23,169
Cleft lip + - Cleft palate	9.3	22,681
Congenital heart disease	7.1	17,316
Hypospadias	5.0	12,194
Tracheoesphageal fistula	3.7	9,023
Diaphragmatic hernia	2.6	6,341
Anorectal atresia/stenosis	2.4	5,853
Microcephaly	2.2	5,365
Cleft palate alone	1.7	4,146

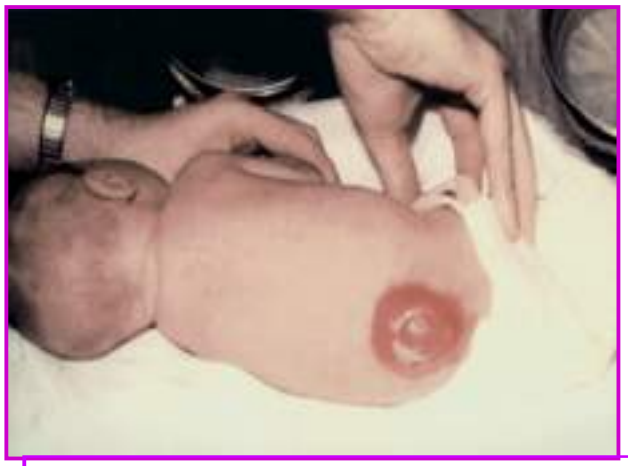
Verma IC (2000) Burden of genetic disorders in India *IndianJPediatr* 67: 893–898.

Neural Tube Defects (NTD)

In the 2nd week of pregnancy (gastrualtion), specialized cells on the dorsal side of the fetus begin to fuse and form the neural tube.
When the neural tube does not close completely, an NTD develops.

- Serious birth defects , Spina Bifida and anencephaly are commonest
- 1 of 1,000 pregnancy
- ~300,000 yearly worldwide
- Caused by failure of neural tube, to close duringneurulation in 21-28 embryonic days.
- Increased consumption of folic acid can prevent 50%70%

Spina Bifida

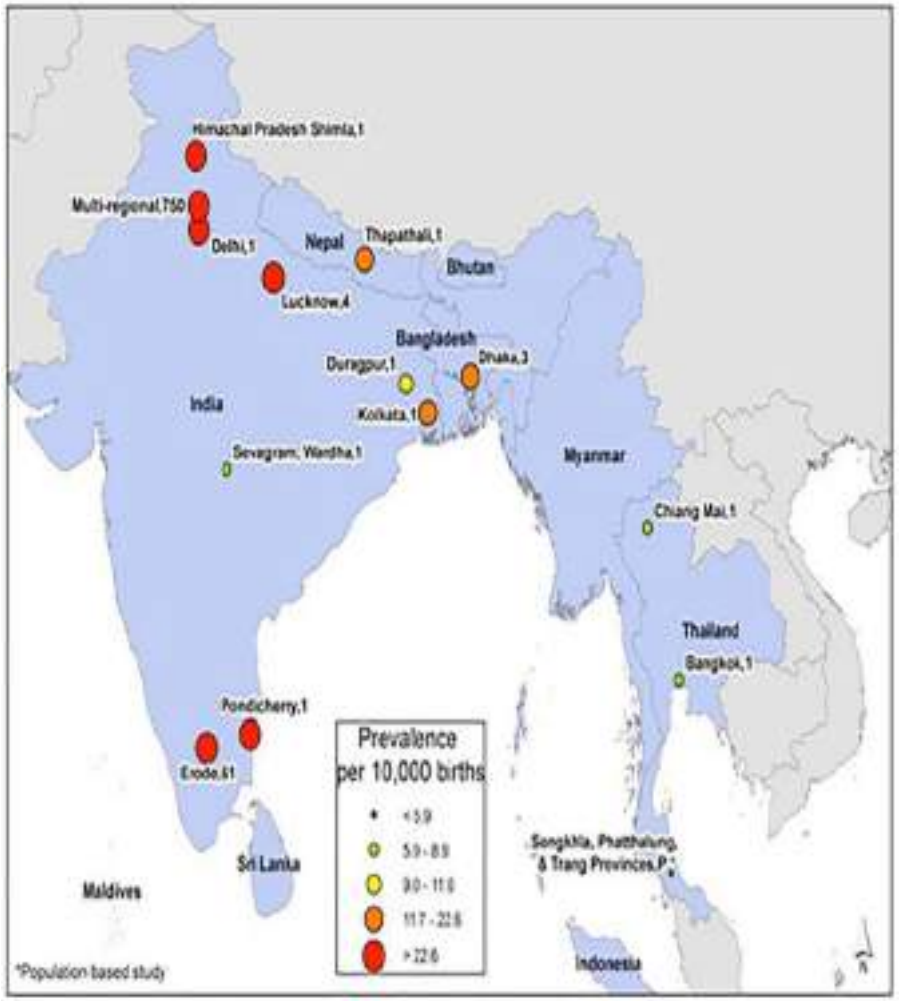


Anencephaly (without brain)

Encephalocele

Frequency of neural tube defects (NTDs)

Urban/ Rural	Frequency
Hospital-based records from major cities of India(a quarter of the population resides)	3·9 to 8·8 per 1000 births
Balrampur District in Uttar Pradesh, a region ranked as the least-developed area in India. The data showed that the incidence of NTDs was 6·57–8·21 per 1000 live births, which is among the highest worldwide.	6·57–8·21 per 1000 live births



Zaganjor et al.,2016

Cherian A et al., 2005

MTHFR and Neural Tube Defects

Metab Brain Dis
DOI 10.1007/s11011-014-9575-7

REVIEW ARTICLE

"Polymorphisms in folate metabolism genes as maternal risk factor for neural tube defects: an updated meta-analysis"

Upendra Yadav · Pradeep Kumar ·
Sushil Kumar Yadav · Om Prakash Mishra ·
Vandana Rai

OR (95%CI),p
TT vs. CC: OR=1.59(1.38–1.82),<0.0001
Association= Yes

- **Folic acid derivatives are essential for the DNA synthesis, DNA methylation, cell division, and tissue growth (Blount et al. 1997; Morrison et al. 1998; James et al. 2003; Pogribny et al. 2004). All these process are important for normal fetal development**.

- Methylation enables proper gene expression and chromosome structure maintenance, both of which are critical for fetal development (Razin and Kantor 2005).

- Low levels of folate and MTHFR C677T polymorphism, associated with hyperhomocysteinaemia, have been found in mothers of children with NTD.
- Maternal nutritional factors, especially folic acid intake, are known to make a substantive contribution to reduce the probability of occurrence or recurrence of the birth of a child with NTD (van der Put et al. 1995, 1996; Lacasana et al. 2012).

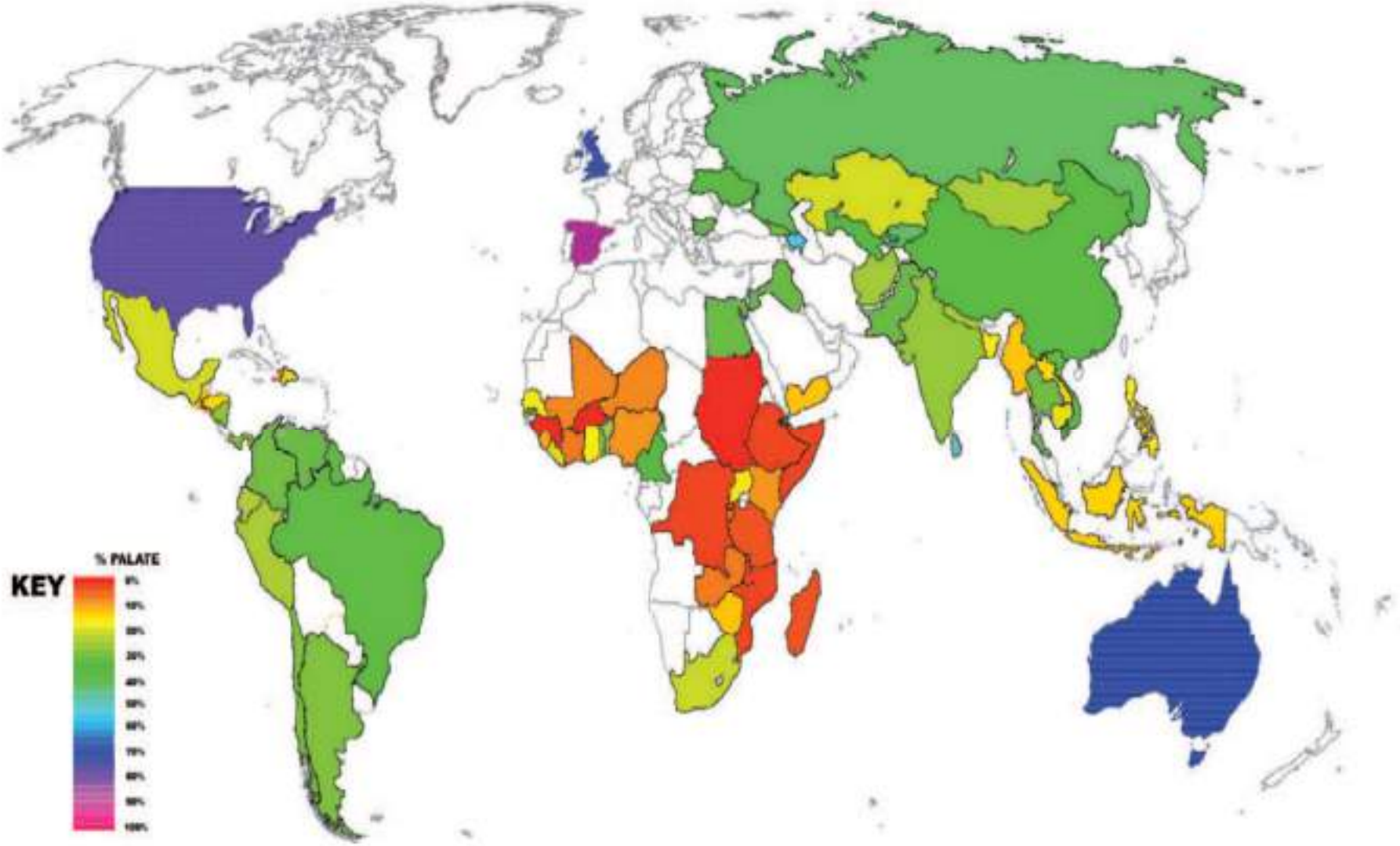
Orofacial Clefts(OFC)
(Cleft Lip and Palate)

- **Cleft lip** (*cheiloschisis*) and **cleft palate** (*palatoschisis*), which can also occur together as **cleft lip and palate**, are variations of a type of clefting caused by abnormal facial development during gestation.

It is one of the most common congenital malformation with the global prevalence ranging between 1 in 300- 2000 birth depending upon geographical origin, ethnicity, and socioeconomic status (Croen et al., 1998; Vanderas,1998; Clark et al., 2003).

A cleft is a fissure or opening—a gap. It is the non-fusion of the body's natural structures that form before birth.

A cleft lip or palate can be successfully treated with surgery, especially so if conducted soon after birth or in early childhood.



Percentage of cleft repair as a proportion of all primary cleft procedures in the 77 countries studied depicted on a global map

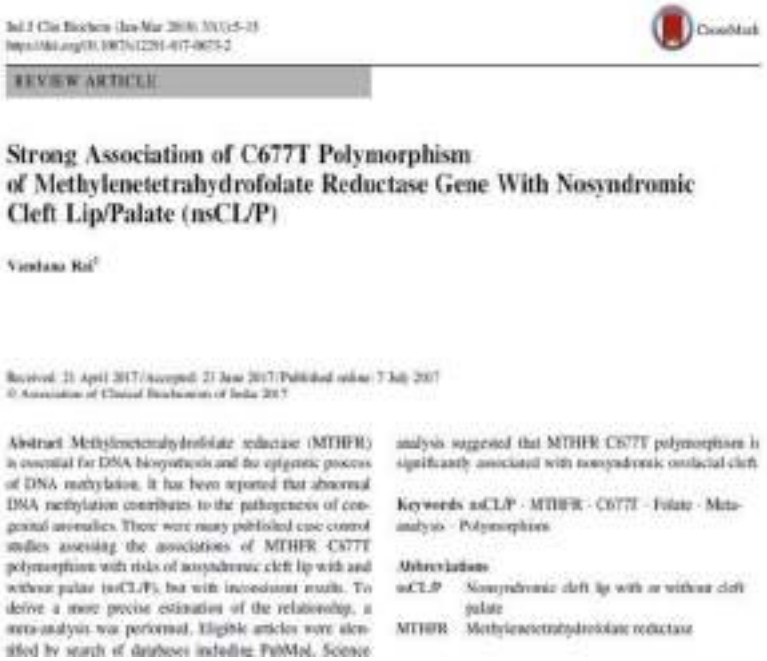
https://en.wikipedia.org/wiki/Down_syndrome

Cleft lip and palate

- Supplemental intake of folic acid and multivitamins around conception is suggested to provide protection against nsCL/P birth defects (Tolarova, 1987; Tolarova and Harris, 1995; van Rooij et al., 2004; Krapels et al., 2006; Badovinac et al., 2007; Chevrier et al., 2007).

- Hyperhomocysteinemia might be directly or indirectly disrupt a number of important cellular processes including cellular proliferation, apoptosis and DNA synthesis, all are important processes in the development of lip and palate (Knott et al., 2003; Brauer and Tierney, 2004; Zetterberg, 2004).

- High level of plasma homocysteine was found in the mothers of chidren with a cleft abnormality (Wong et al., 1999; Knott et al., 2003; Rubini et al., 2005; Verkleij-Hagoort et al., 2007).



REVIEW ARTICLE

Strong Association of C677T Polymorphism of Methylenetetrahydrofolate Reductase Gene With Nosyndromic Cleft Lip/Palate (nsCL/P)

Vandana Rai

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Abstract Methylenetetrahydrofolate reductase (MTHFR) is essential for DNA biosynthesis and the epigenetic process of DNA methylation. It has been reported that abnormal DNA methylation contributes to the pathogenesis of congenital anomalies. There were many published case control studies assessing the associations of MTHFR C677T polymorphism with risks of nonsyndromic cleft lip with and without palate (nsCL/P), but with inconsistent results. To derive a more precise estimation of the relationship, a meta-analysis was performed. Eligible articles were identified by search of databases including PubMed, Science analysis suggested that MTHFR C677T polymorphism is significantly associated with nonsyndromic orofacial cleft

Keywords nsCL/P · MTHFR · C677T · Folate · Meta-analysis · Polymorphism

Abbreviations
nsCL/P Nonsyndromic cleft lip with or without cleft palate
MTHFR Methylenetetrahydrofolate reductase

REVIEW ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Maternal methylenetetrahydrofolate reductase (MTHFR) gene A1298C polymorphism and risk of nonsyndromic Cleft lip and/or Palate (NSCL/P) in offspring: A meta-analysis

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Submitted: 22-04-2014 Revised: 14-05-2014 Published: 30-08-2014

ABSTRACT

Objective: Methylenetetrahydrofolate reductase (MTHFR) A1298C polymorphism has been reported a risk factor for nonsyndromic cleft lip/palate (NSCL/P) in several published articles but results were inconclusive. To confirm the association between maternal MTHFR A1298C polymorphism and NSCL/P risk, a meta-analysis was conducted. Method: Case control articles for maternal MTHFR A1298C polymorphism and NSCL/P risk were identified by search of databases including PubMed, Google Scholar, Elsevier and Springer Link for the period up to December, 2013. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated

Access this article online
Website:

OR (95%CI),p

TT vs. CC: OR=1.24, 95% CI =1.1-1.4, 0.0006

Association= Yes

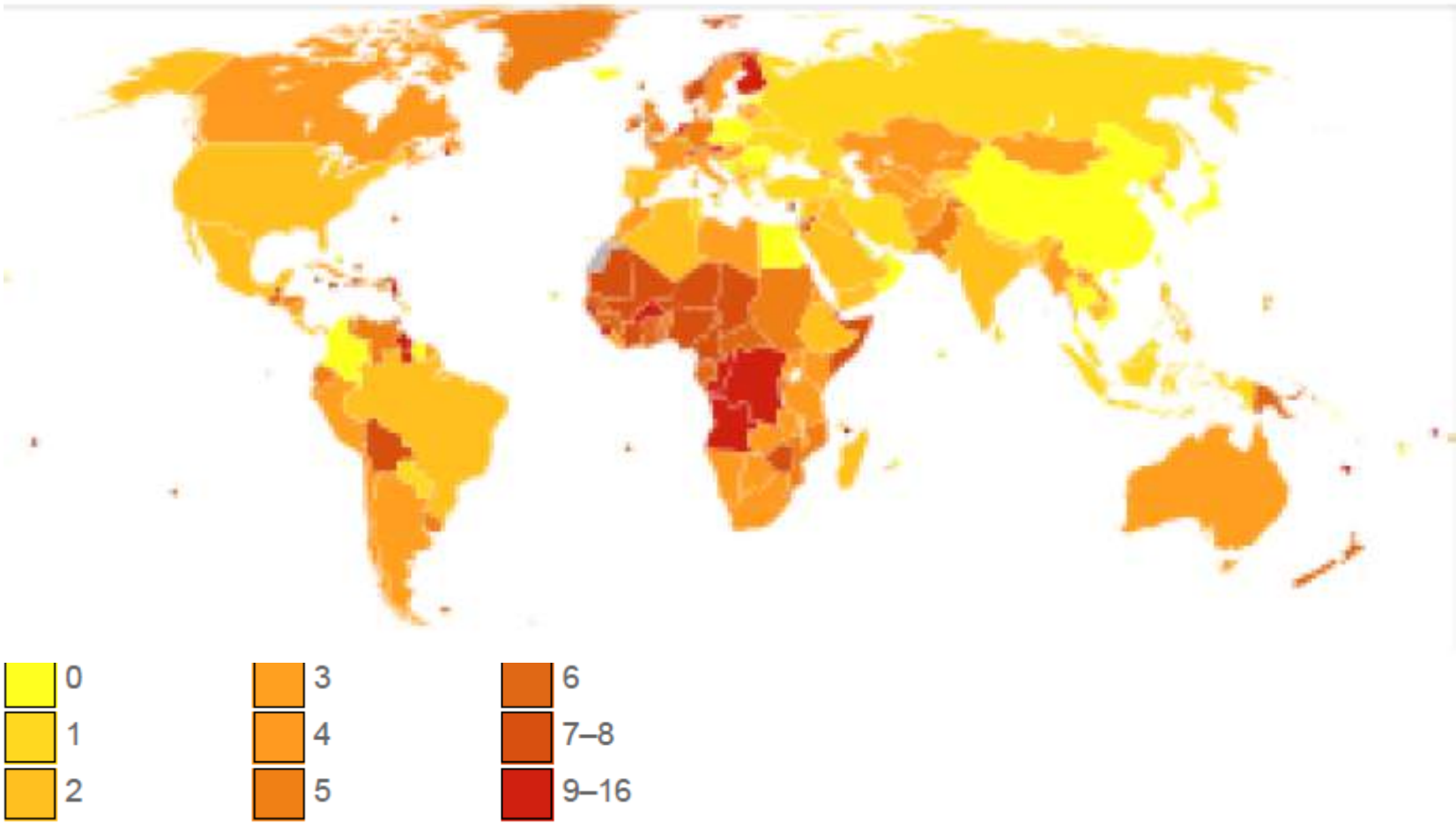
Down Syndrome (DS)

Trisomy 21: 3 copies of chromosome 21
Mental retardation
Narrow eye openings
Up-slanting eyes
Arched eyebrows
Arched palate (cleft)
Flat nose bridge
Bow shaped mouth
low set ears
Short neck
Sloping shoulders
Prevalence rate ~ 1/700 live birth

Word Downs syndrome Day- 21st March

GLOBAL
DOWN SYNDROME FOUNDATION

Death due to Down syndrome per million persons in 2012



https://en.wikipedia.org/wiki/Down_syndrome

MTHFR and Down Syndrome

OPEN ACCESS Freely available online

PLOS ONE

Maternal Methylenetetrahydrofolate Reductase C677T Polymorphism and Down Syndrome Risk: A Meta-Analysis from 34 Studies

Vandana Rai[1*], Upendra Yadav[1], Pradeep Kumar[1], Sushil Kumar Yadav[1], Om Prakesh Mishra[2]

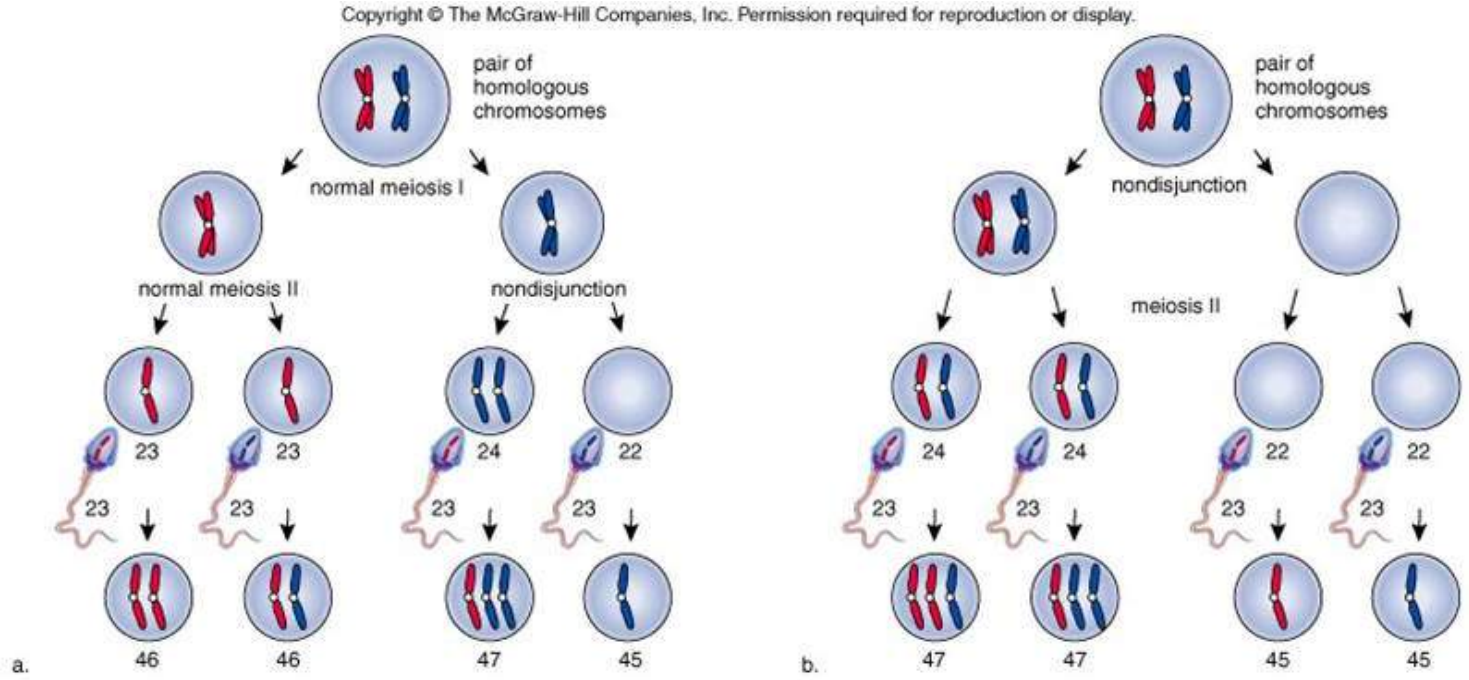
OR (95%CI),p

TT vs. CC: OR = 1.49(1.13–1.97), 0.008

Association= Yes

Down Syndrome

- Several studies performed on human cell cultures, *in-vivo* studies in humans and studies involving animal models have demonstrated that folate depletion from the media, or inadequate folate dietary intake, result in DNA hypomethylation, chromosome breakage, and aneuploidy (Fenech, 2001).
- **Impairments in folate metabolism due to genetic polymorphisms of MTHFR enzyme could predispose women to abnormal chromosome segregation (DNA hypomethylation in centromeric DNA) and act as risk factors for a DS pregnancy (James et al., 1999).**
- Hypomethylation of pericentromeric region of chromosome is responsible for mis-segrgation of chromosomes in meiosis.



Cancer

- Folate functions as methyl donor in the one carbon metabolism pathway, an essential process in DNA synthesis, repair and methylation and dysregulation of the folate metabolic pathway either due to deficiency of folate or MTHFR C677T polymorphism could result in carcinogenesis (Choi and Mason,2001; Jackson et al., 2013).
- **Breast Cancer**
- **Lung Cancer**
- **Colorectal cancer**
- **Esophageal cancer**
- **Ovary Cancer**

cancer

•There are two important mechanisms by which folate deficiency /C677T polymorphism may

influence the risk of cancer:

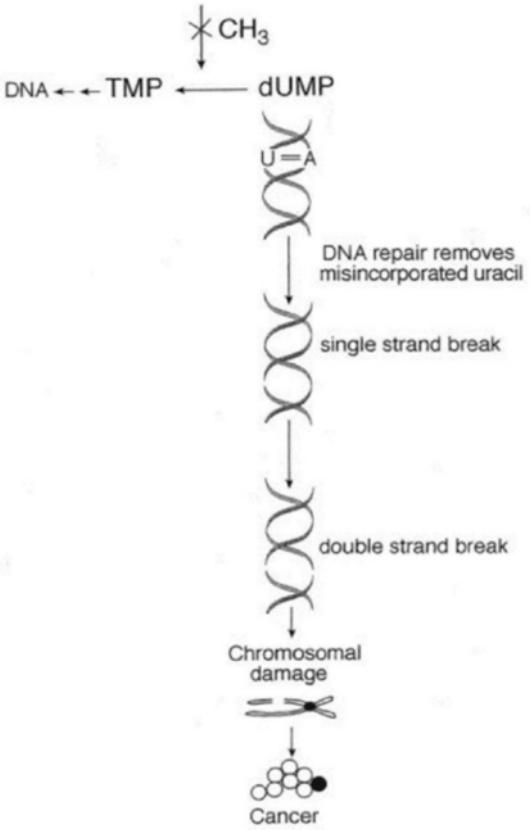
(i) by inducing misincorporation of uracil into DNA, which can lead to chromosomal breaks and

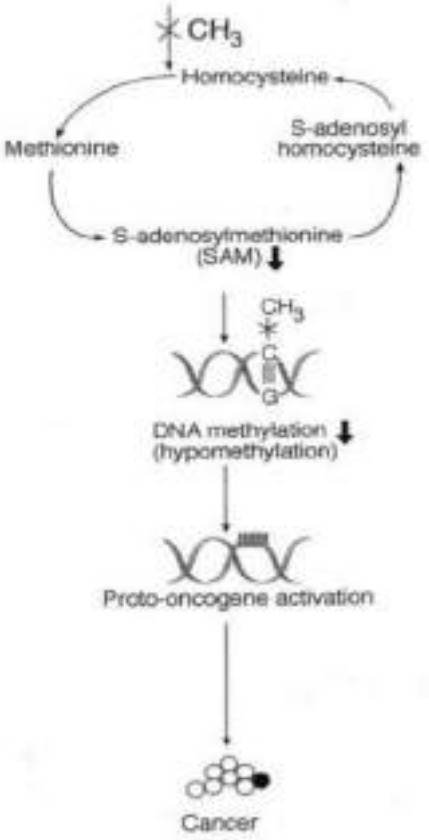
mutations (Kim,2000 ; Duthie,2011), and/or

(ii) by causing aberrant DNA methylation, resulting in altered expression of critical proto-

oncogenes and tumor suppressor genes.

Mechanism of carcinogenesis





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TT vs CC: OR=1.25 (1.01-1.30),
<0.0001
Association=Yes

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journal homepage: www.sciencedirect.com

Review

MTHFR C677T polymorphism and risk of esophageal cancer: An updated meta-analysis

Pradeep Kumar, Vandana Rai *

Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur 222 003, UP, India

Recommended Dietary Allowance for Folate in Dietary Folate Equivalents (DFEs)

Life Stage	Age	Males (µg/day)	Females (µg/day)
Infants	0-6 months	65 (AI)	65 (AI)
Infants	7-12 months	80 (AI)	80 (AI)
Children	1-3 years	150	150
Children	4-8 years	200	200
Children	9-13 years	300	300
Adolescents	14-18 years	400	400
Adults	19 years and older	400	400
Pregnancy	all ages	-	600
Breast-feeding	all ages	-	500

Folic acid Intake : Indian Scenario

- **The National Pilot Programme on Control of Micronutrient Malnutrition estimated that daily intake of folic acid in rural areas of various Indian states (north and north-east) ranged between 75·0 g and 167·7 g, which is far lower than the 400 g necessary to prevent birth defects.**

Salvi VS, Damania KR. Neural tube defects in India—time for action. Lancet 2005; 366: 871–72.