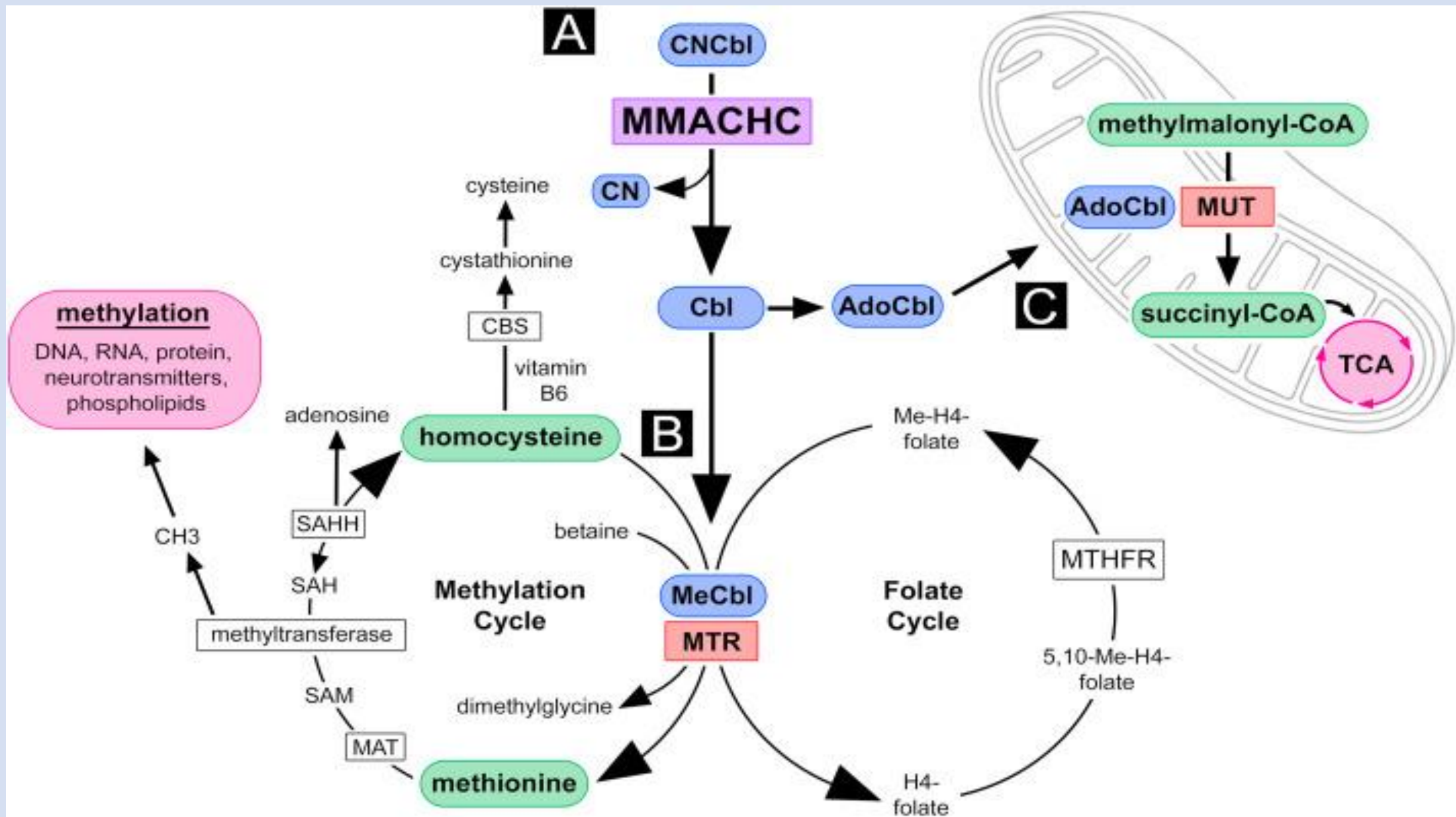
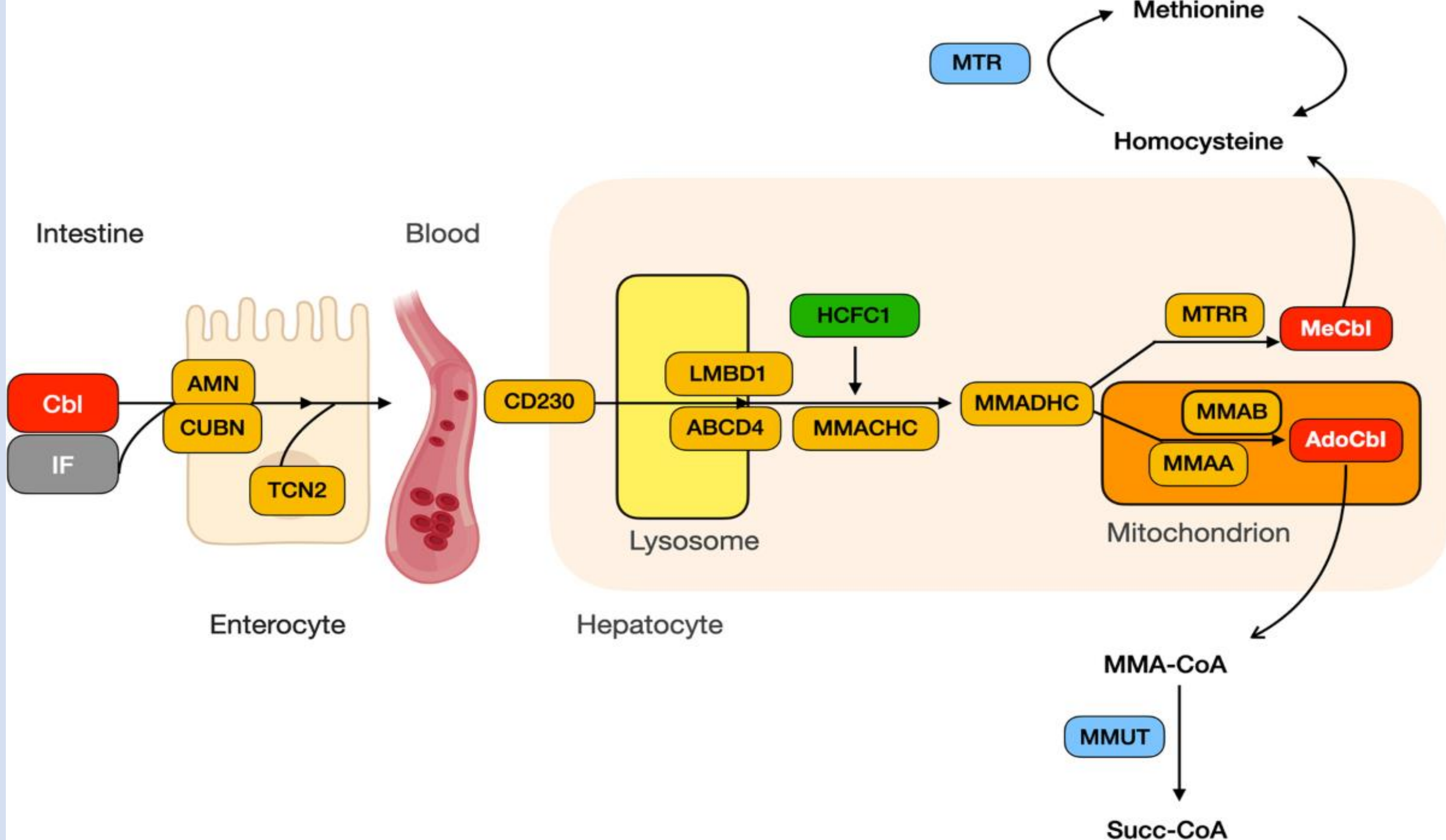


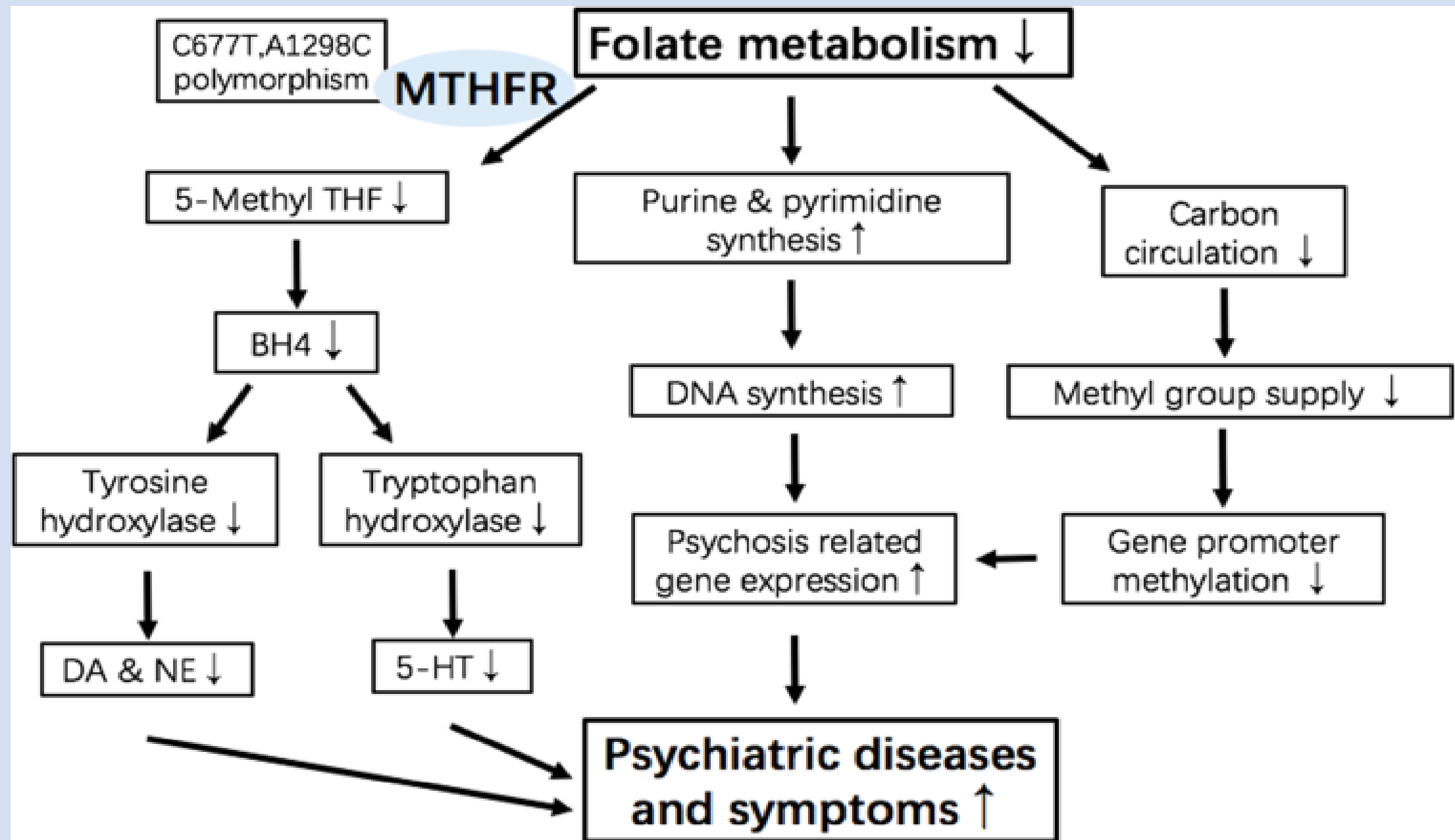
From Ataxia to Hearing Loss: Variable Treatment Responses in MTHFR-Related Homocystinuria

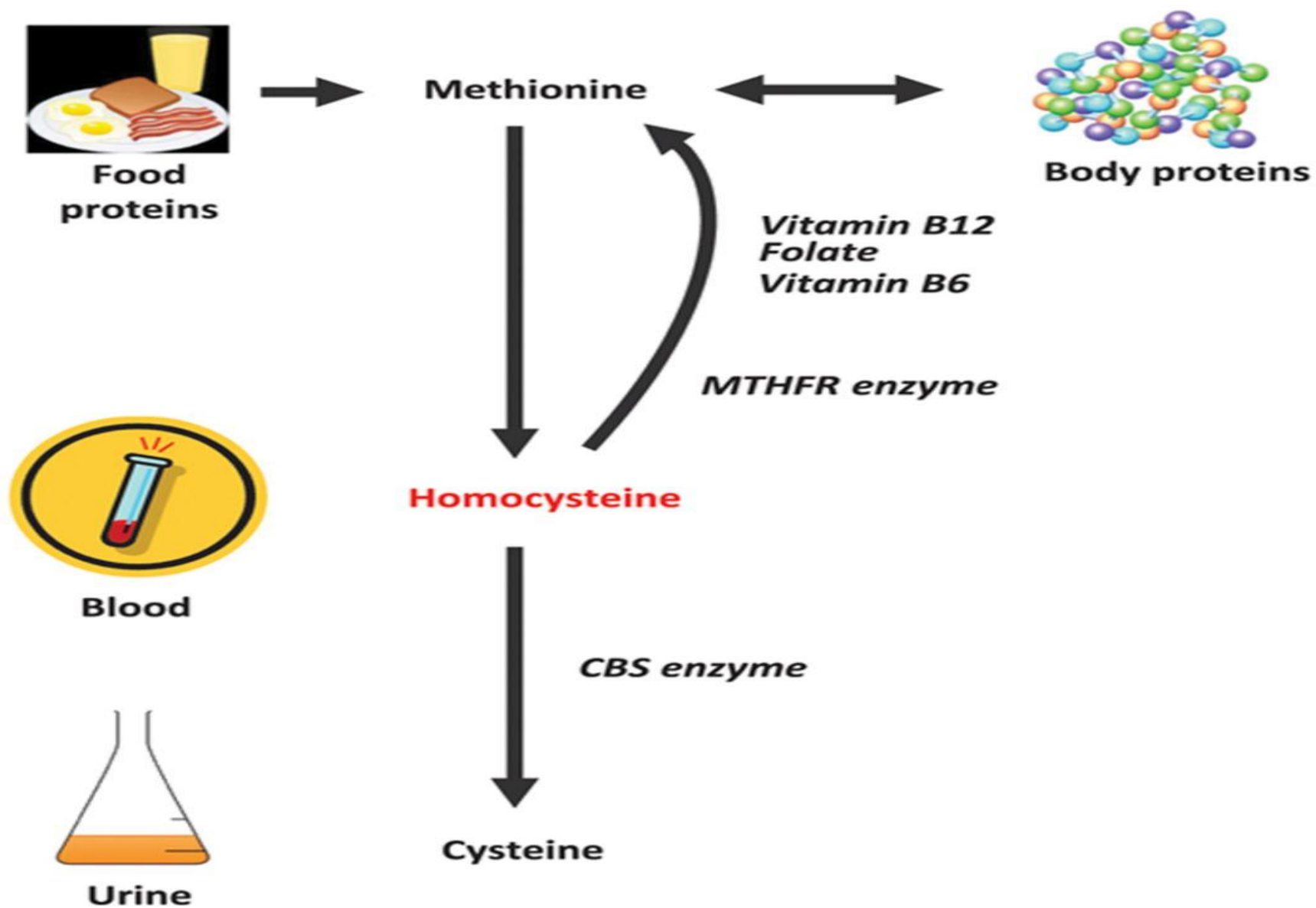
Amirkashani Davood

Iran university of medical sciences

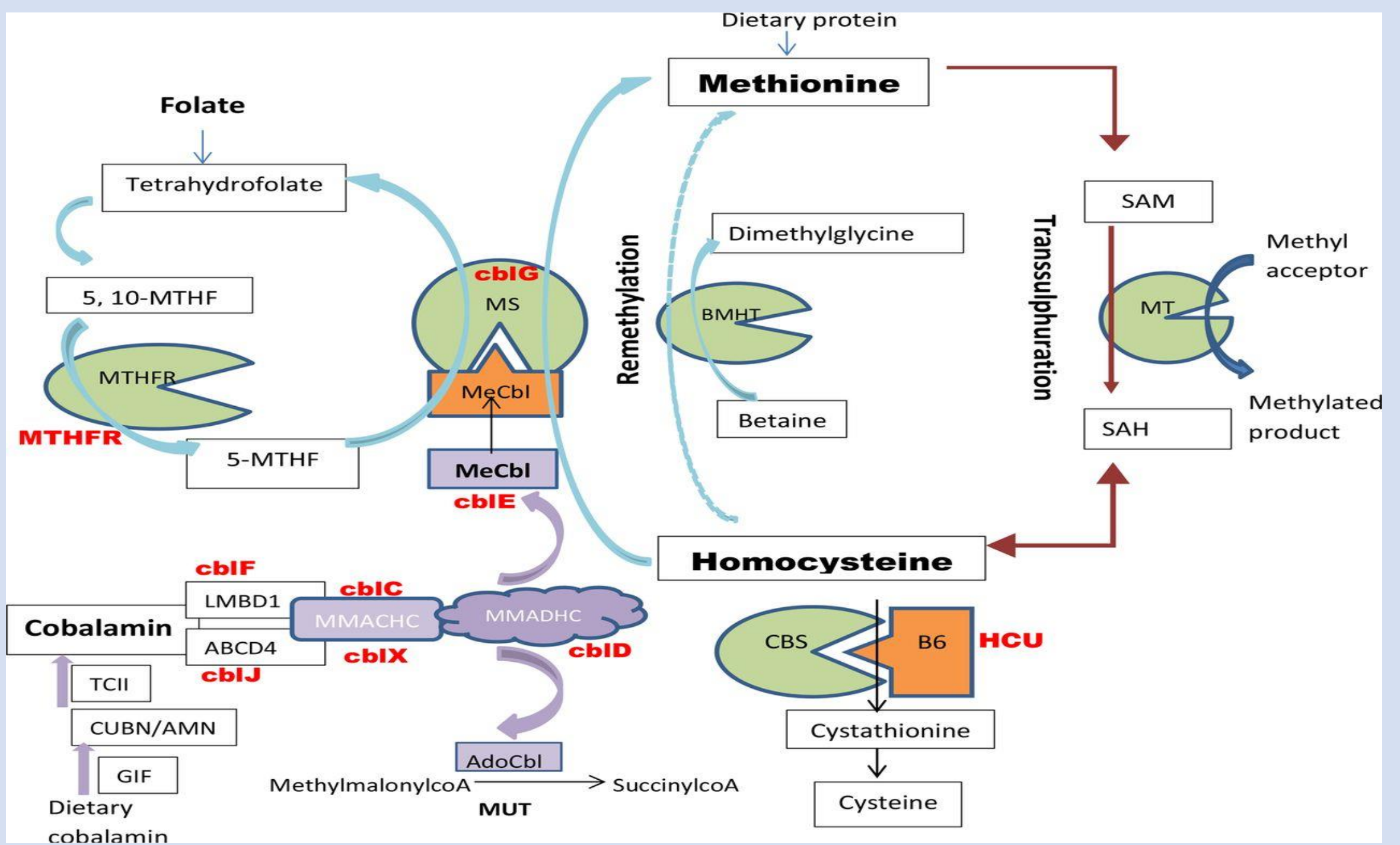






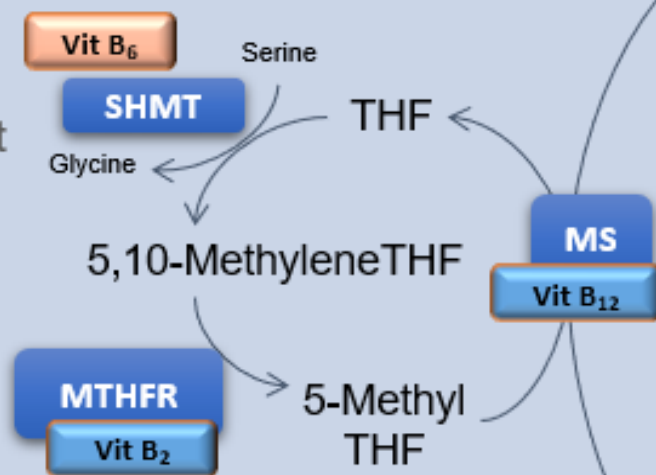


MTHFR = Methylene-Tetra-Hydro-Folate-Reductase; CBS = Cystathionine-beta-synthase



Folate dependent remethylation pathway

- Present in all cells
- Dependent on vitamin B₁₂



Folate non-dependent remethylation pathway

- Limited to liver and kidney
- Dimethylglycine

BHMT

Betaine

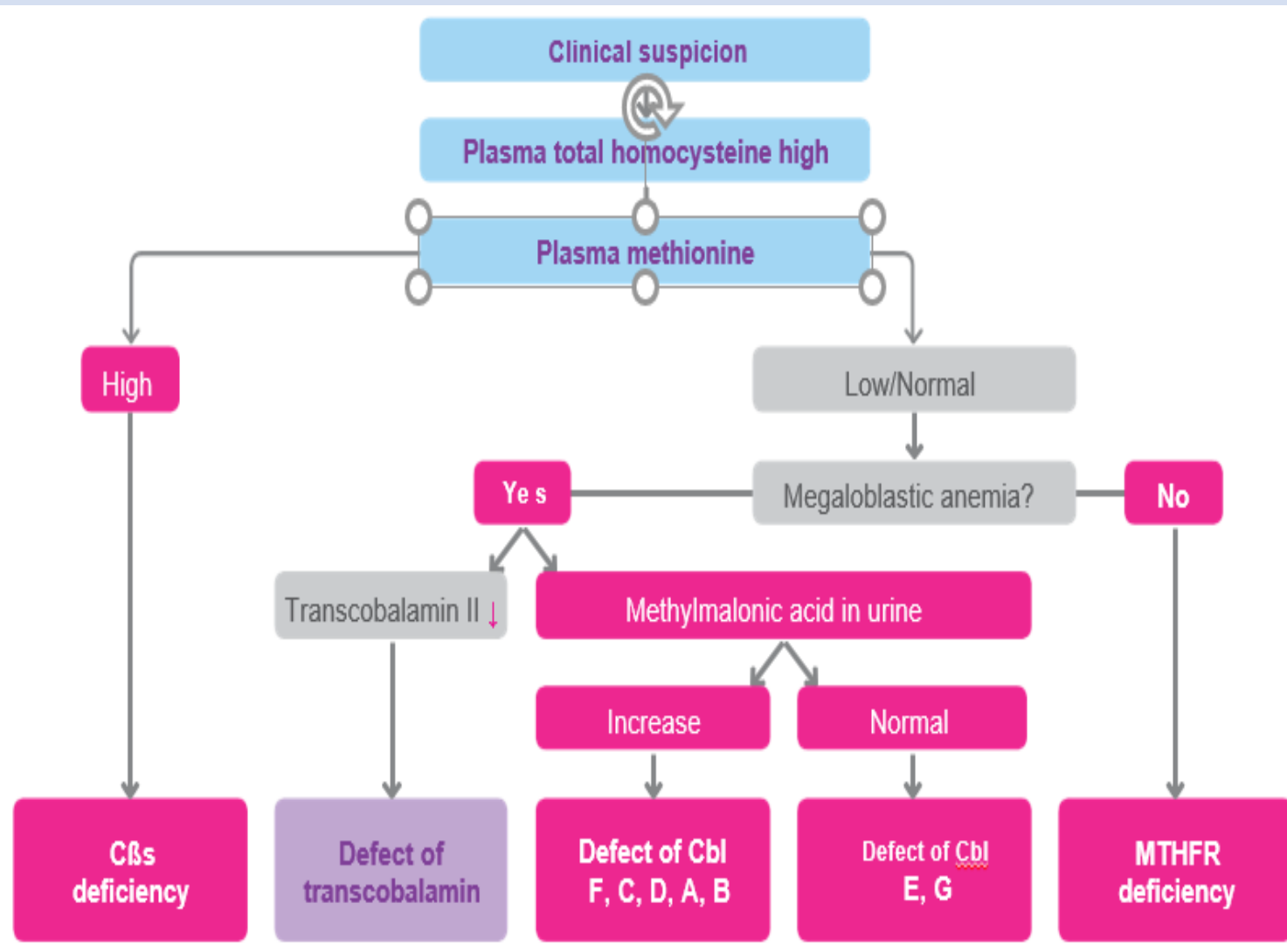
Homocysteine

CβS
Vit B₆
Cystathionine

γ-Cystathionase
Vit B₆
Cysteine

MAT: Methionine adenosyltransferase
SAHH: S-adenosylhomocysteine hydrolase
SAM: S-adenosylmethionine
SAH: S-adenosylhomocysteine
CβS: Cystathionine β-synthase
MS: Methionine synthase
SHMT: Serine-homocysteine methyltransferase
BHMT: Betaine-homocysteine methyltransferase
MTHFR: 5,10-methylenetetrahydrofolate reductase

HOW TO DIAGNOSE THESE 3 TYPES OF HOMOCYTINURIAS?

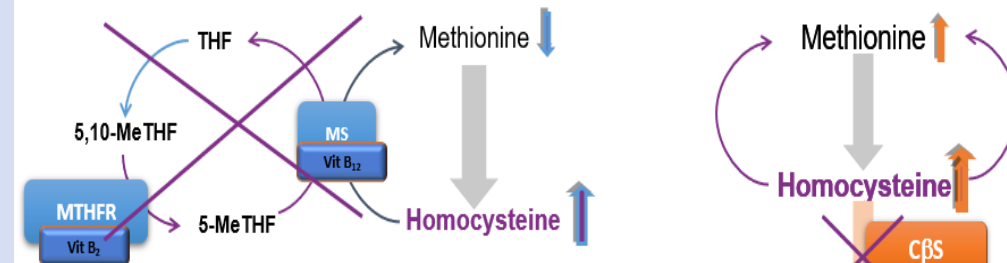


Biochemical hallmarks

Remethylation defects

versus

Transsulphuration defect

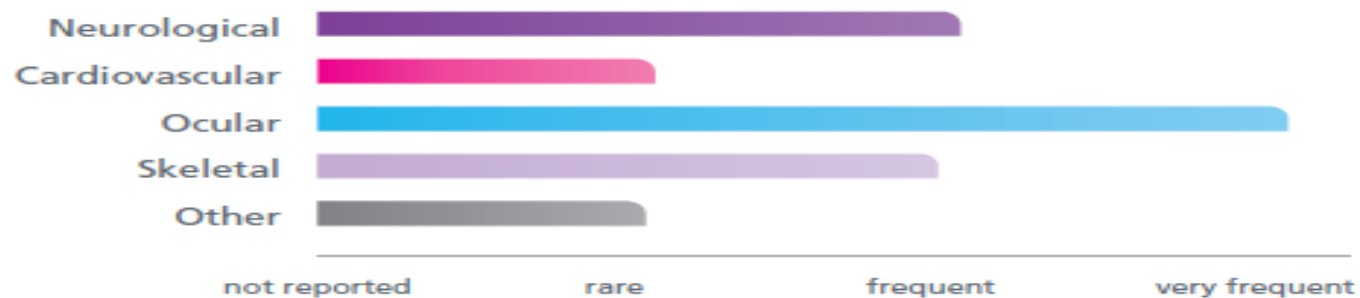


Defects	Methionine	Homocysteine	Cysteine
Transsulphuration	↑↑	↑↑	↓↓
Remethylation	Normal - ↓	↑↑	Normal - ↑

FREQUENCY OF THE CBS DEFICIENCY SYMPTOMS

Transsulphuration defects^{3,4}

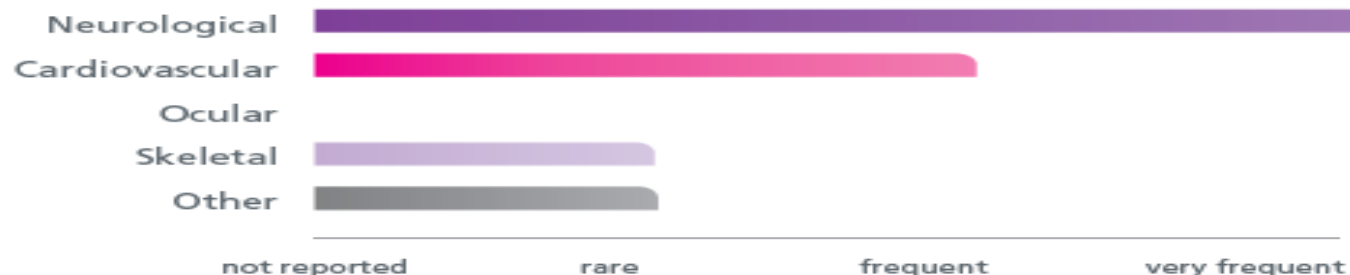
Symptoms:



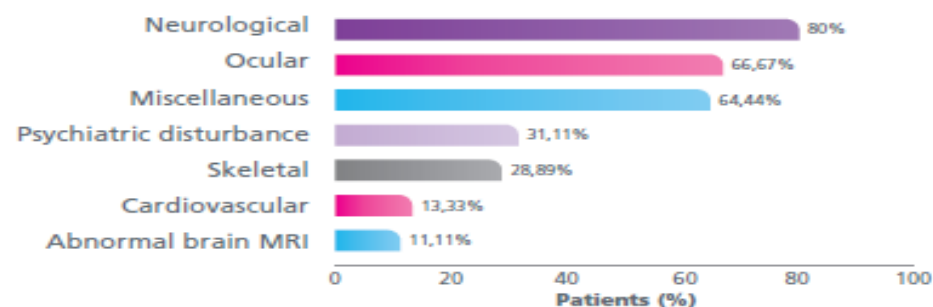
FREQUENCY OF THE MTHFR DEFICIENCY SYMPTOMS

Remethylation defects⁷⁻⁸

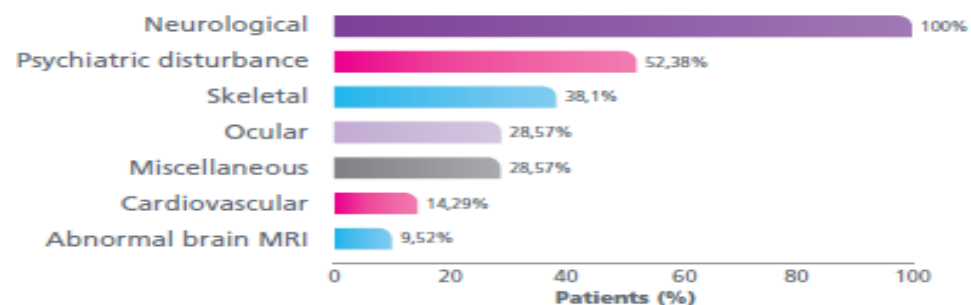
Symptoms:



Cbl metabolism defects (n=45)



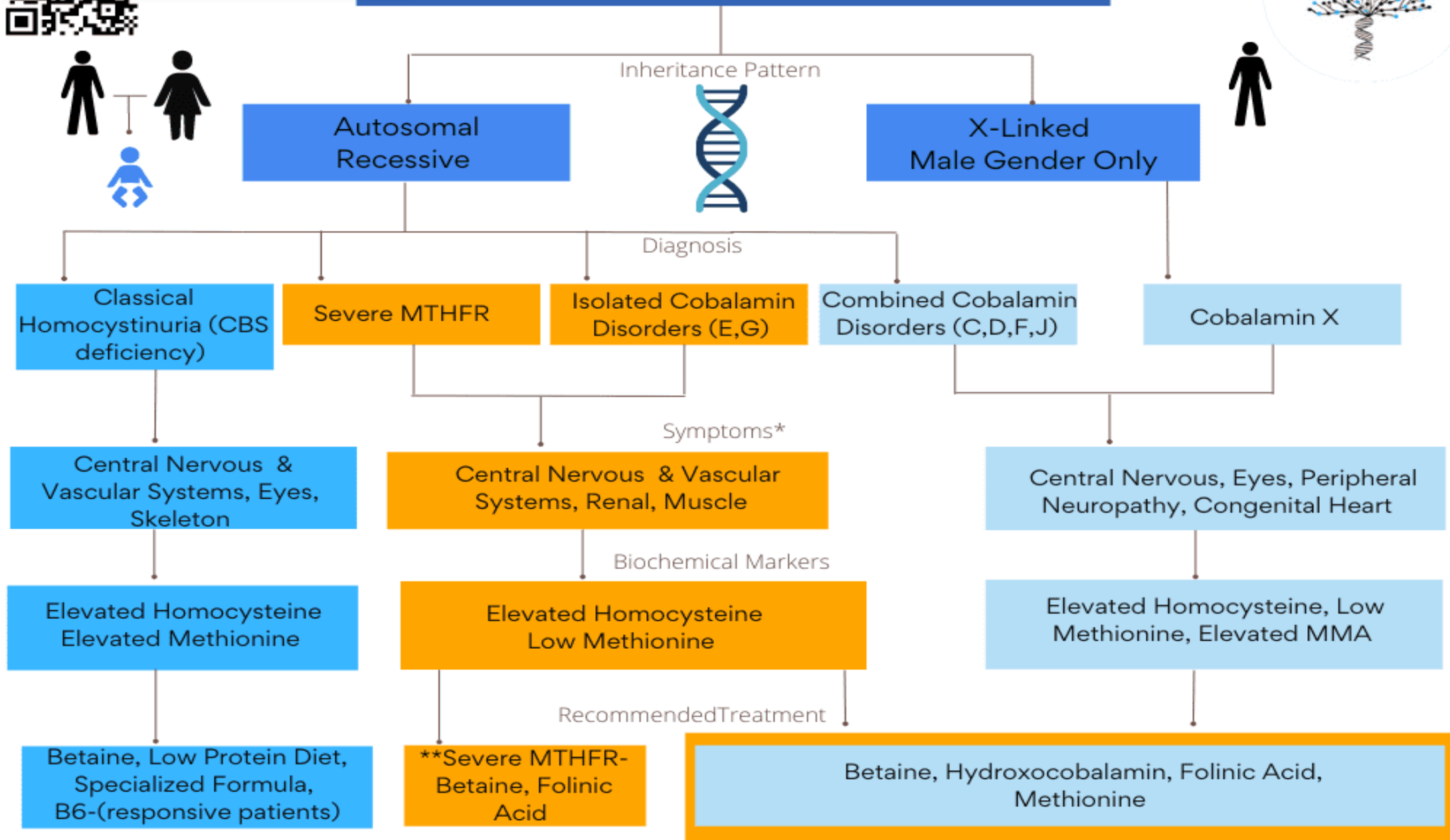
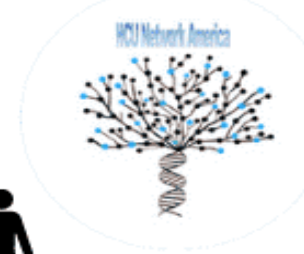
MTHFR deficiency (n=21)





Contents of the Clipboard.

Homocystinuria

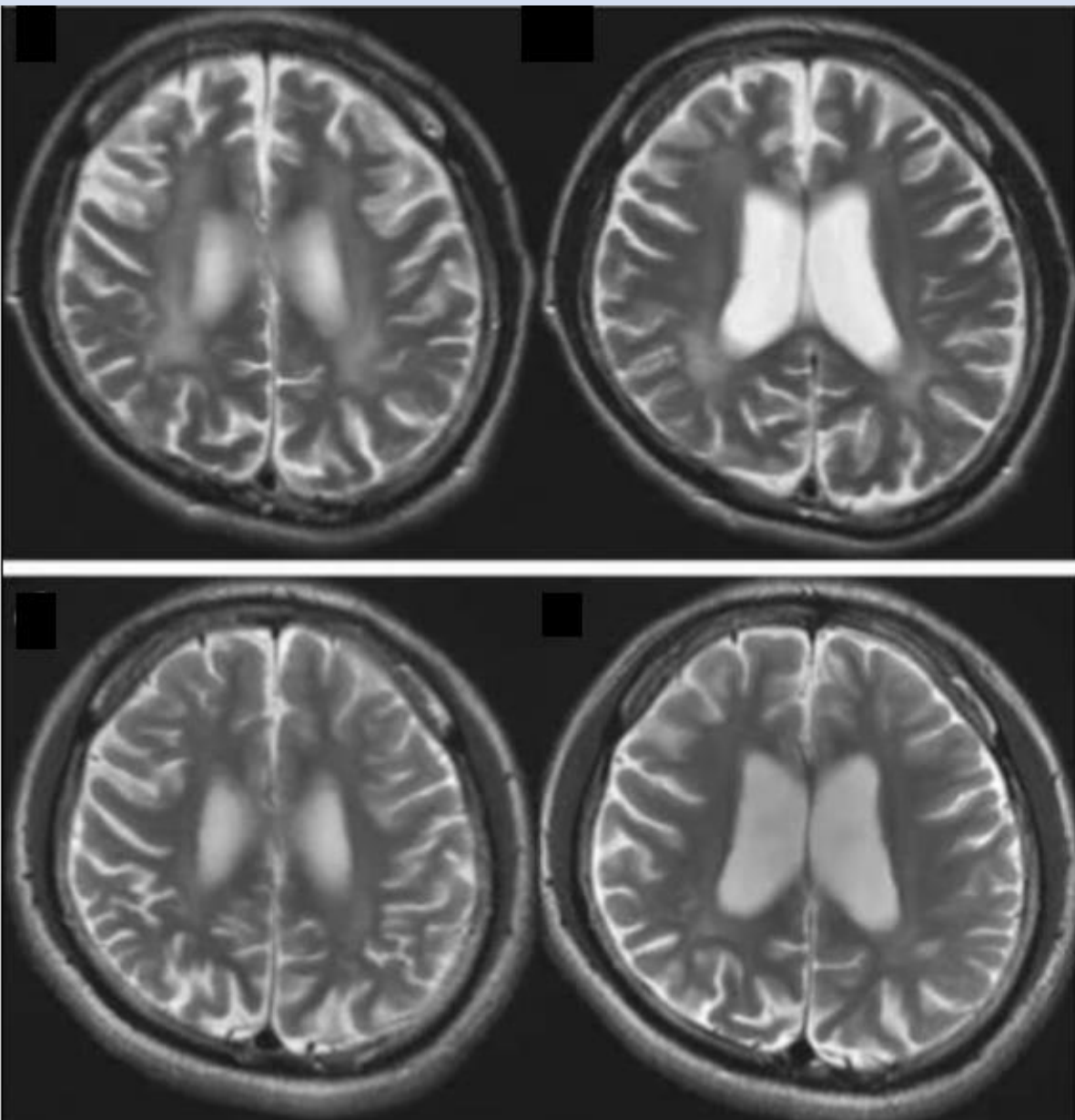


Case Presentation 1:

A 12-year-old girl from Ilam province (recently residing in Tehran) presented with an **one-year history** of :

- progressive **ataxia**
- generalized **dystonia**
- seizures** (starring & clonic)

Despite being under neurological care during this period, her symptoms persisted except seizures.



Obtained MRI images (FLAIR) from patient showed a **high-intensity area in the white matter** around the **posterior horns** of the lateral ventricles.

High-intensity areas in the white matter **around the posterior horns** of the lateral ventricles, as seen on MRI, are most commonly attributed to white matter hyperintensities (WMHs), a **hallmark of small vessel disease**.

Other possible causes include:

periventricular cap formation
demyelination (as seen in **MS** or other demyelinating diseases)
inflammation or **infection**.

Treatment History

She was started on anticonvulsant therapy (Levetiracetam and carbamazepine), which successfully controlled her seizures.

However, she continued to experience **persistent gait ataxia** and **generalized dystonia**, predominantly affecting her lower limbs.

Previous history

Over the past one year, the patient's only complaint has been **migraine** headaches, despite having no family history of migraines in their parents or close relatives!!!.

Examination Findings

At the time of evaluation:

Ataxia was evident, particularly impairing her balance while walking.

Dystonia remained prominent, with noticeable involvement of the lower extremities.

Metabolic work up had been done by ped. neurologist, but all result had not shown anything.

Normal profile in (acyl carnitin & plasma amino acids & urine organic acids) had been reported.

Test(s) requested: Whole Exome Sequencing		
Clinical Indication: A 12-years-old girl with speech delay, ataxia, seizure, developmental delay. The parents are consanguineous.		
Quality of sample: Good		
Sequencing process: Whole Exome Sequencing	Read Length (bp): 100	Coverage: 100X
Single/paired-end sequencing: Paired-end sequencing	Platform: Illumina NovaSeq6000	
Method: Genomic DNA was extracted from the peripheral blood. Whole Exome Sequencing was used to enrich all exons of protein-coding genes as well as some important other genomic regions in DNA. Next-generation sequencing was performed to sequence close to 100 million reads on an Illumina Sequencer. In general, the test platform examined >95% of the targeted regions with a sensitivity of above 99%. In this test, point mutations, and micro-insertion/deletions and duplication (<20bp) can be simultaneously detected. Bioinformatics analysis of the sequencing results was performed using international databases and standard bioinformatics software.		
Interpretation:		
This sample is Homozygous for Likely Pathogenic variant in the MTHFR gene. This variant, <u>(c.1130G>A, p.Arg377His)</u> has not been reported for its clinical evidence as Pathogenic . Mutations in this gene have associated with Neural tube defects, susceptibility to, Schizophrenia, susceptibility to, Thromboembolism, susceptibility to and Homocystinuria due to MTHFR deficiency.		

genetic study was shown :likely pathogenic variant in the **MTHFR** gene. (c.1130G>A,p.Arg377His)

With this genetic result, we expected the laboratory test report to be different
and
to have normal or low methionine levels
in addition to
high homocysteine levels along with normal C3.

“Error origin in metabolic laboratory”

we started

Folinic acid 15 mg/day

A high-dose oral therapy with **betaine** (10 g/day)

Hydroxocobalamin (5mg/ day for 3 3 consecutive days and then every other day)

B6 & Methionine.

Significant improvements in motor and cognitive functions were observed within 4–6 days of therapy.

Early treatment with Betaine(a methyl donor) has been shown to prevent mortality and improves psychomotor development even in pediatric cases with severe enzyme deficiency.

We strongly recommend betaine treatment in individuals with MTHFR deficiency; it **improves the outcome** and **prevents disease** when given early.



Severe MTHFR deficiency usually occurs shortly after birth or in early childhood with

failure to thrive
muscle hypotonia
Hydrocephalus
apnea.

Late onset is characterized by predominantly

psychiatric or cognitive symptoms
Seizures
&
in nearly all cases—gait disorders

NEUROLOGICAL	
EARLY ONSET	LATER ONSET
• Recurrent apnea • Microcephaly • Convulsions	• Mental regression • Ataxia • Psychiatric disorders, e.g. schizophrenia • Symptoms related to cerebrovascular events

MTHFR		Could it be Homocystinuria?
Common Variants		Rare Variants
• MTHFR deficiency (C667T and A1298C) • Found in 30-40% of the U.S. population		• Homocystinuria due to severe MTHFR deficiency • Fewer than 100 documented cases worldwide
• MTHFR enzyme activity only mildly or moderately reduced		• <1% MTHFR enzyme activity
• Homocysteine - normal or slightly elevated; normal range is 5-15 (µmol/L) • Methionine - normal		• Homocysteine - higher than normal; often over 100 (µmol/L) in untreated individuals • Methionine - usually lower than normal
• No treatment typically recommended		• Treatment must be prescribed by a doctor
• Symptoms Typically not symptomatic		• Symptoms Early-Onset: Lethargy, Feeding Problems, Seizures, Global Delays Late-Onset - Cognitive Deficits, Behavior Problems, Seizures

MTHFR deficiency leads to a partial or complete **lack of methylation** of homocysteine to methionine resulting in highly elevated plasma total homocysteine and low plasma methionine.

Especially **low methionine** level causes a depletion of S-adenosylmethionine which plays an important role as a **donor** in methylation reactions in the central nervous system.

At **6-week**-follow-up, she showed :

an almost **complete recovery** of ataxia and almost complete regeneration in cognitive abilities

&

Her gait improved significantly, enabling her to walk 20 m without help or assistance.

Homocysteine level decreased to 66.0 $\mu\text{mol/l}$.

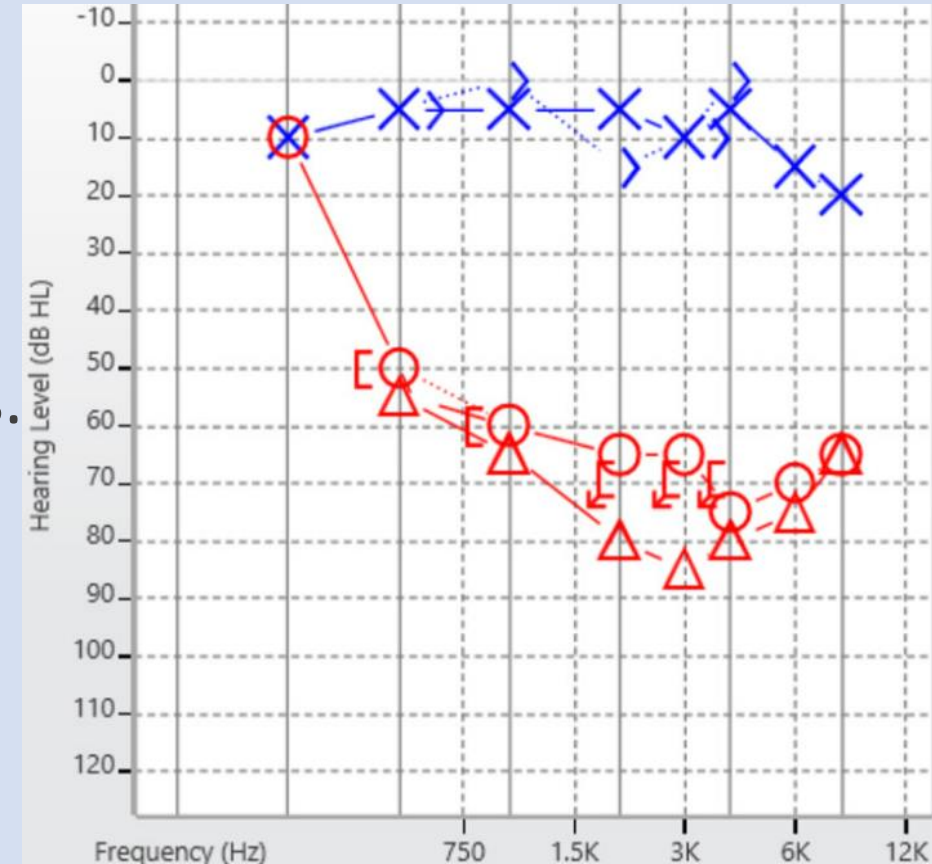
Three months follow-up MRI surprisingly showed a **notable reduction of paraventricular leukoencephalopathy** with regressive hyperintense T2 lesions

In one literature published in 2019, reported that most patients with MTHFR deficiency, are symptomatic in the **first year of life**, but one patient was not diagnosed **until age 21 years** and carried a **misdiagnosis of multiple sclerosis** .

Case presentation 2

A 3-year-old child was referred for developmental delay. Surprisingly, the patient's deafness had gone unnoticed until I incidentally suspected hearing loss.

A subsequent hearing test confirmed sensorineural hearing loss.



Previous neurological evaluations attributed the findings to asphyxia, leading to the assumption that a metabolic disorder was unlikely.

Consequently, I initially ordered only the most accessible metabolic tests.

Primary lab data showed :

Elevated Homocysteinuria
Low Methionine

Advanced lab data was done:

Beside elevated plasma homocysteine level & low methionine level , C3 acylcarnitine was normal and also undetectable urine methylmalonic acid .

Therefore the most probable diagnosis was Homocysteinuria due to MTHFR mutation

Association between the methylenetetrahydrofolate reductase gene C677T polymorphism and sudden sensorineural hearing loss: a meta-analysis

Jingcheng Shu · Shihua Yin · An-Zhou Tan ·
Meirong He

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doi:10.1017/S002221511000099X

Main Article

Abstract
Methylenetetrahydrofolate reductase C677T gene mutation as risk factor for sudden sensorineural hearing loss: association with plasma homocysteine, folate and cholesterol concentrations

Methylenetetrahydrofolate reductase C677T gene mutation as risk factor for sudden sensorineural hearing loss: association with plasma homocysteine, folate and cholesterol concentrations

E J LEE, Y J CHO, Y J YOON

Abstract

Objective: Impaired cochlear perfusion appears to be the most important event in the development of sudden sensorineural hearing loss. Methylenetetrahydrofolate reductase gene mutations at nucleotide 677 cause reduced methylenetetrahydrofolate reductase enzyme activity, resulting in vascular impairment.

Methods: Thirty-three patients and 68 control subjects underwent audiological and haematological investigation

There was a significant association between the C677T polymorphism and sudden sensorineural hearing loss. The association was more significant in patients with plasma homocysteine, folate and cholesterol concentrations

Impaired cochlear perfusion (ischemic) is the most widely accepted hypothesis for the pathogenesis of sudden SNHL.

studies have contributed to the:

1) **vascular theory** of sudden SNHL by investigating thromboembolism, blood factor disturbances and cochlear microcirculation alteration

2) **Vascular derangement**, caused by cochlear injury or dysfunction due to anoxia or hypoxia, may cause sudden SNHL.

3) **endothelial dysfunction**, high levels of haemostatic factors and blood flow disturbance – the fundamental features of thromboembolic disease – can impair cochlear microcirculation.

Treatment was started with

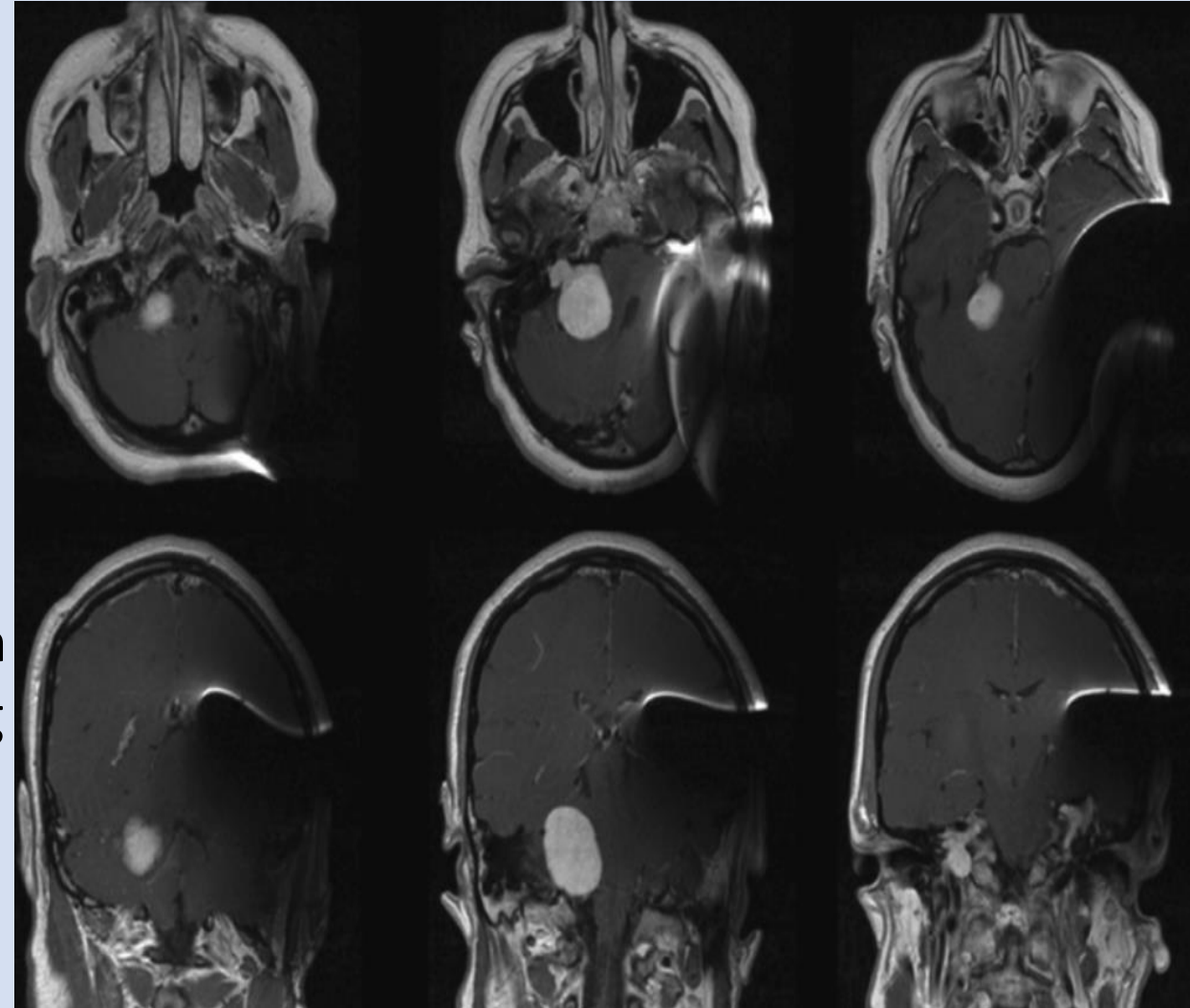
Folinic acid 15 mg/day

Betain 4g/day

Hydroxocobalamin

Pyridoxin 3 Tab/day.

- Plasma Homocystein level and clinical condition got better surprisingly but sensory neural hearing loss remain intact. And he underwent **brain stem implant.**



The folate cycle is a biochemical pathway that processes folate (vitamin B9) to support critical functions like DNA synthesis, cell division, and methylation
(a process vital for gene regulation and neurotransmitter production).

1-Folate Uptake

Dietary folate (from leafy greens, legumes) or synthetic folic acid enters cells via transporters like the reduced folate carrier (RFC).

2. Activation:

- Natural folate is converted to *tetrahydrofolate (THF)* via enzymes like dihydrofolate reductase (DHFR).
- Folic acid must first be reduced to *THF* by DHFR, which can be inefficient in some people.

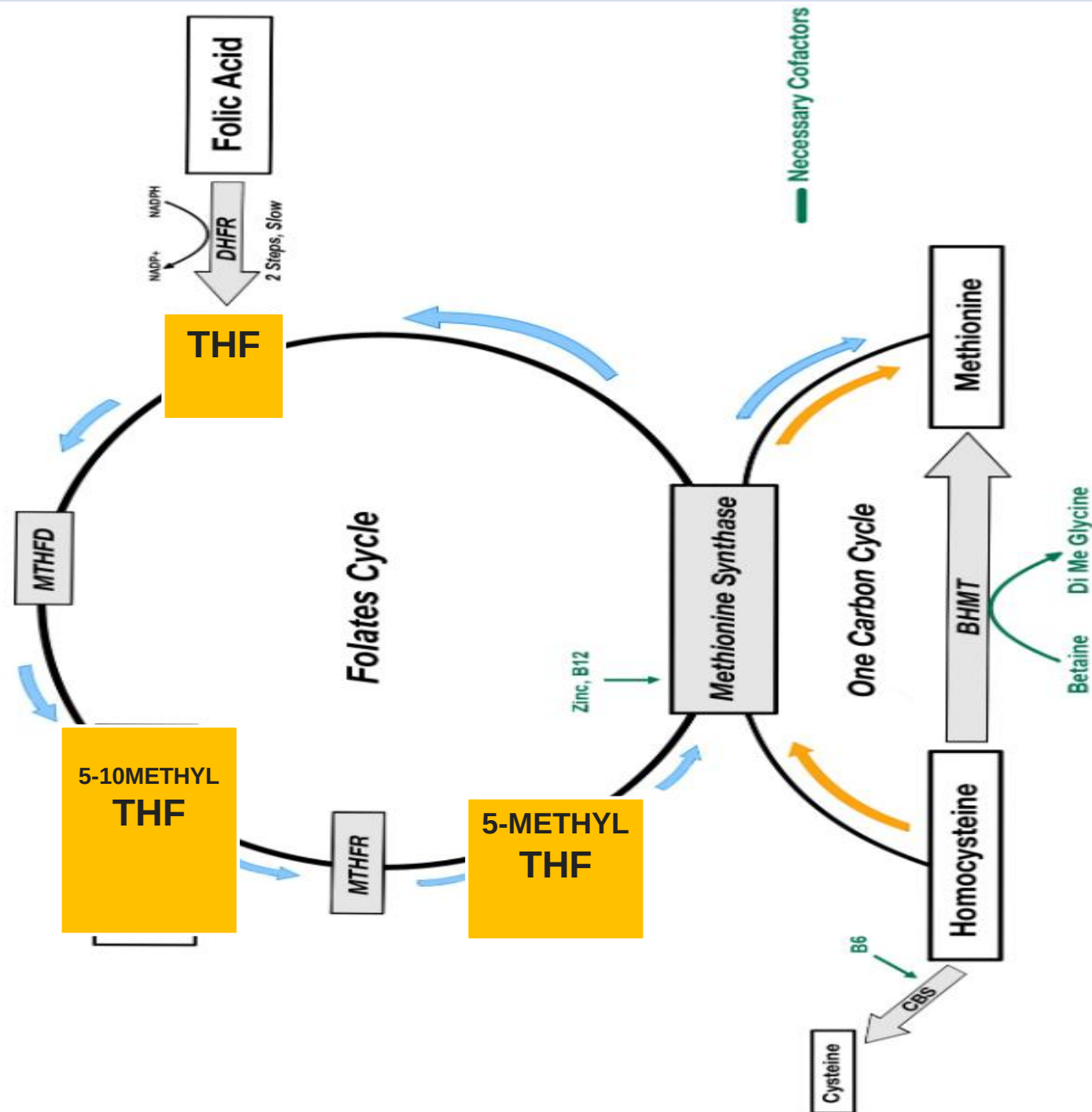
3. One-Carbon Transfers

THF carries "one-carbon units" (e.g., methyl groups) to:

- DNA synthesis

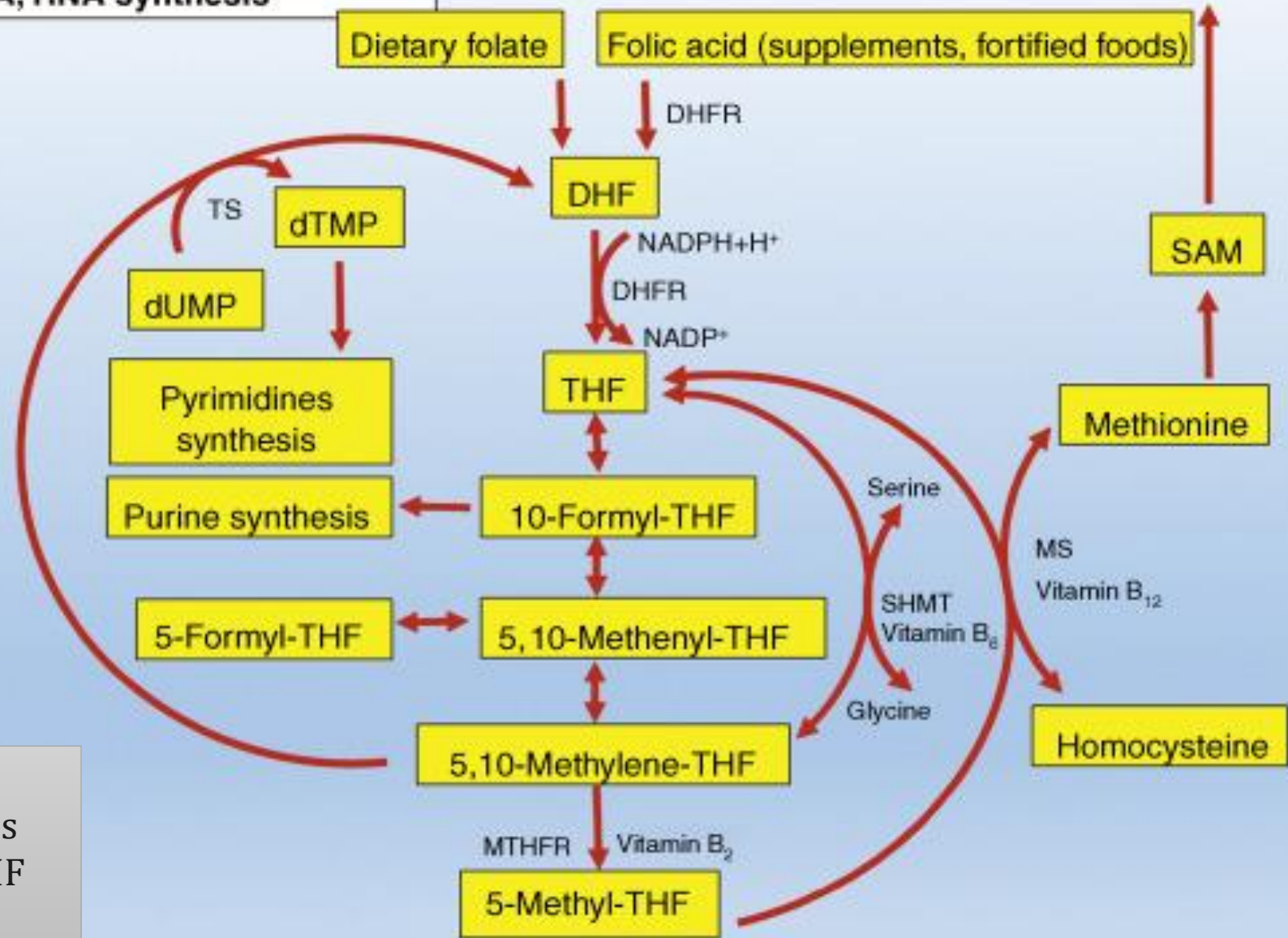
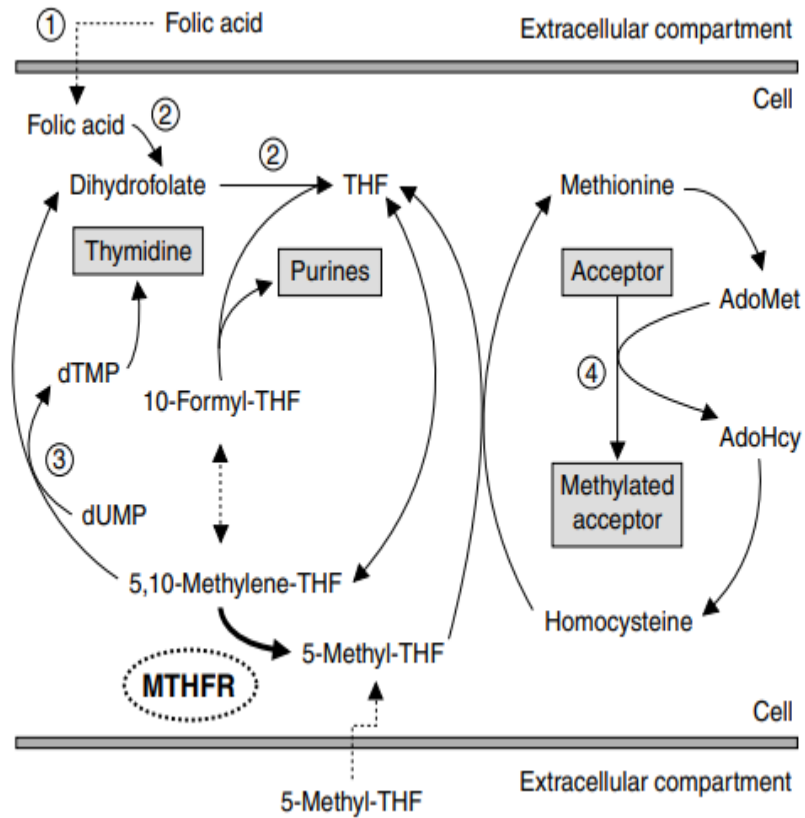
4. Methylation

5-methyl-THF donates a methyl group to vitamin B12, forming methionine and eventually SAME (the body's master methylator).

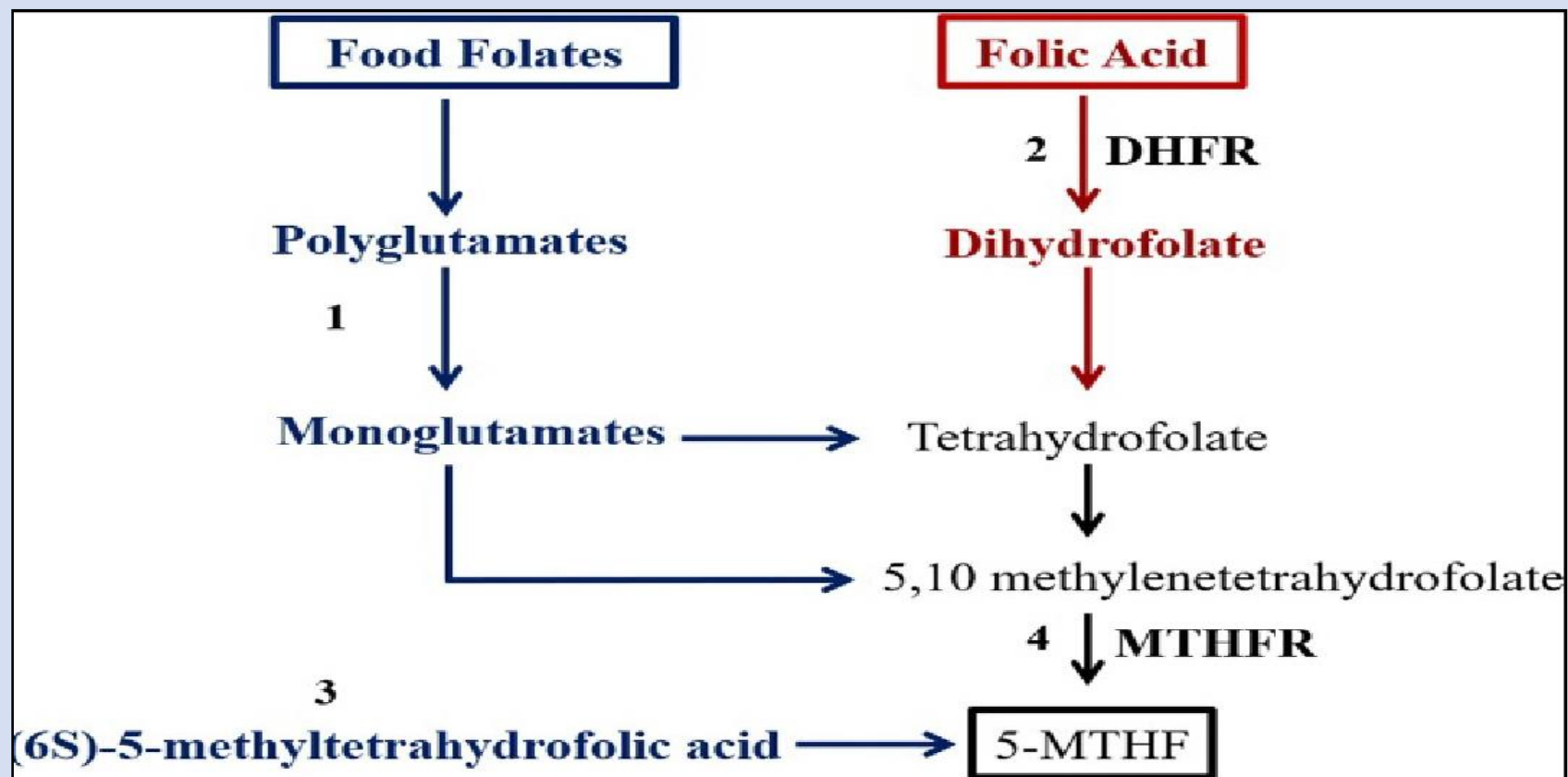


Nucleotide synthesis → DNA, RNA synthesis

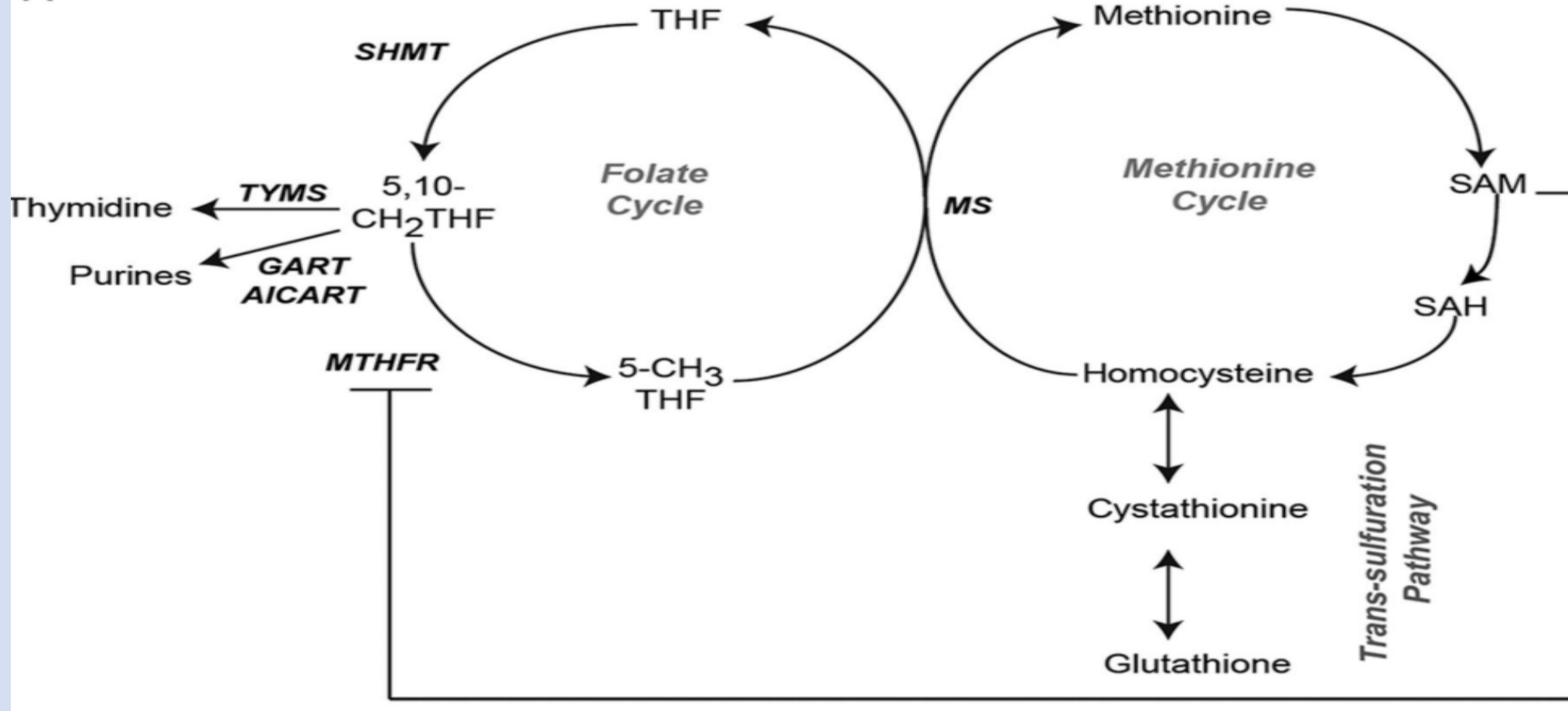
DNA, RNA, protein, lipids methylation



Folinic acid (Leucovorin) is also a synthetic molecule, a 5-formyl derivative of THF that is readily converted to 5-10 MTHF and 5-MTHF without requiring the action of DHFR.



Feature	Folic Acid	Folinic Acid (Leucovorin)
Form	Synthetic, oxidized form of B9	Natural or semi-synthetic, reduced form (5-formyl-THF)
Metabolism	Requires DHFR and MTHFR enzymes to activate (inefficient in MTHFR mutations)	Bypasses DHFR; directly converted to active folate forms without MTHFR
uses	Pregnancy (prevents neural tube defects)	



Methylation is involved in the production of myelime.

Deficiencies in methylation-related pathways can lead to impaired myelination, affecting nerve function and cognitive health.

Since **SAM** is the primary **methyl donor** in the body has role in numerous biochemical reactions, including **DNA methylation** and **neurotransmitter synthesis**, its **accumulation** signals that sufficient methylation is occurring, leading to **MTHFR inhibition** to prevent excessive methionine synthesis.

SAM binding alters the orientation of flavin adenine dinucleotide (**FAD**), an essential **cofactor for MTHFR**, thereby reducing its catalytic efficiency.

This inhibition helps regulate the balance between folate metabolism & methionine cycle, ensuring proper methylation processes without excessive homocysteine conversion. If MTHFR function is impaired due to genetic mutations, it can lead to elevated homocysteine levels and reduced SAM production and SAM/SAH ratio , potentially can affecting methylation-dependent processes

Folate is crucial for nucleotide synthesis, particularly for:

1. Purine Synthesis

Folate derivatives (like 10-formyl-THF) donate **one-carbon units** required for building the purine ring structure (positions C2 and C8).

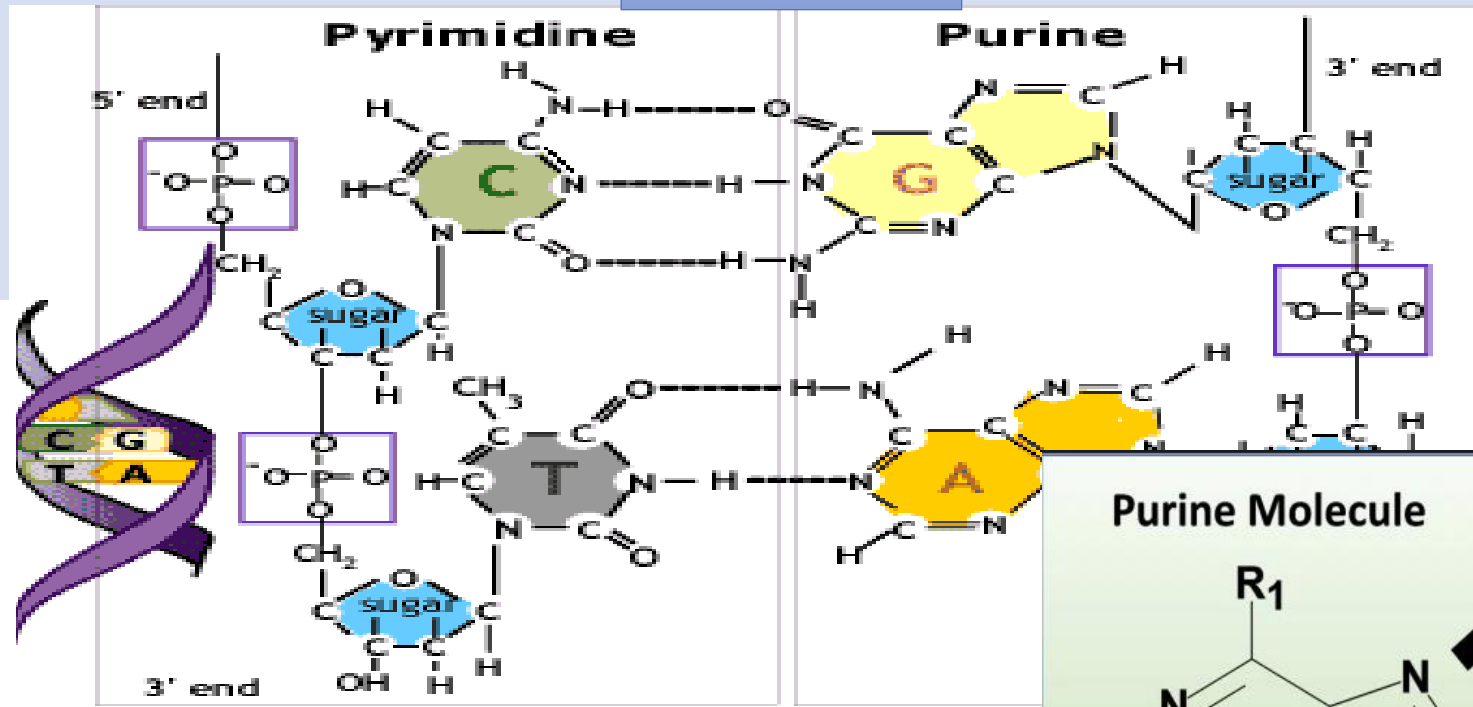
2. Pyrimidine Synthesis (dTMP Production)

Folate (as 5,10-methylene-THF) is essential for converting **dUMP (deoxyuridine monophosphate)** into **dTMP (deoxythymidine monophosphate)**, a critical step mediated by thymidylate synthase.

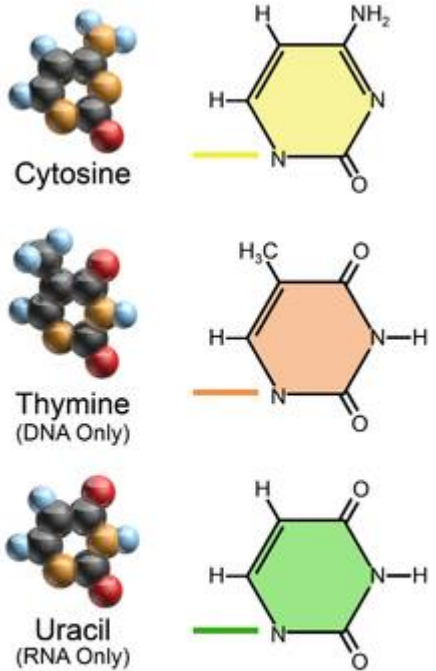
3. Methionine and S-Adenosylmethionine (SAM) Synthesis

1. Folate helps regenerate **methionine** (via homocysteine remethylation), which is needed for **SAM**, a methyl donor for DNA methylation and gene regulation.

DNA structure



Purines
(adenine and guanine) are
essential **nitrogenous
bases** that form part of the
DNA structure.



Pyrimidines

Purine Molecule

Guanine: Target of genetic mutations.

Adenine (nucleoside or nucleotide form):
-Effects on cell signalling as ligand.
-Structural core for compound design with controlled effects.
-Regulation of metabolism, anti-cancer immune modulation, etc.

S-Adenosylmethionine (SAM):
Methyl donor that acts as a cancer protector.

R₁ = -NH₂, R₂ = -H and R₃ = -H (Adenine). R₁ = -NH₂, R₂ = -H and R₃ = -Ribose (Adenosine).
R₁ = =O, R₂ = -NH₂ and R₃ = -H (Guanine). R₁ = =O, R₂ = -NH₂ and R₃ = -Ribose (Guanosine).
R₁ = -NH₂, R₂ = -H and R₃ = -Ribose-5'-L-methionine (SAM).

Folate (vitamin B9) plays a critical role in DNA synthesis by providing the necessary one-carbon units

needed to build DNA bases (purines and pyrimidines)

Brain development and its maturation also depend on **correct lipid methylation**,
and
continued neuronal function depends on **biogenic amines** that **require methylation for their synthesis**.

All methylation processes are **carried out via a methyltransferase** enzyme and its unique **co-factor** S-adenosylmethionine (**SAM**); the transfer of a methyl group to a target molecule results in the release of SAH (SA homocysteine), and then homocysteine (Hcy).

Both of these molecules are **toxic, inhibiting methylation** in a variety of ways, and Hcy recycling to methionine is imperative; this is achieved via the **one carbon cycle**, supported by the **folates cycle**.

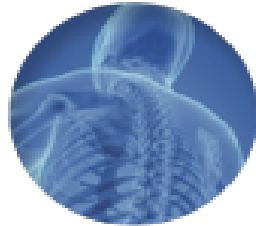
Folate deficiency causes hyperhomocysteinaemia, with several associated diseases; during early pregnancy, deficiency interferes with closure of the neural tube at the fourth week of gestation, and nutraceutical supplementation has been routinely prescribed to prevent neural tube defects, mainly involving B vitamins, Zn and folates

HAVE YOU EVER SEEN THOSE SYMPTOMS?¹



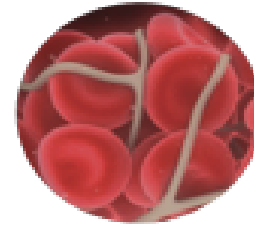
OCULAR

- Lens :
 - Progressive severe myopia
 - Iridodonesis
 - Lens dislocation (ectopia lentis)
 - Cataract
- Glaucoma resulting from untreated ectopia lentis
- Retinal detachment secondary to ectopia lentis



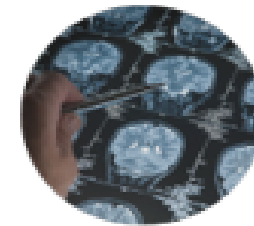
SKELETAL

- Osteoporosis
- Biconcave vertebrae
- Scoliosis
- Increased length of long bones
- Irregular widened metaphysis
- Metaphyseal spicules
- Abnormal size and shape of epiphysis
- Growth arrest lines
- Pes cavus (high arched feet)
- High-arched palate



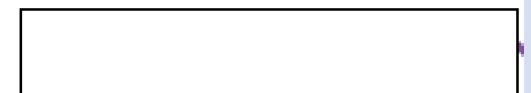
CARDIOVASCULAR

- Thrombo-embolism
→ Vascular occlusion may appear at any age



NEUROLOGICAL

- Intellectual disability
- Psychiatric disturbances
- Extrapyraxidal signs
- Seizures



12/13	Age: 4 month	WES accession: : PGWES3014
Referred by: Dr. kashani	Sample type: Peripheral Blood	Clinic number: CCGS-14549
	Source: DNA	Date Collected: 403/06/03
		Date Reported: 1403/09/17

Test(s) requested: Whole Exome Sequencing

Clinical Indication: A 4-month-old boy with hyper methioninemia and developmental delay. The parents are consanguineous.

Quality of sample: Good

Sequencing process: Whole Exome Sequencing	Read Length (bp): 100	Coverage: 100X
Single/paired-end sequencing: Paired-end sequencing	Platform: Illumina NovaSeq6000	

Method: Genomic DNA was extracted from the peripheral blood. Whole Exome Sequencing was used to enrich all exons of protein-coding genes as well as some important other genomic regions in DNA. Next-generation sequencing was performed to sequence close to 100 million reads on an Illumina Sequencer. In general, the test platform examined >95% of the targeted regions with a sensitivity of above 99%. In this test, point mutations, and micro-insertion/deletions and duplication (<20bp) can be simultaneously detected. Bioinformatics analysis of the sequencing results was performed using international databases and standard bioinformatics software.

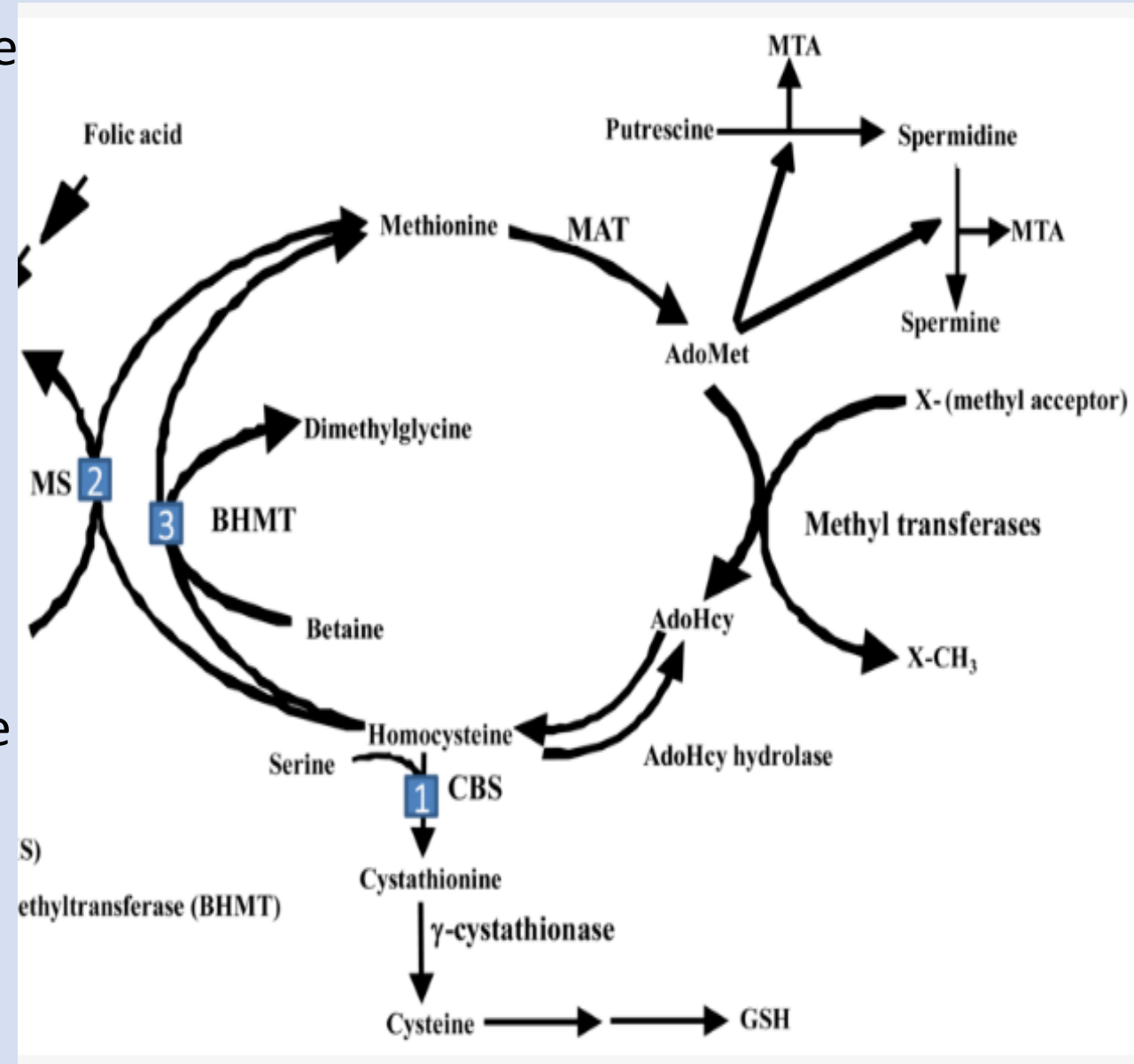
Interpretation:

This sample is **Homozygous** for VUS variant in the *MATIA* gene. This variant, (c.428C>T, p.Thr143Ile) has not been reported for its clinical evidence in Clinvar. Mutations in this gene have associated with **Methionine adenosyltransferase deficiency, autosomal recessive**.

MAT I/III deficiency is caused by mutations in the MAT1A gene, which encodes the enzyme responsible for converting methionine to [S-adenosylmethionine](#) (SAM).

Methionine adenosyltransferase (MAT) I/III deficiency, also known as **Mudd's disease**, is a rare inherited metabolic disorder caused by mutations in the MAT1A gene.

It results in **elevated levels of methionine** in the blood (hypermethioninemia). While many individuals with MAT I/III deficiency are **asymptomatic**, some experience **neurological problems**, including brain demyelination, developmental delays, and intellectual disability.



A methionine-restricted diet may be recommended for those with elevated methionine levels (above 500-600 $\mu\text{mol/L}$) to help manage the buildup.

In some cases, supplementation with SAM may be considered, although its benefits are not fully established.