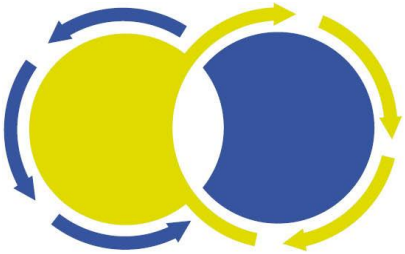


E-HOD

European Network and Registry for
Homocystinurias and Methylation Defects

Homocystinurias: diagnosis, management and role of E-HOD in improving patient care

Viktor Kožich

Institute of IMD, Charles University-First Faculty of Medicine, Prague

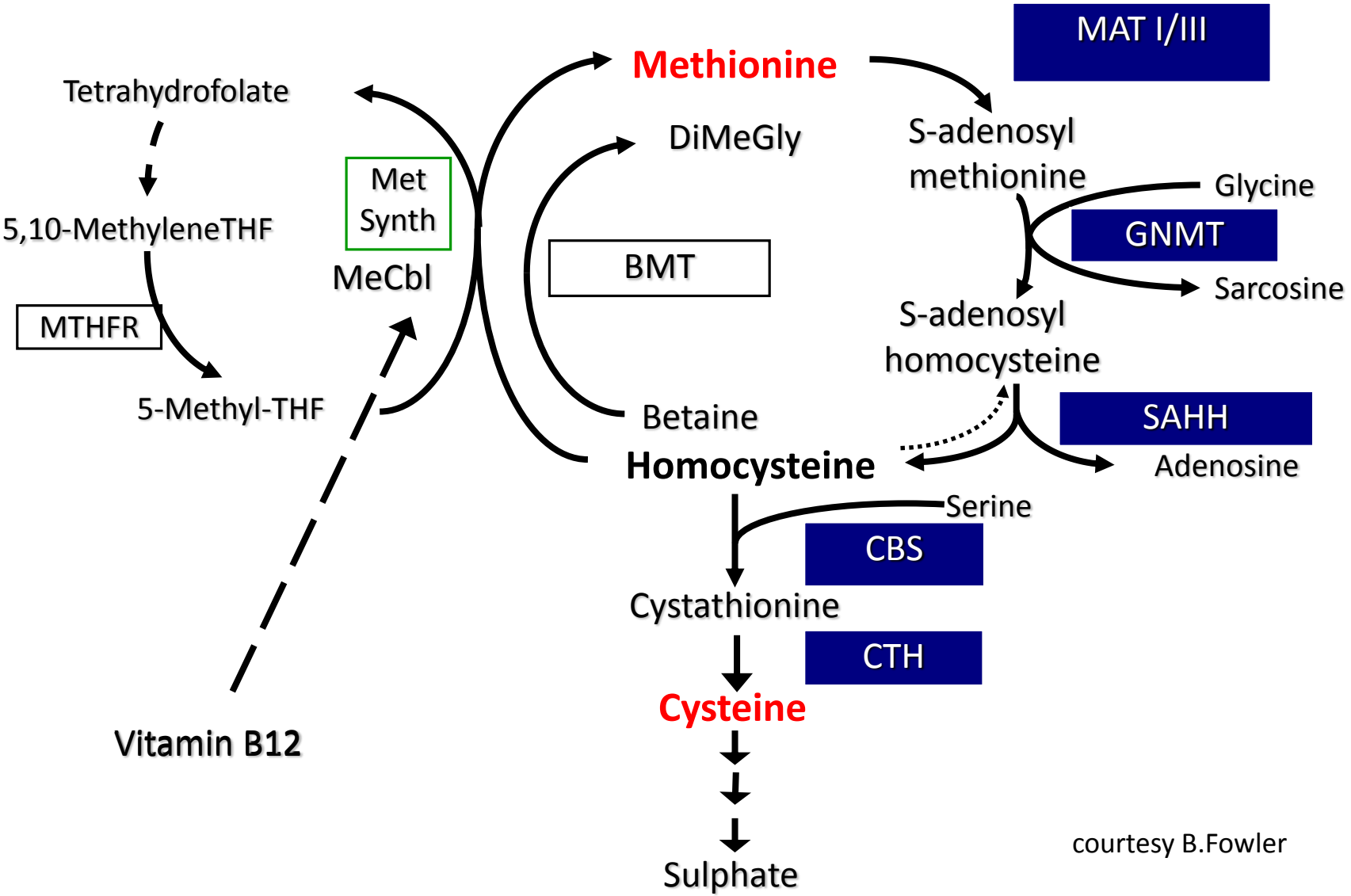
Rare disease day 2015, Vilnius

Co-funded by
the Health Programme
of the European Union

Outline

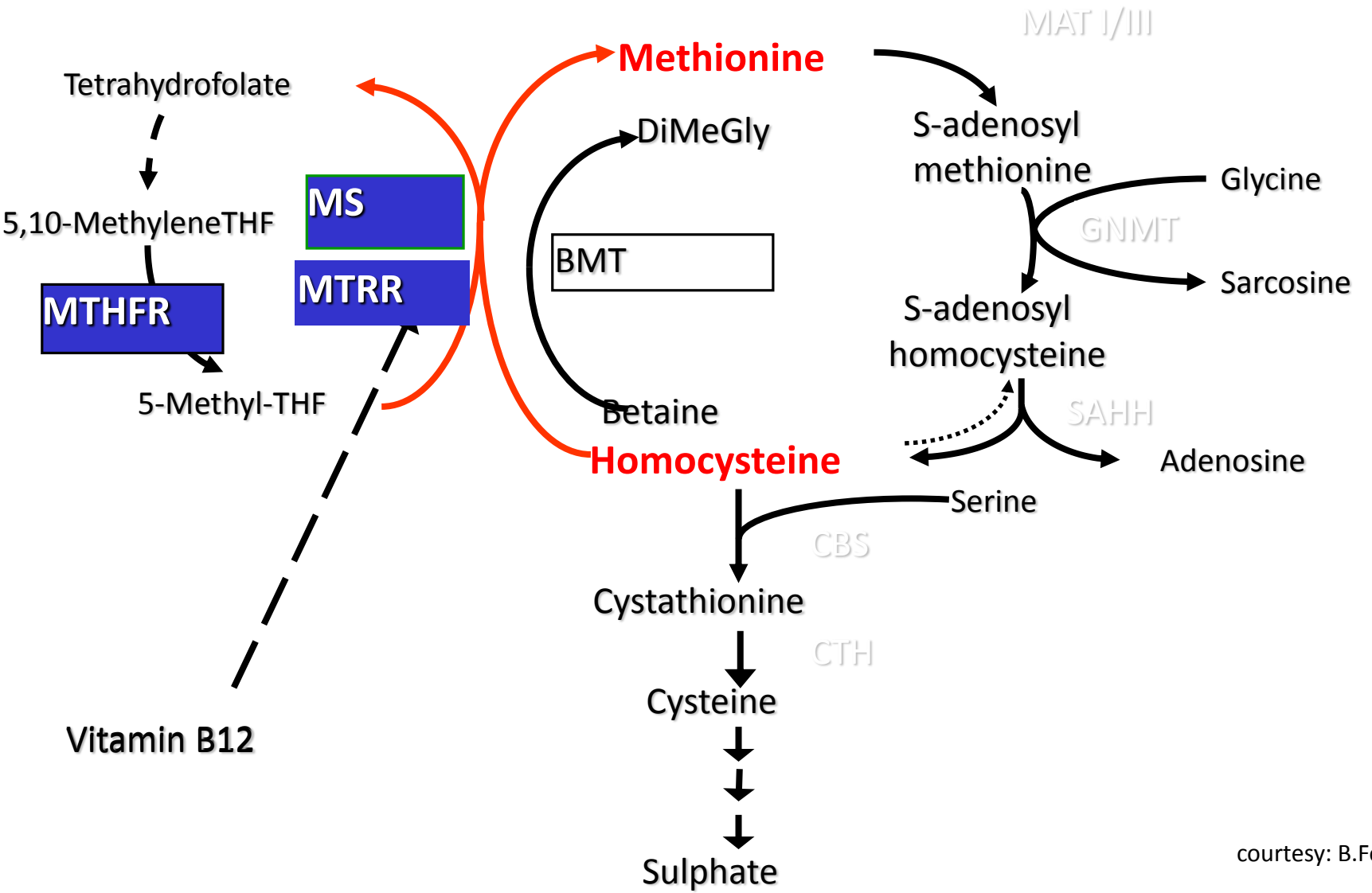
- **Metabolism of sulfur amino acids**
- Homocystinurias
- Improving care with help of E-HOD
- Guidelines: NBS for homocystinurias
- Expanded NBS in Czech Republic

Transsulfuration Met→Cys



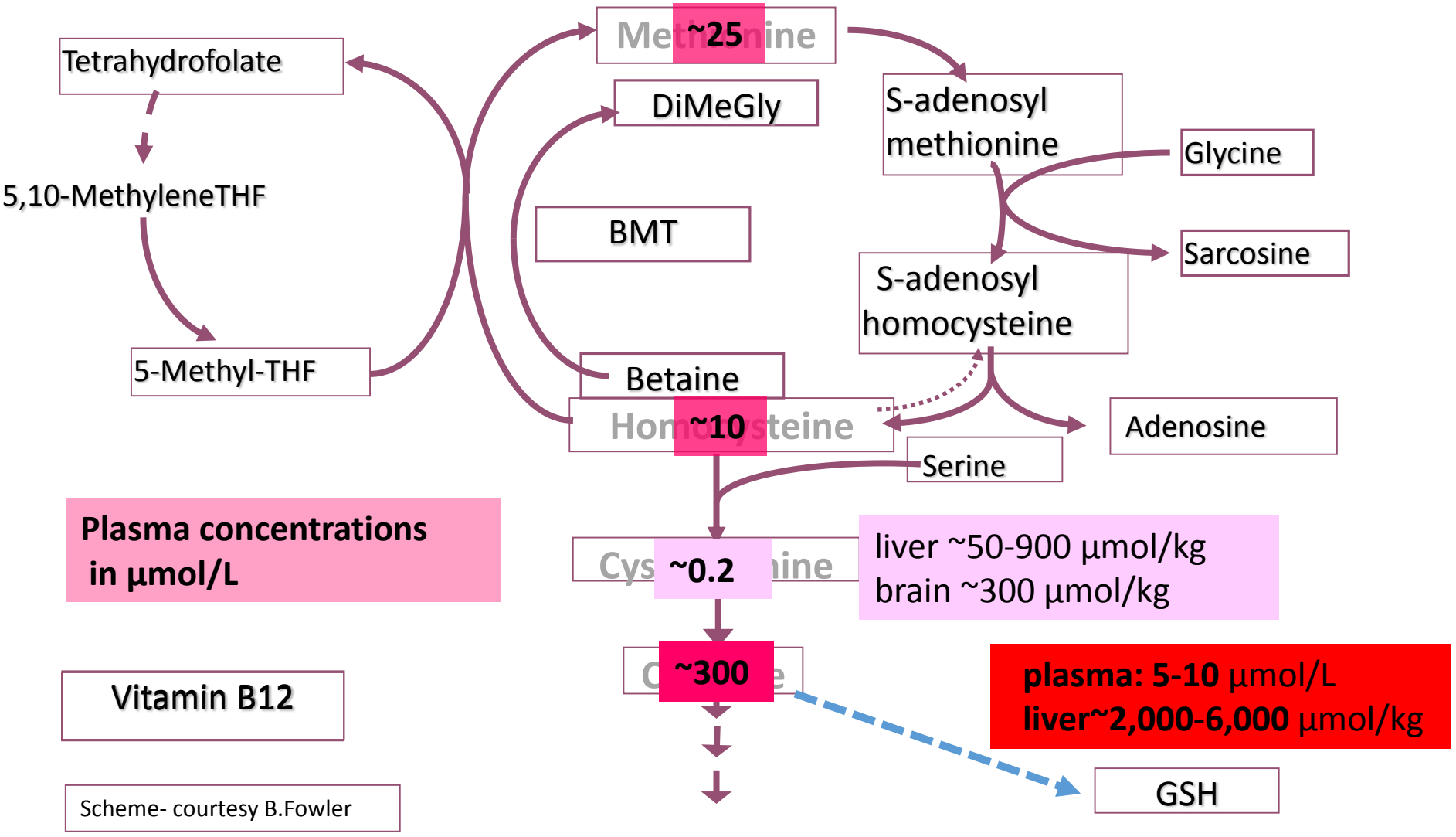
courtesy B.Fowler

Remethylation Hcy→Met

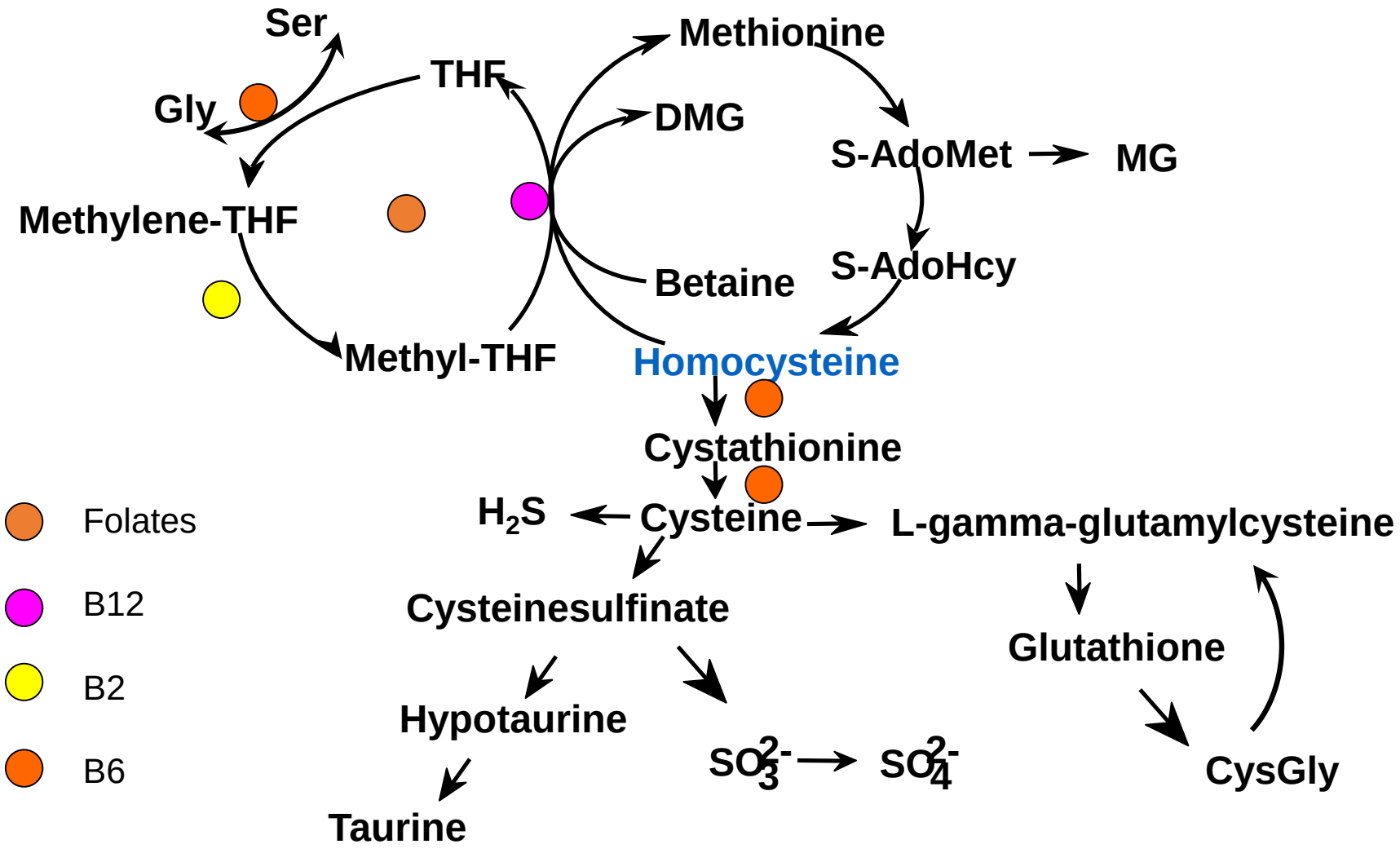


courtesy: B.Fowler

Concentration of metabolites



Vitamins and Hcy metabolism



Outline

- Metabolism of sulfur amino acids
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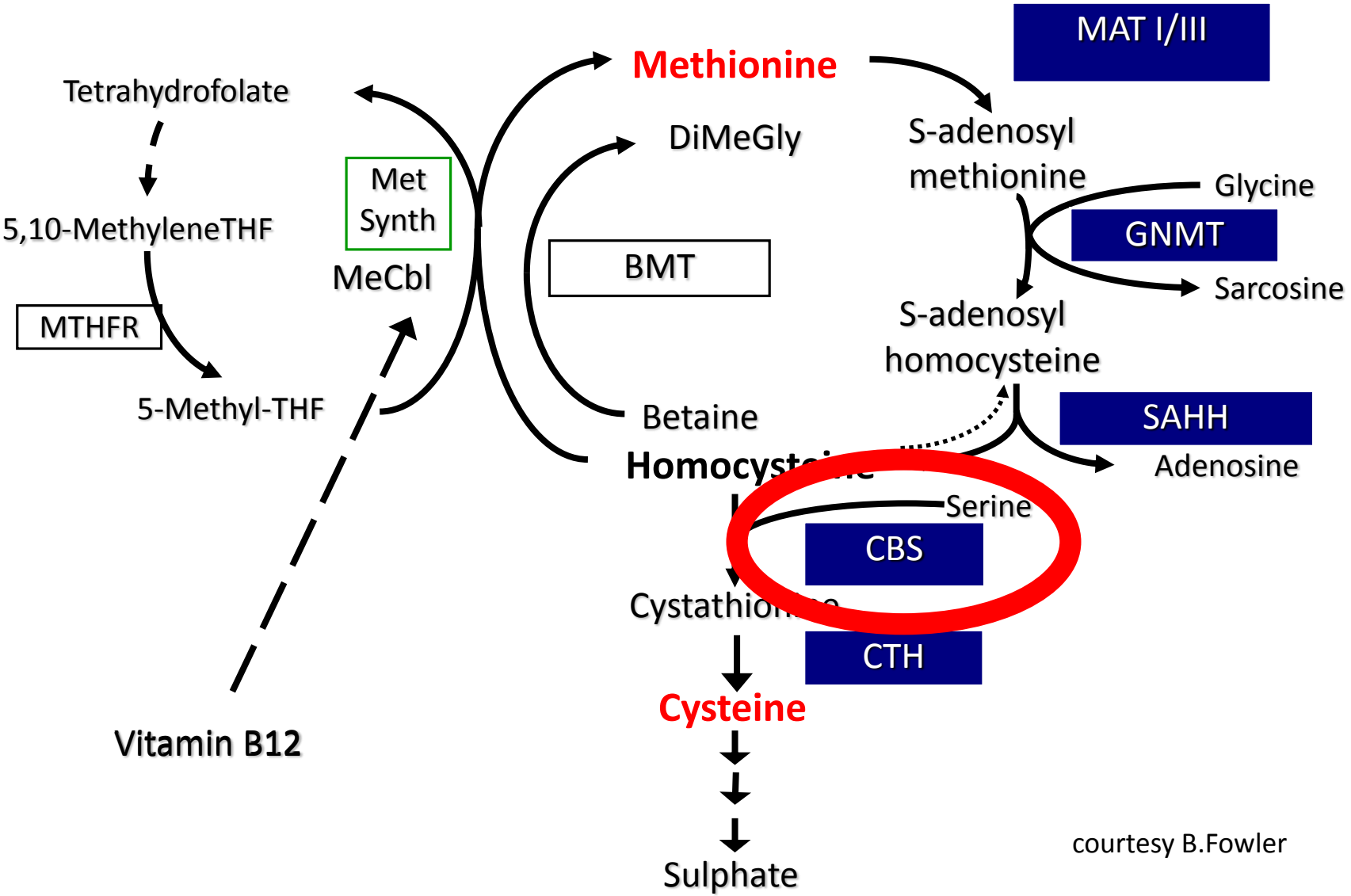
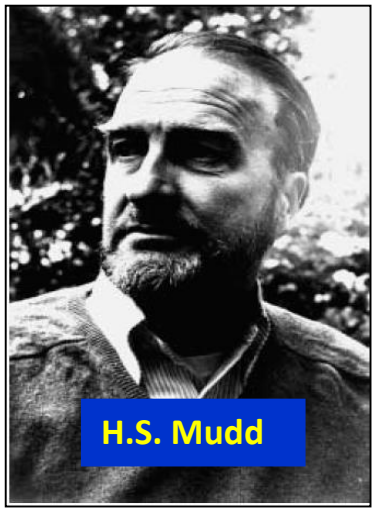


courtesy B.Fowler

Plasma metabolites in transsulfuration defects

Deficiency	MAT	GNMT	SAHH	CBS	CTH
Methionine	↑↑	↑↑	↑↑	↑↑	→
AdoMet	→	↑↑	↑↑	(↑)	
Sarcosine	→	→	↑	↑	
AdoHcy	→	→	↑↑	↑	(↑)
tHcy		→	(↑)	↑↑	(↑)
Cystathionine				↓	↑↑

CBS deficiency-history



- **1962: 2 sibs reported**
  Carson et al,
  Arch Dis Child, 1963
- **1964: enzyme defect described**
  Mudd et al,
  Science, 1964

- **1970s enzymatic studies**
- **1980s Czech patients diagnosed**
  in Mudd et al
  AJHG, 1985

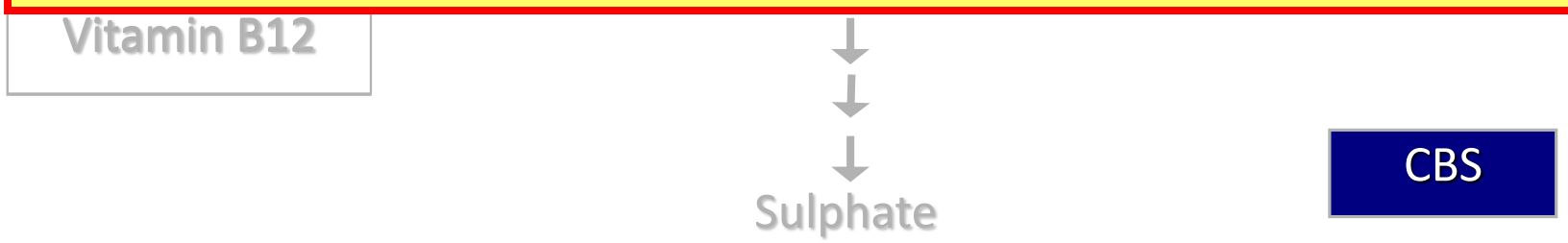
- **1992: first mutations described**
  Kozich and Kraus
  Hum Mutat 1992
- **1998: gene structure**
  Kraus et al
  Genomics 1998

Human *CBS* gene

- chromosome 21q22.3, 23 exons, 30 kbp
- mRNA 1.8-2.1 kbp → protein of 551 amino acids
- major expression in liver, pancreas, kidney, brain
- over 160 mutant alleles known

CBS enzyme

- Cystosolic enzyme
- Cofactors: heme, SAM, PLP
- Tetramer, subunit ~ 60 kDa
- Proteolytic clevage via TNF- formation of dimer
- Km (L-Hcy) 0.017- 5 mM, Km (L-Ser) 2-5 mM



CBS MUTATION DATABASE

Mutation database

Back

CBS allele database statistics

Total number of alleles (records) in CBS allele database: 925

Total number of mutations in CBS allele database: 164

The most frequent mutations are:

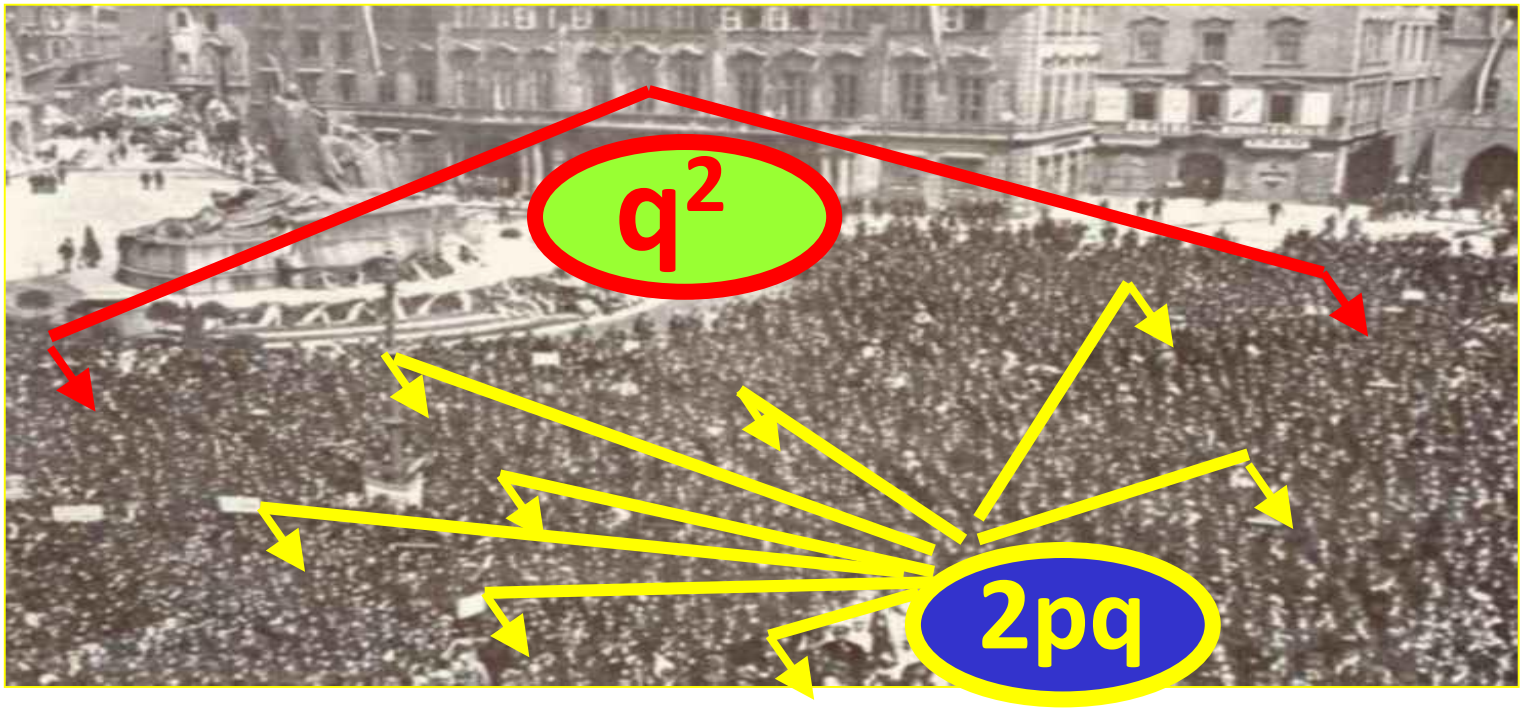
I278T = 153

T191M = 149
G307S = 88
R336C = 83
del ex 12 = 25
W323X = 20

Pyridoxine responsiveness

Pyridoxine non-responsiveness

Expected frequency of homocystinuria



q^2c.833 T>C
(p.I278T)

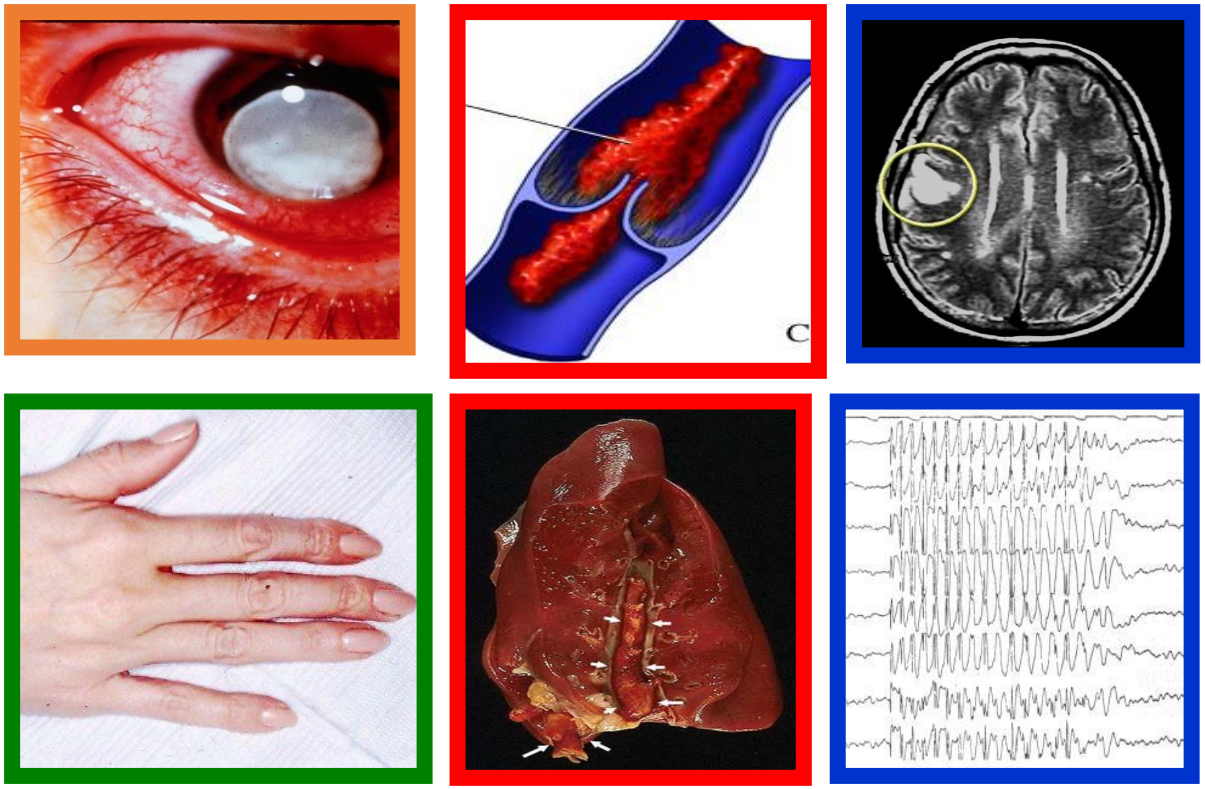
1:20,500 (DK)
1:17,800 (D)
1:83,000 (CZ)

q^2 (c.833 T>C + c.1105 C>T+O)
(p.I278T and p.R369C and other)

1:6,400 (N)
1: 15,500 (CZ)

data B.Janošíková
J.Sokolová

Clinical picture of CBS deficiency



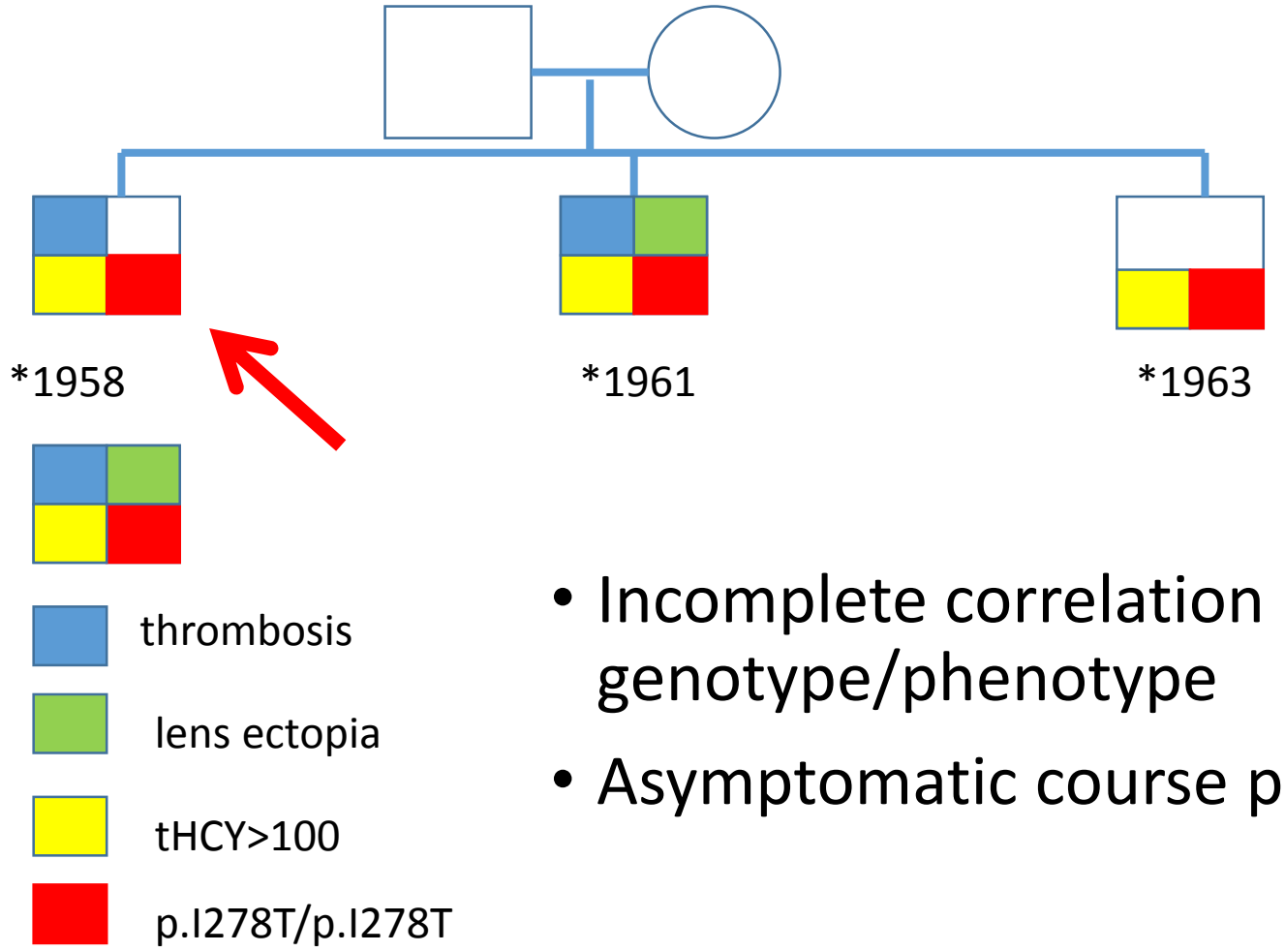
connective tissue **vasculature** **brain**

n=629 patients, Mudd et al, Am J Hum Genet 37, 1985

Case report-B_6 sensitivity

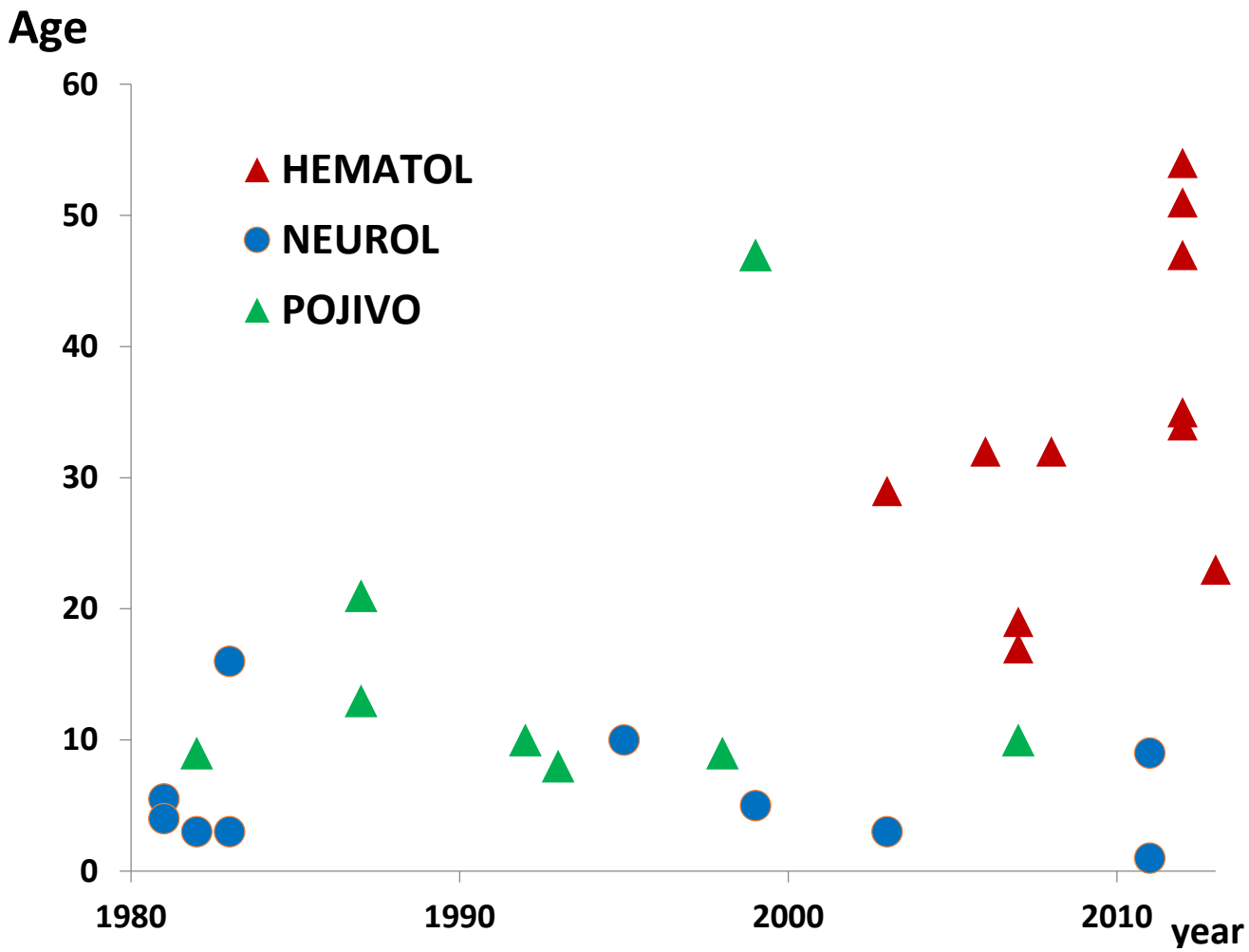
- Woman born 1968, at 19 yr thrombosis+ pulmonary embolia
- FU by hematologist, tHcy >50 umol/l, warfarin and vitamins (ac.folicum 10 mg + pyridoxine 40 mg)
- **Visit on vitamins: tHcy 14.1 (ref .range<15)!!!**
- Visit 2 weeks later (after pyridoxine elimination):
  - tHcy **125 umol/l** (N<15)
  - cystathionine **51 nmol/l** (N>80)
  - CBS activity in plasma **60 nmol/l/hr** (N>100)
  - DNA: **homozygosity for p.I278T**
- **MESSAGE: homocystinurias should be tested while NOT on vitamins**

Clinical variability within family



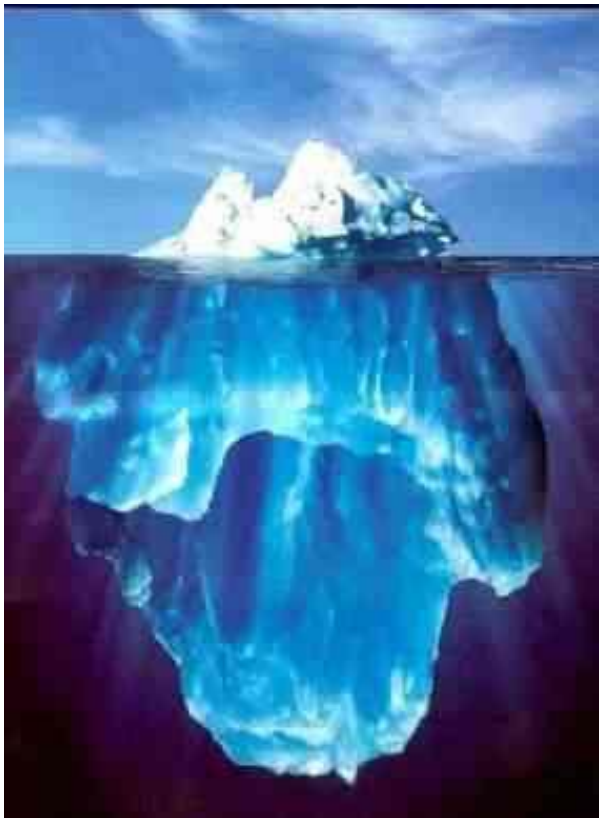
- Incomplete correlation genotype/phenotype
- Asymptomatic course possible

Phenotype at diagnosis (Czech)



Magner et al, J Inher Metab Dis 2011

Phenotype and frequency of CBS deficiency



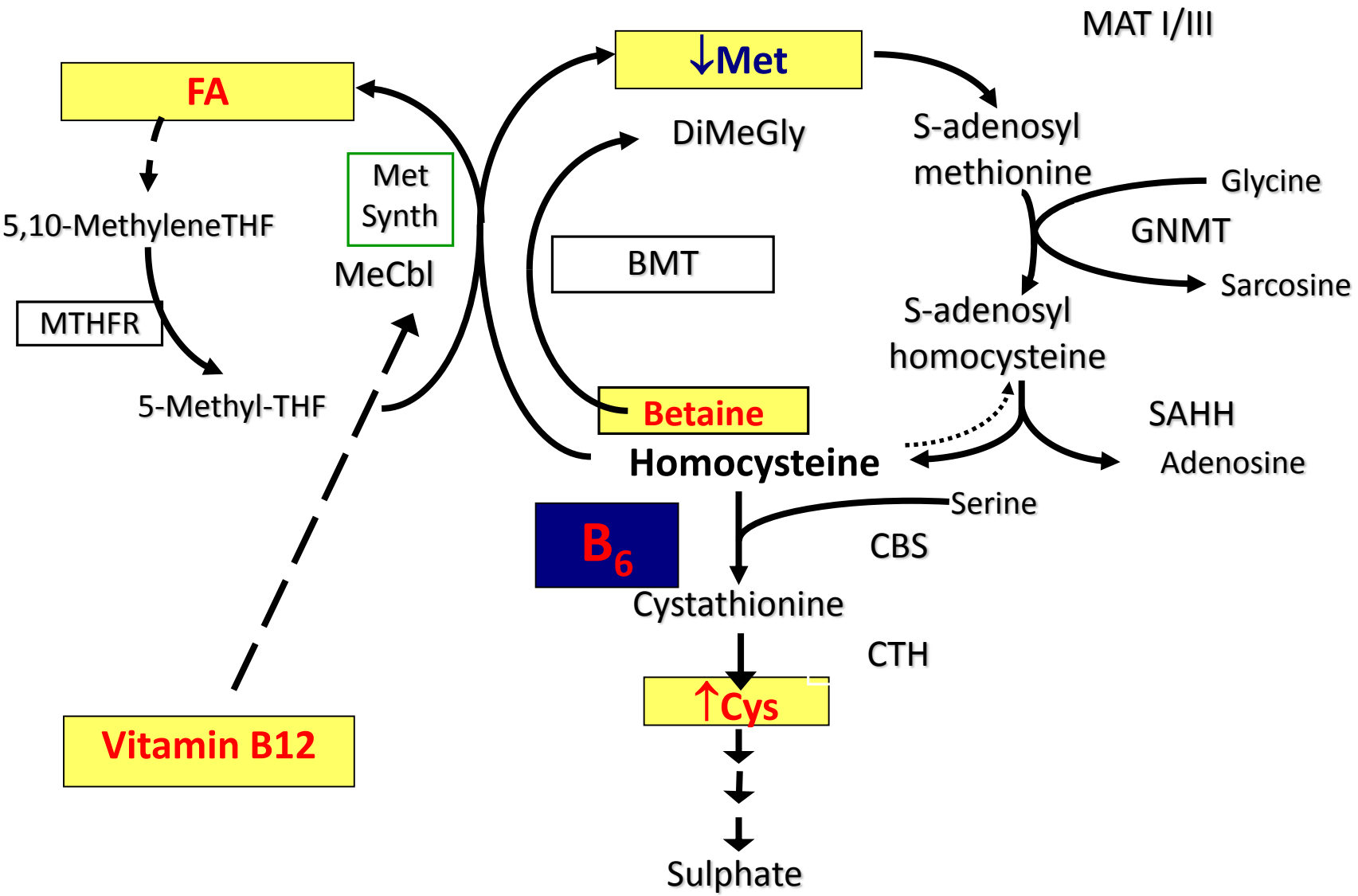
1960s to 1990s

- Marfan-like disease
- rare ~1: 65,000-335,00
- newborn Met screening in Irish

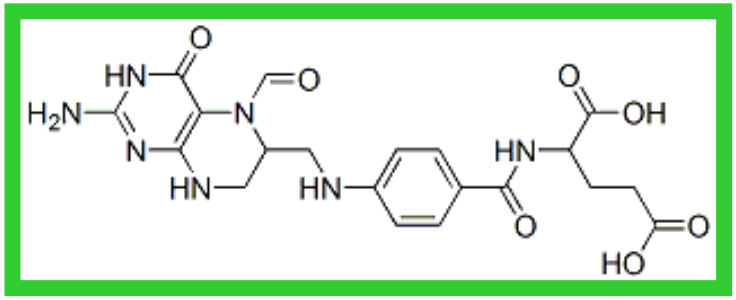
now

- thromboembolism only
- putatively ~1:10,000
- newborn Hcy screening ???

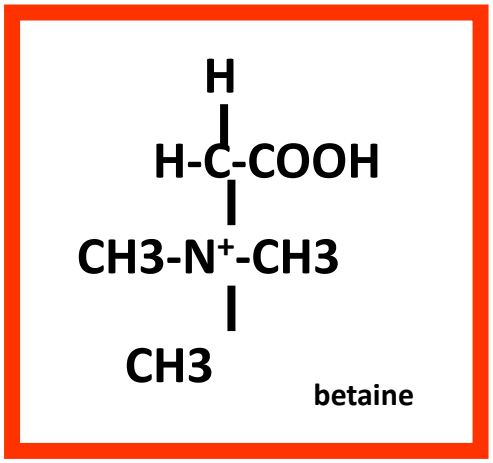
Treatment of CBS deficiency

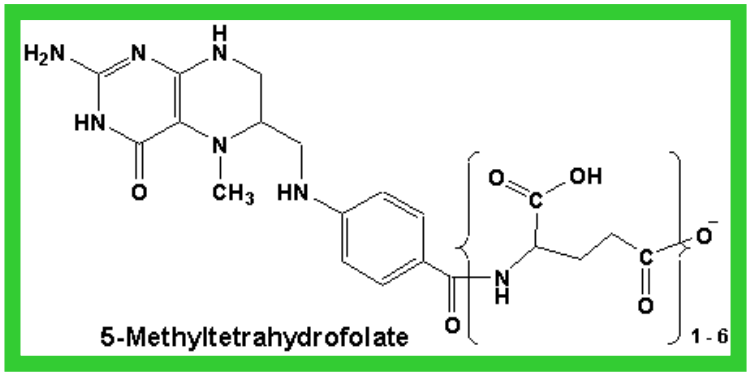


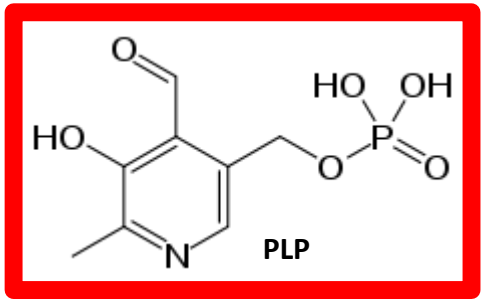
Pharmacological agents



5-Formyltetrahydrofolate (folinic acid)







http://www.aw-bc.com/mathews/MN/MTHF.GIF

Pyridoxine

- Cofactor of CBS
  - After conversion to PLP
  - Mechanism unknown- possiby chaperone
- Dosage
  - 3 doses per day
  - Response in about 50% patients
  - Doses vary 5-500 mg/day/adult
  - Biochemical effect present within about 2 weeks
  - Inexpensive medication
- Side effects
  - Sensorineural deafness

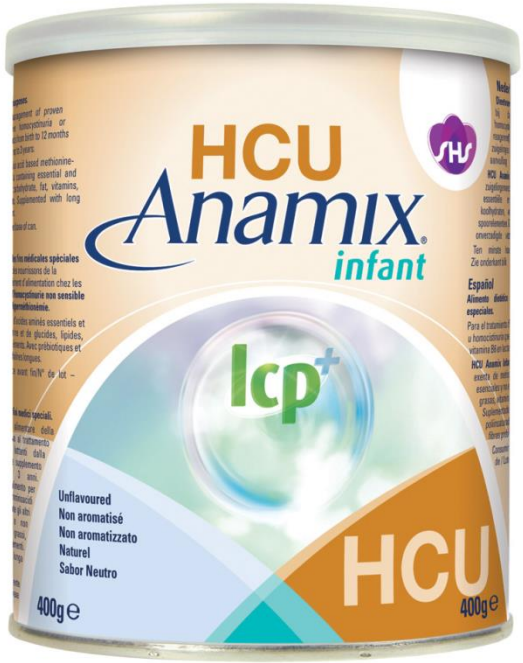
Betaine

- Remethylation of Hcy to Met
  - Supports vicious circle in CBS
  - Corrects block in RM (including SAM restoration)
- Dosage
  - 2 doses per day
  - In children 50 mg/kg in 1 dose as a start
  - Possibly no benefit at > 150mg/kg/day
- Side effects
  - Overdose-brain edema
  - Dimethylglycine may inhibit RM enzymes
  - Fish odor (Rx riboflavin)

Dietary measures in CBS

- Low protein diet with low Met content
- Severe diet
- Dietary records maintained
- May ba as low as only about 20% of natural protein

+



CBS deficiency-summary

- frequency 1:6.000-1:900.000
- clinical triade
  - Connective tissue: marfanoid features, kyfoskoliosis, osteoporosis, lens luxation
  - hemokoagulation: thromboses
  - CNS: cognitive impairment, seizures
- classical and mild forms

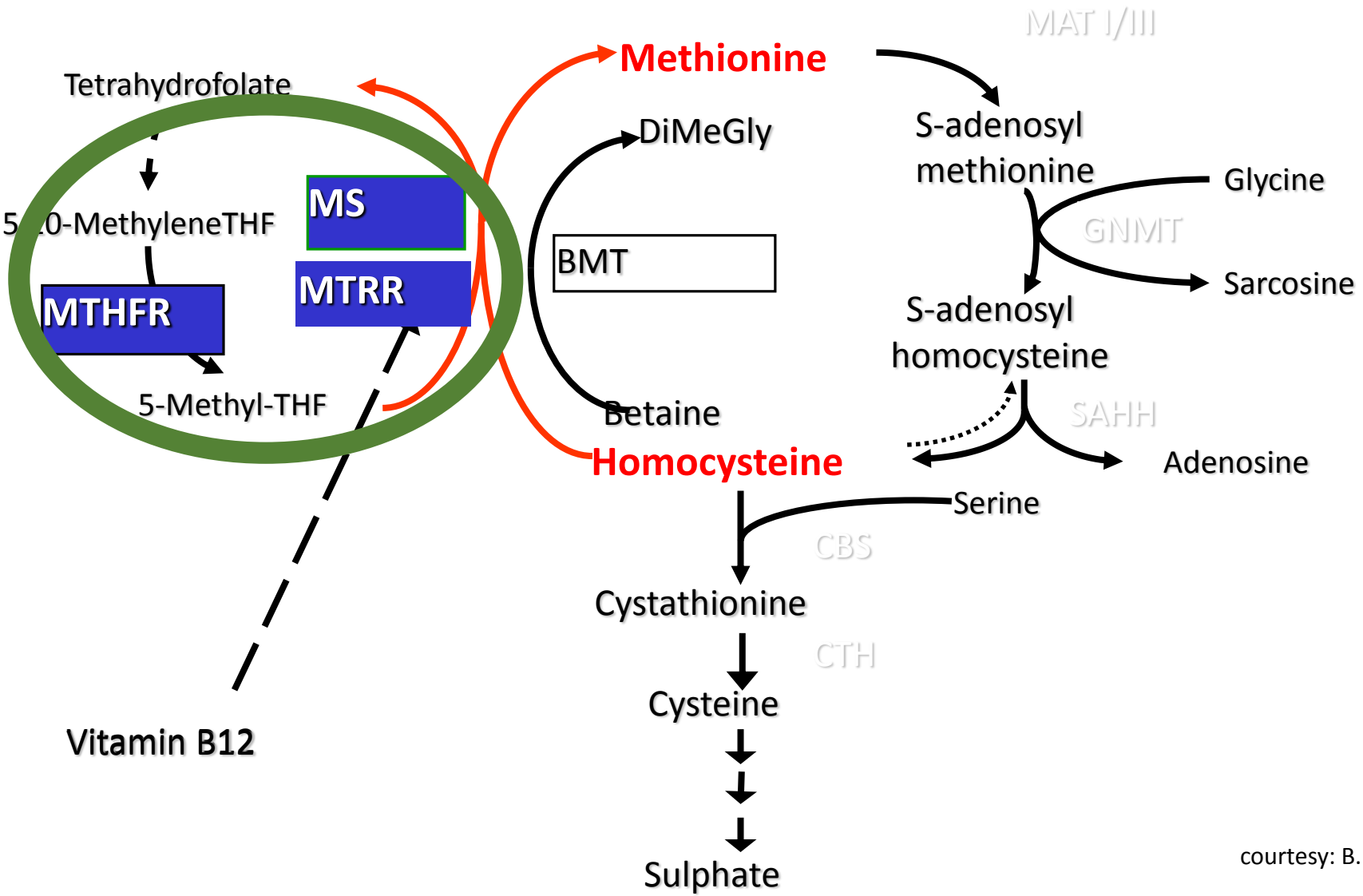
- therapy: low Met/Cys enriched diet, pyridoxine as a chaperone, betaine to enhance remethylation

Proper function of remethylation: vegetables or meat?

http://www.lisburncity.gov.uk/filestore/images/Raw-Meat-1.jpg

http://www.healthier-harvest.com/images/health_063006/fruits_and_vegetables2.jpg

Remethylation Hcy→Met



courtesy: B.Fowler

Plasma (csf) metabolites in RM defects

Deficiency	MTHFR	cblE	cblG
Folates	→ ↓	→	→
Methionine	→ ↓	→ ↓	→ ↓
AdoMet	→ ↓	→ ↓	→ ↓
AdoHcy	→ ↑	→ ↑	→ ↑
tHcy	↑ ↑	↑ ↑	↑ ↑
Cystathionine	↑	↑	↑
B12	→	→	→
MMA	→	→	→

Mutations and patients reported (2014)

Gene	Total mutations	Total patients (estimate)
MTHFR	~100	>100
MTRR	~30	~50
MTR	~35	~50

Symptoms and signs

Onset

- neonatal
- **infancy**
- **childhood**
- adulthood
- asymptomatic

Blood

- only cblE&cblG: megaloblastic anemia, macrocytosis
- (thromboembolia)

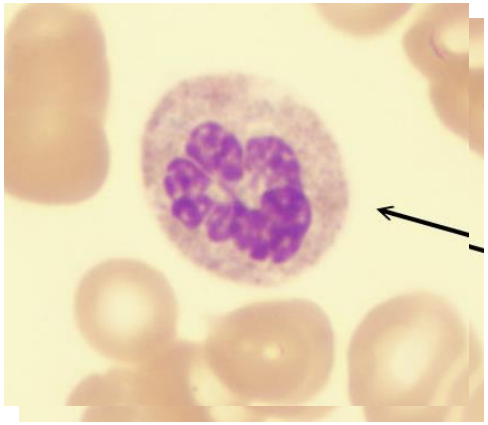
Nervous system

- developmental delay
- abnormal muscle tone
- seizures
- psychosis
- cerebral atrophy (CT)// white matter abnormalities (MRI)

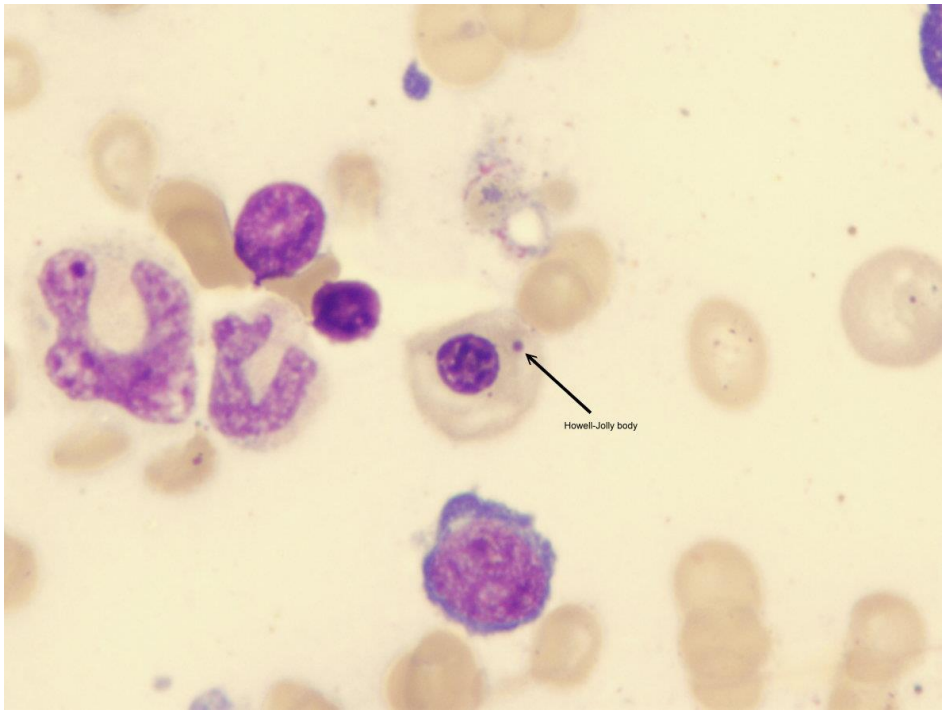
General symptoms

- failure to thrive
- no connective tissue involvement

Hematological abnormalities



hypersegmentation of neutrophils

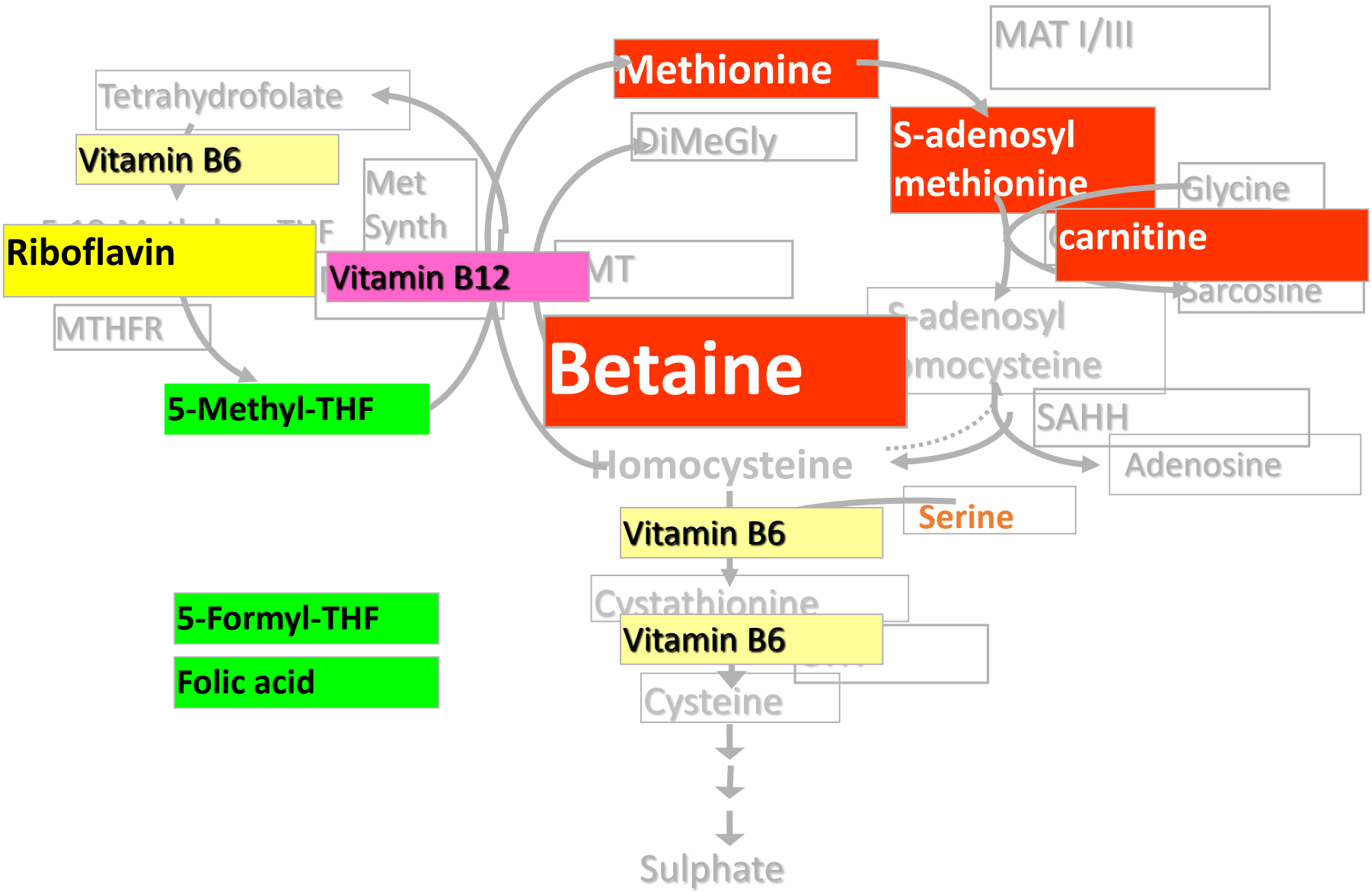


megaloblasts and Howell-Jolly bodies

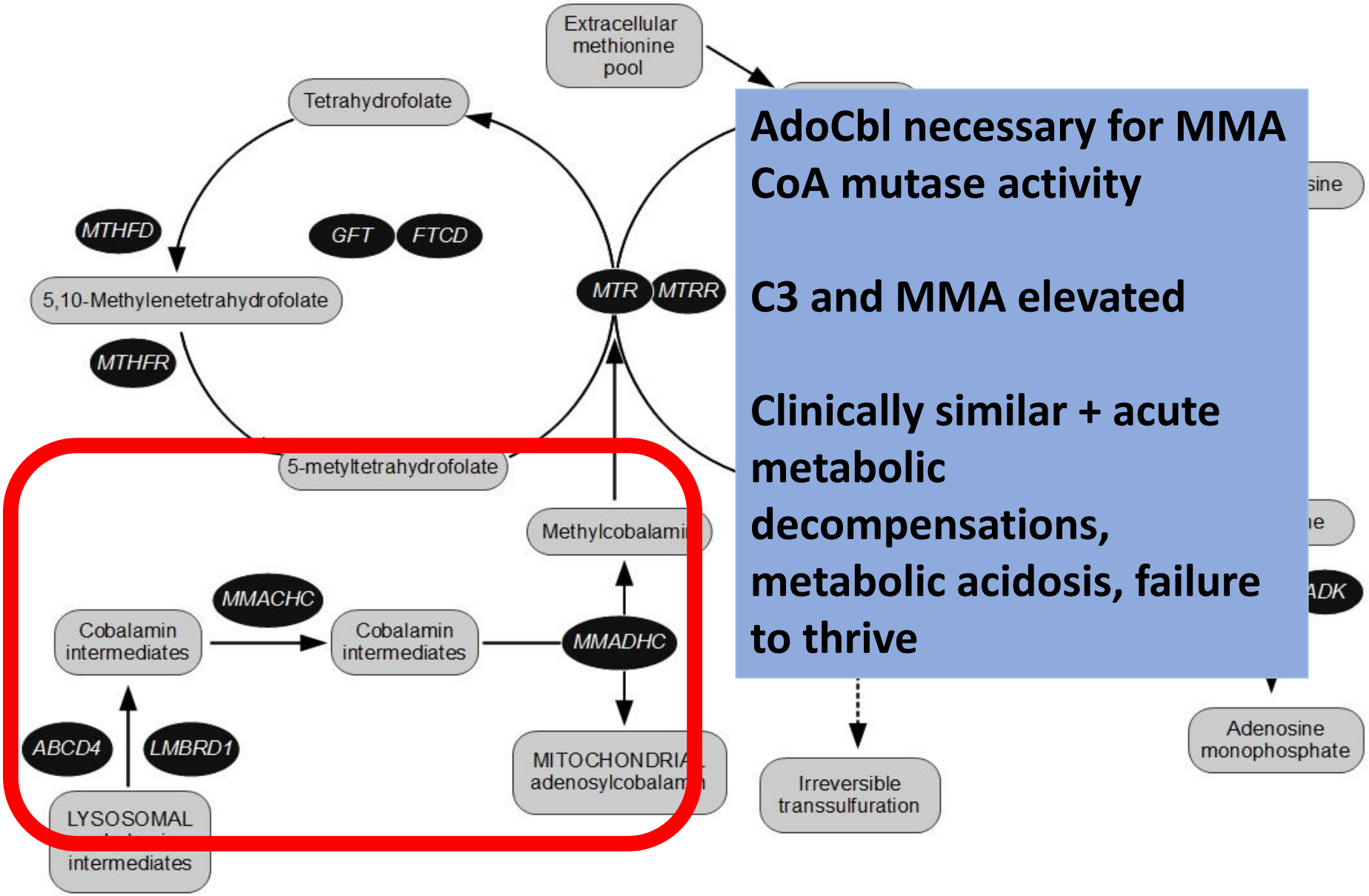
http://www.healthsystem.virginia.edu/internet/hematology/HessImages/pernicious-anemia-orthochromatic-megaloblast-with-howell-jolly-body-website-arrow.jpg

Pathogenesis of anemia (cblE&G)

Treatment modalities

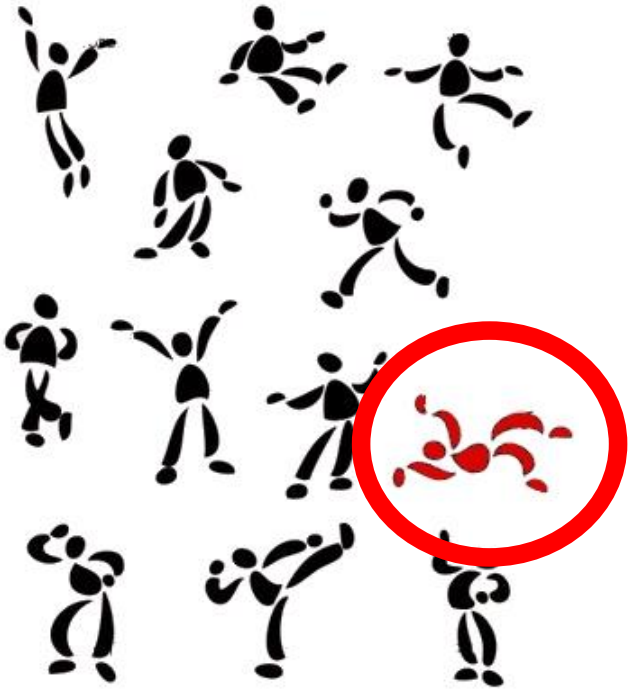


Combined cbl defects



Diagnosis of homocystinurias

Selective screening



Population screening

Laboratory testing

Screening tests

- tHcy in plasma (no vitamins!!!)
- tHcy in DBS
- Met in plasma (poor sensitivity)

Dif dg. and confirmation

- Vitamins-especially B12
- Cystathionine in plasma (LC-MS/MS)
- CBS activity in plasma
- CBS and RM enzymes in fibroblasts
- DNA analysis

Role of DBS in diagnosis

- Preanalytical phase of tHcy determination impractical
- Adherence to treatment is often poor-low frequency of tHcy measurements
- Dry blood spot (DBS) is used for PKU monitoring

Can be DBS used for diagnosis in patients with homocystinurias?

Clinica Chimica Acta 437 (2014) 211–217

Contents lists available at ScienceDirect

Clinica Chimica Acta

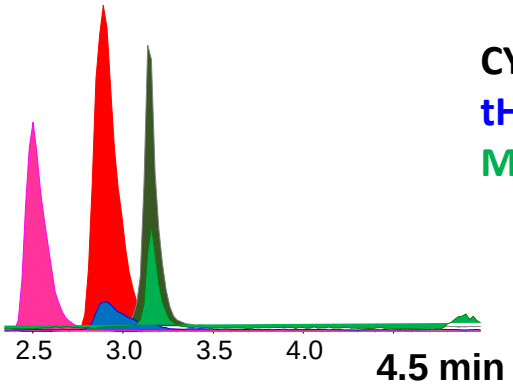
journal homepage: www.elsevier.com/locate/clinchim

Simultaneous determination of cystathionine, total homocysteine, and methionine in dried blood spots by liquid chromatography/tandem mass spectrometry and its utility for the management of patients with homocystinuria

Josef Bártl, Petr Chrastina, Jakub Krijt, Jakub Hodík, Karolína Pešková, Viktor Kožich *

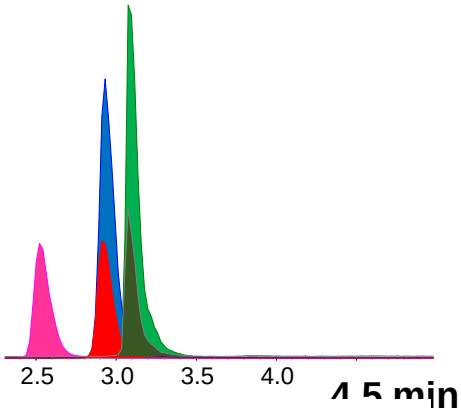
Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Czech Republic

A) Healthy control



CYST 200 nmol/L
tHCY 7 µmol/L
Met 15 µmol/L

B) CBS deficiency

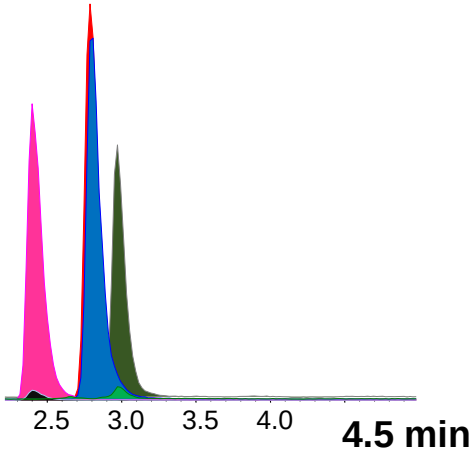


CYST 50 nmol/L
tHCY 150 µmol/L
Met 90 µmol/L

Internal standards:
D4-CYST
D4-HCY
D3-Met

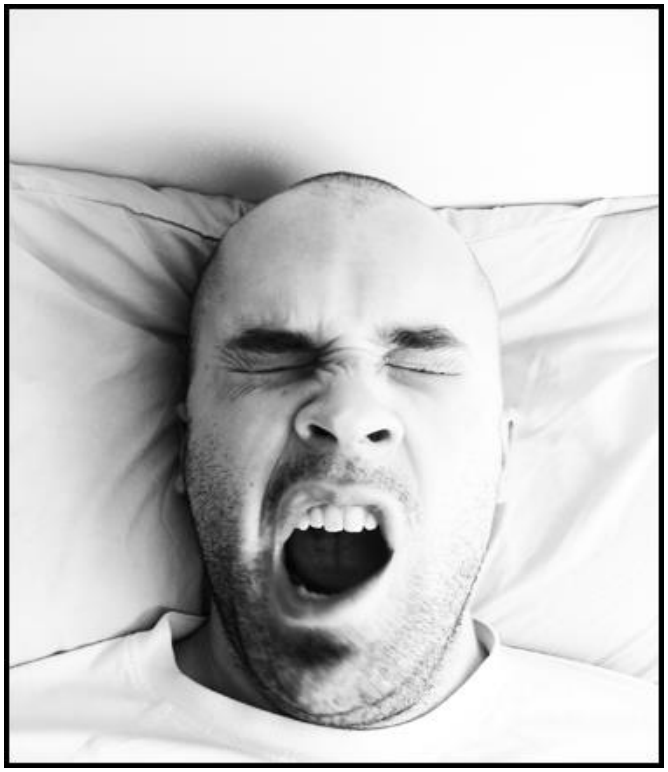
Utility for dif Dx

C) RMD



CYST 3000 nmol/L
tHCY 70 µmol/L
Met 4 µmol/L

MRM scans



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€HOD

European Network and Registry for Homocystinurias and Methylation Defects

Contract number:	EAHC 2012 12 02
Starting date:	15th February 2013
Duration of the project:	39 months
Total amount of the project:	€1.151.870

Co-funded by the Health Programme of the European Union

€ 690.793

Challenges in patient care

- Knowledge on natural history only partially known or lacking
- Efficacy of various treatment regimens poorly investigated
- Guidelines and recommendations on diagnostic procedures and therapy missing
- Systematic analysis of evidence for decisions on neonatal screening missing
- Information for patients and caregivers missing

Co-funded by
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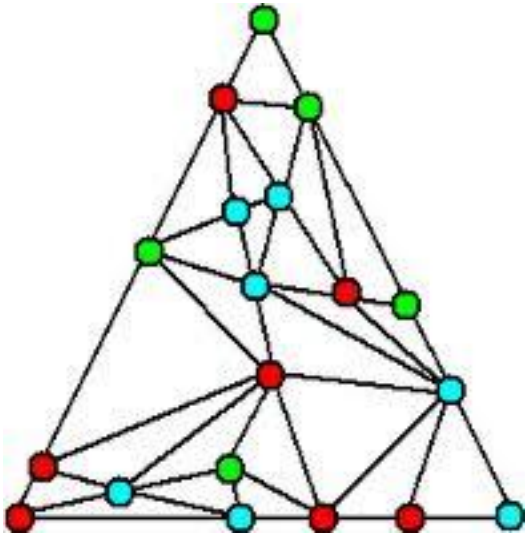
EHOD
European Network and Registry for
Homocystinurias and Methylation Defects

E-HOD Network

Professionals



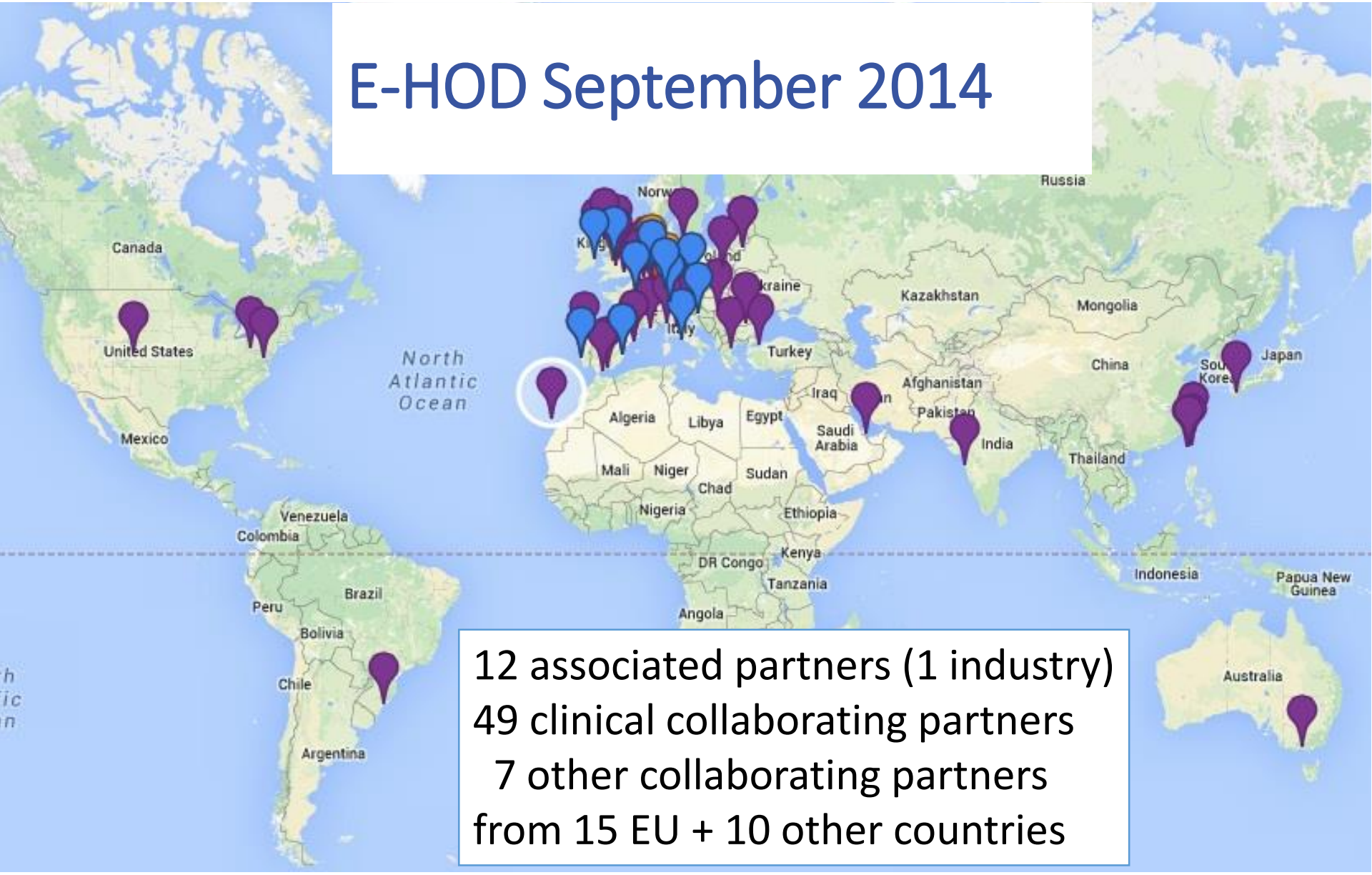
Patient organisations

Public-private partnerships

Co-funded by the Health Programme of the European Union

EHOD
European Network and Registry for Homocystinurias and Methylation Defects

E-HOD September 2014



12 associated partners (1 industry)
49 clinical collaborating partners
7 other collaborating partners
from 15 EU + 10 other countries

Co-funded by
the Health Programme
of the European Union

EHOD
European Network and Registry for
Homocystinurias and Methylation Defects

Structure of WPs

WP1 Coordination

WP lead: **Freiburg** (Blom)

WP2 Dissemination

WP lead: **Prague** (Kožich)

WP3 Evaluation

WP lead:
Manchester (Morris)

WP4 Registry

WP lead:
Heidelberg
(Kölker)

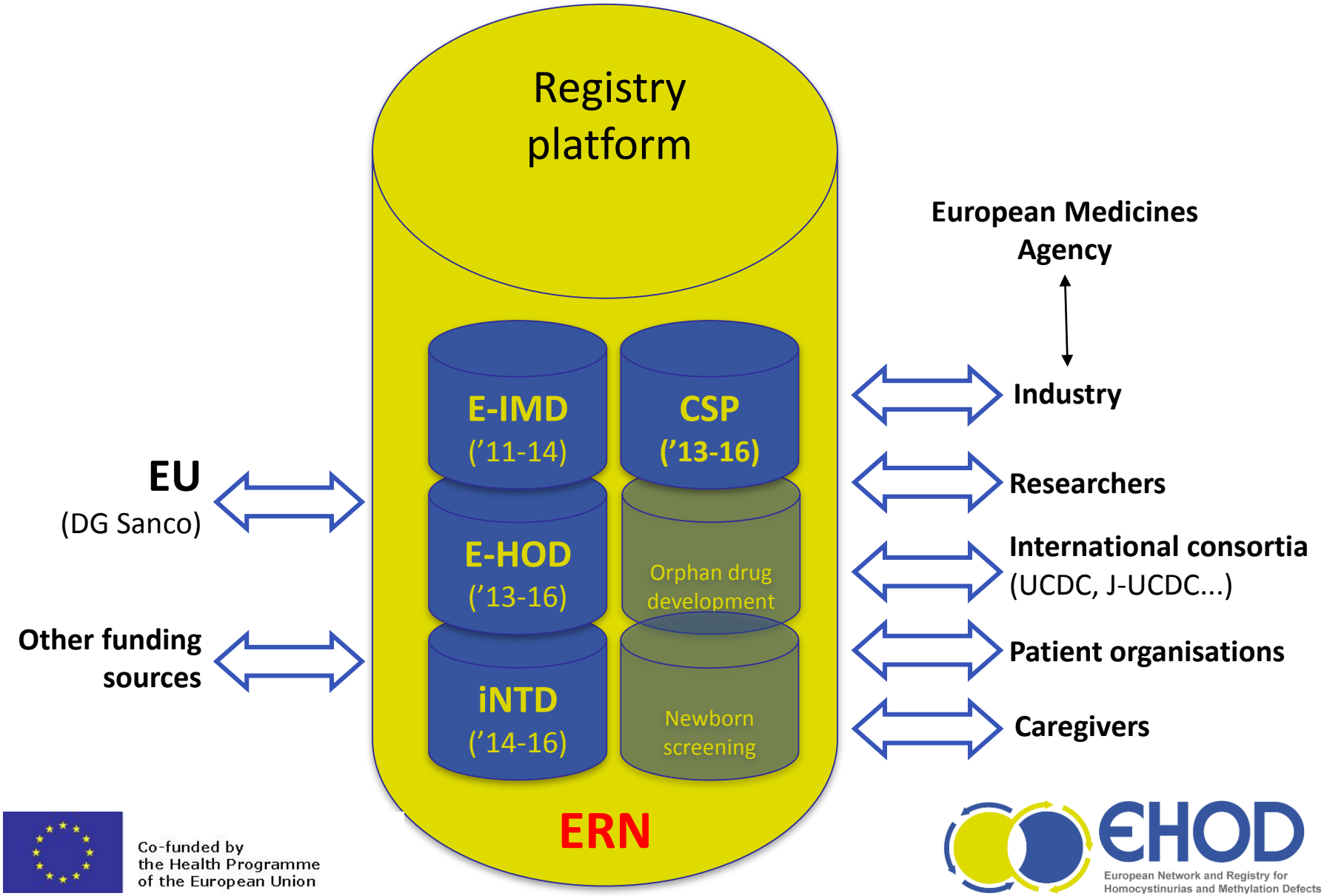
WP5 Guidelines

WP lead:
Rome
(Dionisi-Vici)

WP6 Newborn screening

WP lead:
Bregenz
(Huemer)

E-HOD builds upon E-IMD



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EHOD
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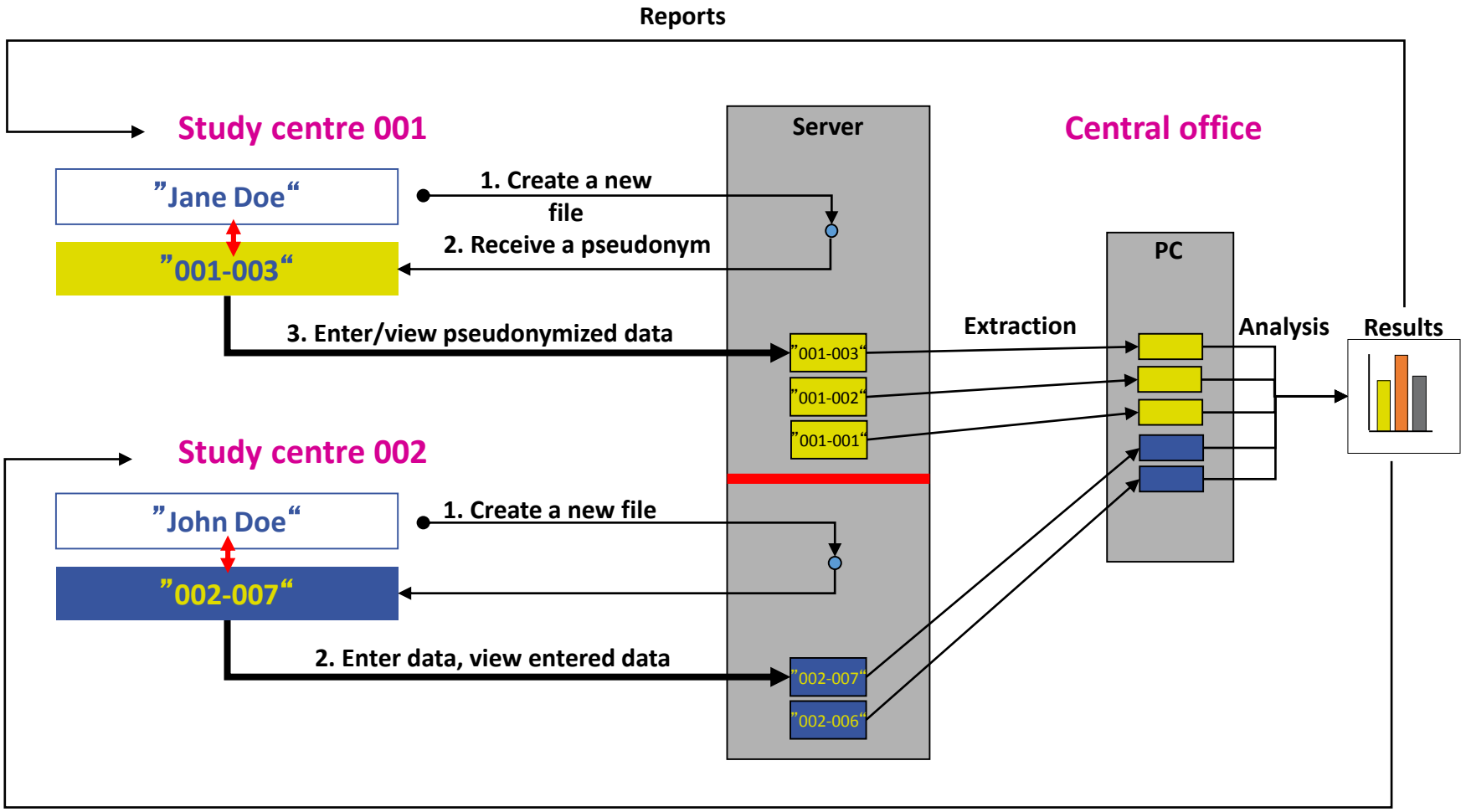
Pseudonymisation of entered data



Co-funded by
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EHOD
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Homocystinurias and Methylation Defects

Types of visits

1. Baseline visit (B)

- **Once at the beginning** for a **new study patient**
- Missing data, e.g. results of pending analyses should be completed asap

2. Regular visit (V_1, V_2...V_n)

- Scheduled visits (inpatient or outpatient)
- At least **once yearly**

3. Emergency (or any other unscheduled) visit (ER_1, ER_2...ER_n)

- **All unscheduled visits** (inpatient or outpatient)
- Due to (impending) metabolic decompensation or any other significant health problem

4. Fatal disease course visit (F)

- **For a known study patient in case of death**
- **For a new study patient if diagnosis was made after death**

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Homocystinurias and Methylation Defects

Visits and forms (E-HOD and CSP)

Forms	BV	RV	ER/UV	Fatal disease course (of a known study patient)	Fatal disease course (if diagnosis was made after death)
0. Eligibility form	X				X
A. Baseline assessment form	X	X*			X
B. Medical history form	X	X*	X*		
C. Physical / neurological examination form	X	X			
D. Emergency visit form			X		
E. Dietary treatment form	X	X			
F. Drug and other treatment form	X	X	X	X	
G. Cystadane™ Surveillance Protocol	X**	X**	X**	X**	
H. Neuropsychological development form H*i*. Parent questionnaire (adapted to BSID-III) H*ii*. Test schedule	 (X) (X)	 (X) (X)			
I. Quality of life form	(X)	(X)			
J. MRI/MRS form (optional)	(X)	(X)	(X)		
K. Laboratory analysis form	X	X	X		
L. Fatal disease form (if applicable)				X	X

* If update is required

** Only for patients participating in the Cystadane™ surveillance protocol

() Only if applicable

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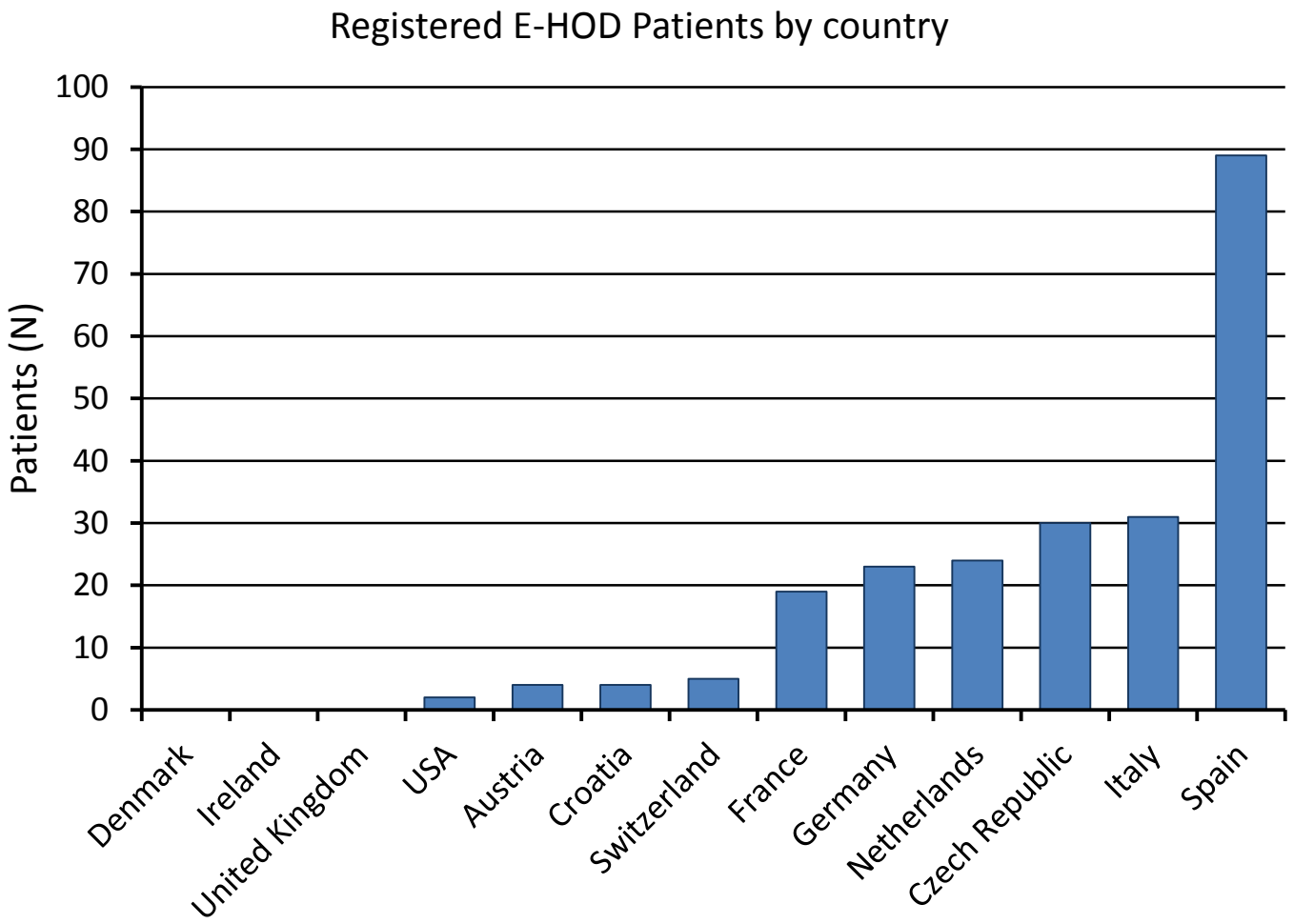
Origin of registered patients IX/2014



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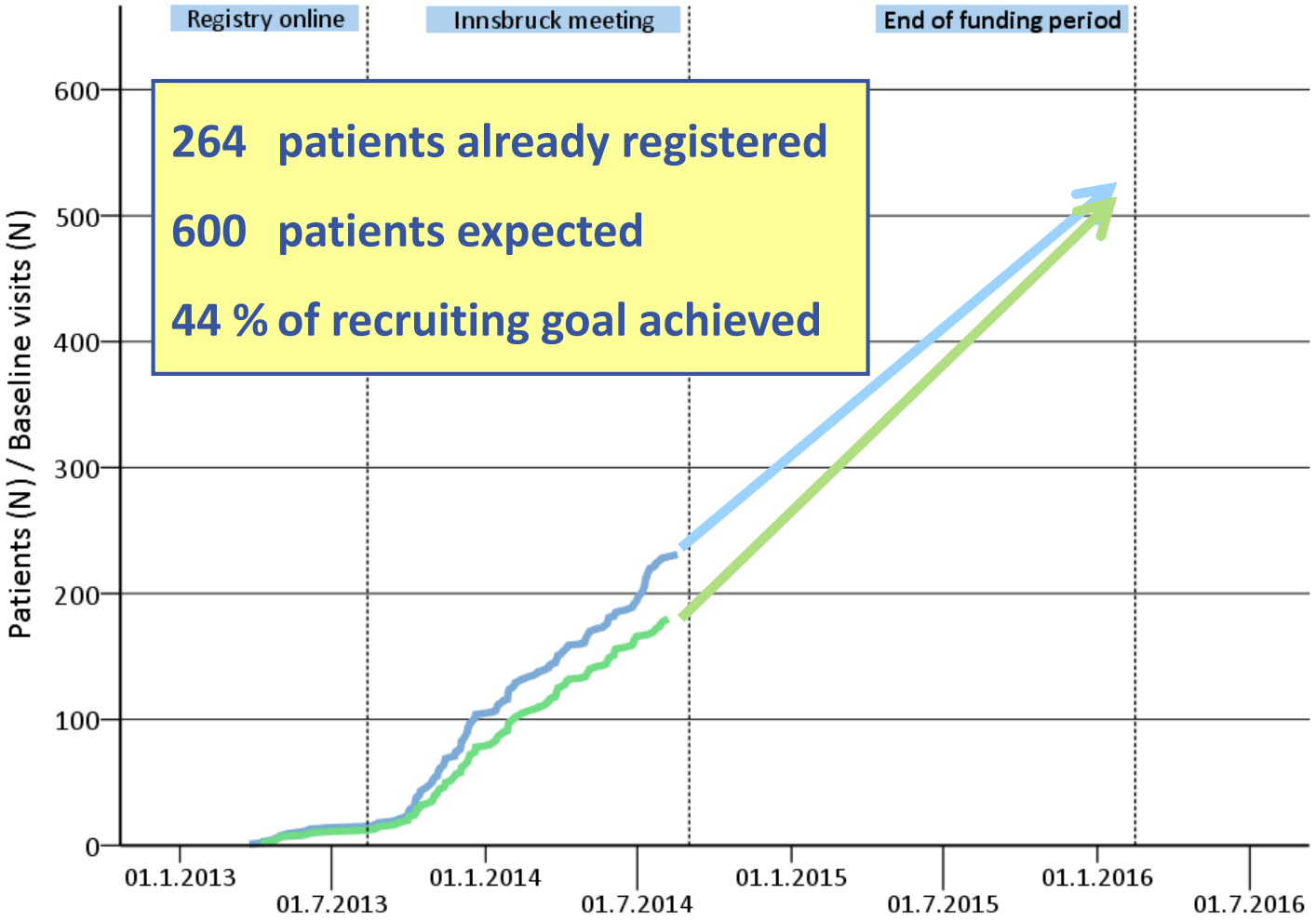
Registry-patients registered XI/2014



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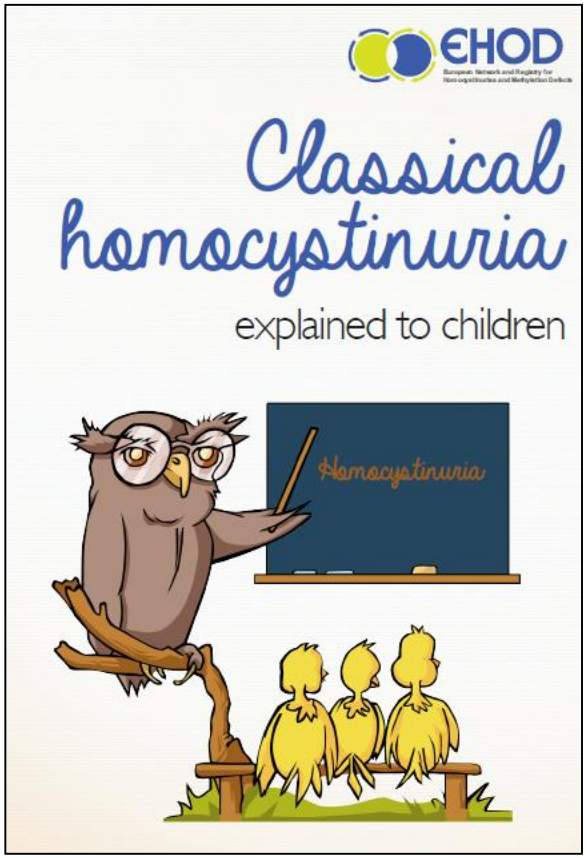
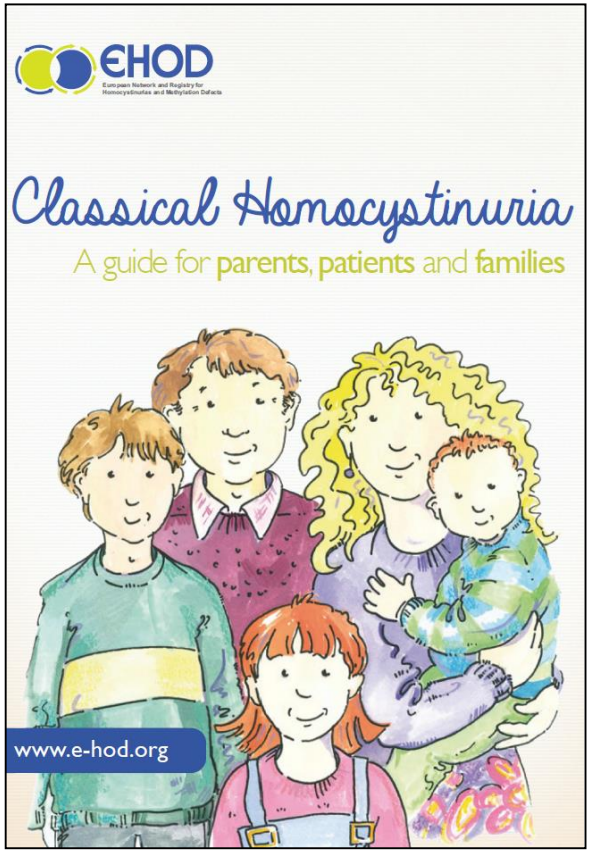
Information for patients





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E-HOD website

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Definition of guidelines

"Guidelines are recommendations intended to assist providers and recipients of health care and other stakeholders to make informed decisions. Recommendations may relate to clinical interventions, public health activities, or government policies."

WHO 2003, 2007

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Guidelines for Guidelines (Schünemann 2008ff)

- Determining which outcomes are important
- Searching evidence, deciding what evidence to include
- Grading
- Cost-effectiveness, affordability, equity, applicability transferability
- Reporting
- Dissemination and implementation
- Evaluation

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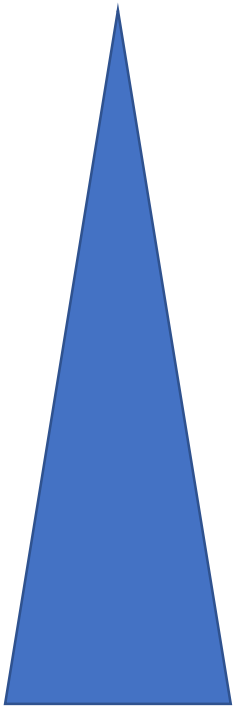
Hierarchy of evidence based on quality

(Schünemann, Ahmed, Morgan 2011)

STUDY DESIGN

BIAS

- Randomized Controlled Trials
- Cohort Studies and Case Control Studies
- Case Reports and Case Series, Non-systematic observations
- Expert Opinion



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E-HOD guidelines development

- **Clinical care guidelines (Dx and Rx)**
  - 3 different guidelines- CBS, remethylation, methylation disorders
  - Experts met in 2014, draft documents written
  - Additional meetings 2015

- **Newborn screening guidelines**
  - Experts met 2x in 2014
  - Additional meeting of main authors
  - Manuscript accepted II/2015

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Guidelines- NBS for homocystinurias

Outline

- Metabolism of sulfur amino acids
- Homocystinurias
- Improving care with help of E-HOD
- **Guidelines: NBS for homocystinurias**
- Expanded NBS in Czech Republic

Guidelines for NBS

Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines

Martina Huemer*, Viktor Kožich*,Piero Rinaldo, Matthias R. Baumgartner, Begoña Merinero, Elisabetta Pasquini, Antonia Ribes, Henk J.Blom

Journal of Inherited Metabolic Disease, *in press*

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NBS guidelines-methodology

- 103 papers reviewed by at least 2 independent evaluators
- Non-published evidence evaluated
- GRADE scoring
- 2 consensus meetings + 1 meeting of main authors
- several rounds of revisions

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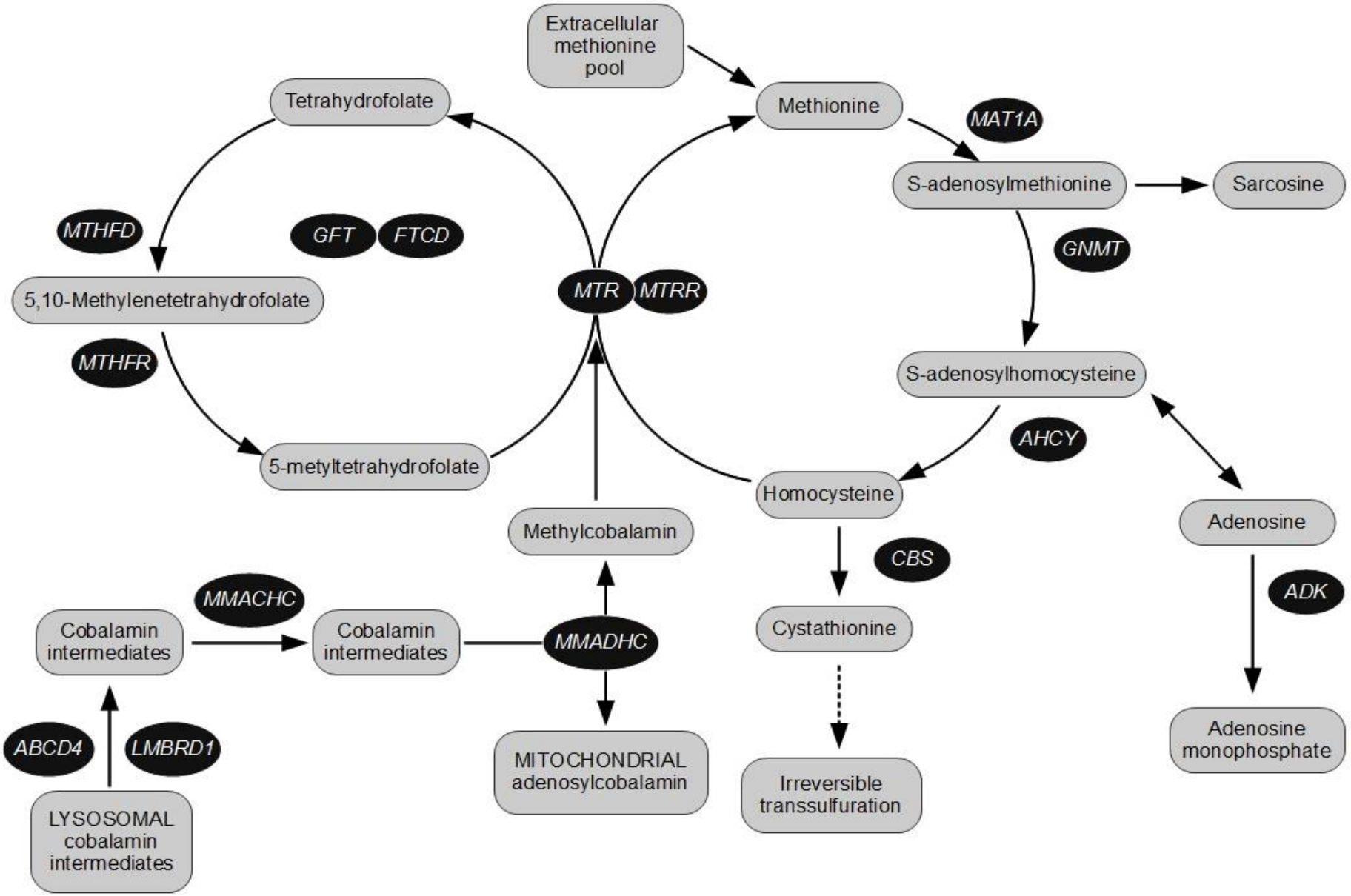
Diseases evaluated



Questions asked for each disease

a) Is the natural course of the disease severe?

b) Is treatment generally beneficial?

c) Is early intervention more effective?

d) Are robust, valid and reliable methods, screening approaches and strategies available?

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To screen or not not screen?

DISEASE	SEVERITY	EFFICACY RX	MARKER	SCREENING
CBS	YES	YES	Met, Met/Phe + tHcy	**YES**
MAT I/III	YES/NO	±	Met, Met/tHcy	MAYBE
MTHFR	YES	YES	Met, Met/Phe + tHcy	**YES**
cblC	YES	±	C3, C3/C2 (Met)	YES
cbl	YES	±	(C3, C3/C2, Met)	NO
GNMT, SAHH, ADK	NO YES	NO	UNLCEAR	NO NO

How to screen?

- A. Methionine (Met)
  - low sensitivity and specificity
  - efficacy depends on Met cut-off (Met 134 to 67 umol/l)
- B. Total homocysteine (tHcy)/DNA
  - good sensitivity and specificity (unknown for B6 responders)
  - expensive for massive screening
- C.Two-tier approach Met/tHcy
  - bottom and top Met percentiles analyzed for tHcy

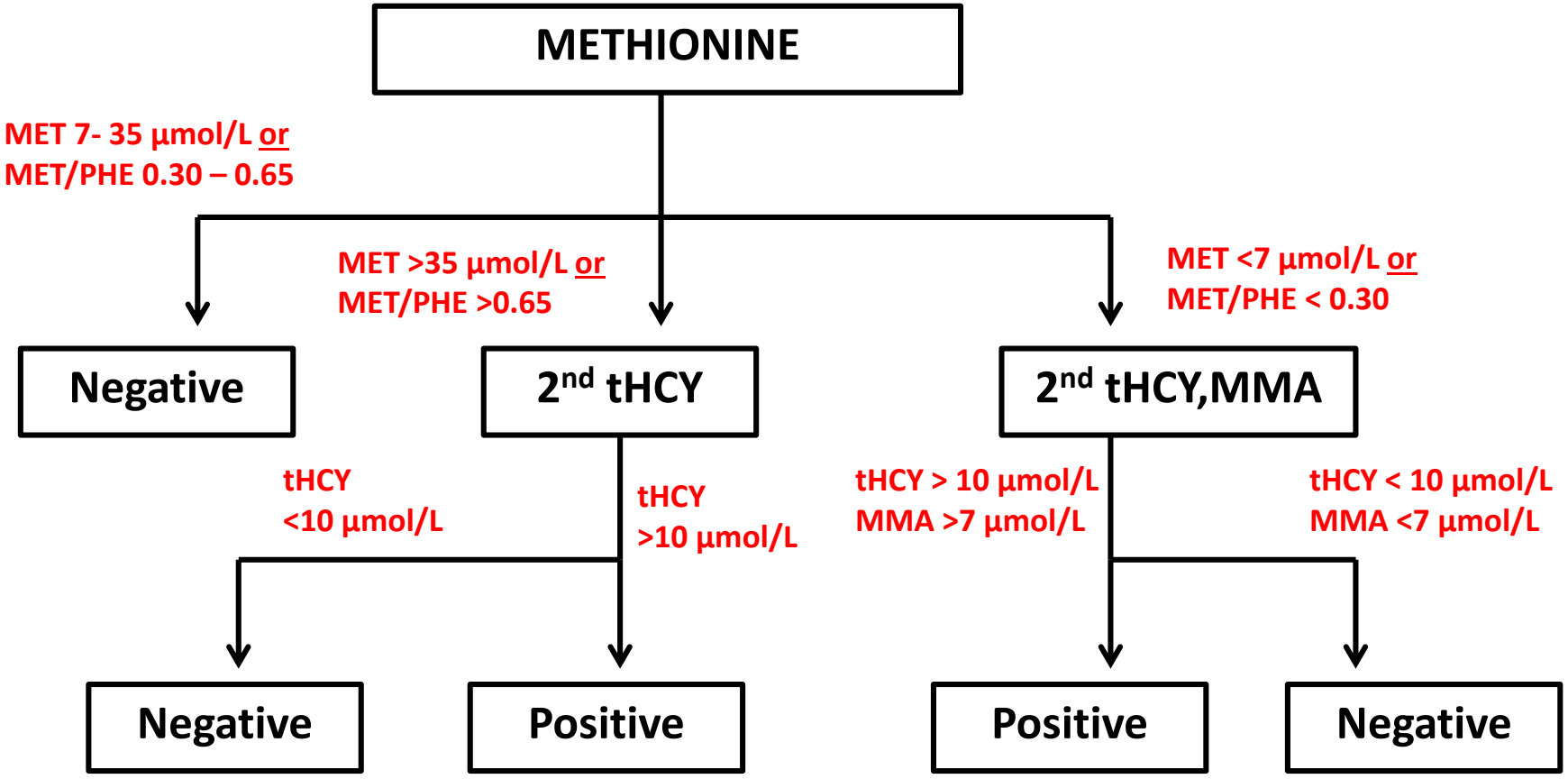
Peterschmitt MJ et al, N Engl J Med. 341:1572-6, 1999 Gan-Schreier H et al J Pediatr.156:427-32, 2010
Turgeon CT et al Clin Chem 56:1686-95, 2010 Tortorelli et al, J Pediatr 157, 271-5, 2010

Estimated screening efficacy (R4S)

	Met >35	Met/Phe >0.65	tHcy >10	R4S (n=)
CBS	99%	99%	100%	107
MAT I/III	99%	99%	N/A	141

	Met <7	Met/Phe <0.3	tHcy >10	R4S (n=)
cblC/cblD	75%	75%	100%	173
MTHFR cblE/cblG	50%	99%	100%	18
Maternal B12 def	N/A	N/A	75%	33

Proposed algorithm-CZ program

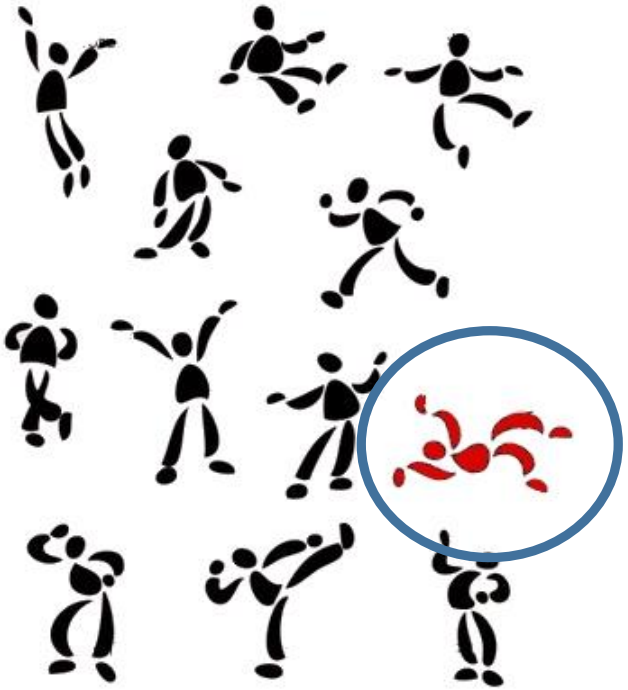


Outline

- Metabolism of sulfur amino acids
- Homocystinurias
- Improving care with help of E-HOD
- Guidelines: NBS for homocystinurias
- **Expanded NBS in Czech Republic**

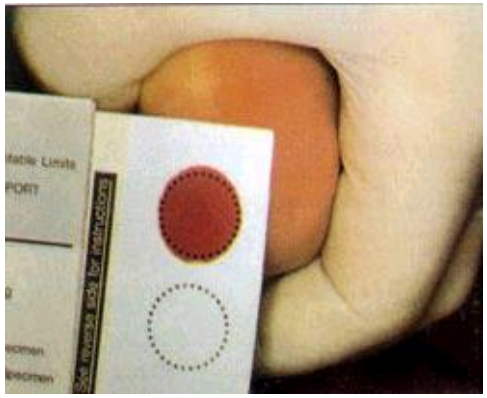
Genetic testing

Selective screening



Population screening

NBS in Czech Republic



Imunoanalytical labs (TSH, 17OHP, IRT)

DNA testing CF

MS/MS labs

VFN: Coordination Center for NBS

MINISTERSTVO ZDRAVOTNICTVÍ
ČESKÉ REPUBLIKY

Věstník

Ročník 2009

MINISTERSTVA ZDRAVOTNICTVÍ

ČESKÉ REPUBLIKY

Částka 6 | Vydáno: 12. SRPNA 2009 | Cena: 294 Kč

METODICKÝ NÁVOD K ZAJIŠTĚNÍ CELOPLOŠNÉHO NOVOROZENECKÉHO LABORATORNÍHO SCREENINGU A NÁSLEDNÉ PÉČE

Thirteen disorders screened since 10/2009

(2) V rámci novorozeneckého laboratorního screeningu jsou ze suché kapky krve vyšetřovány níže uved onemocnění:

Endokrinní onemocnění (EO):

a) kongenitální hypotyreóza (CH)
b) kongenitální adrenální hyperplazie (CAH)

Dědičné poruchy metabolismu (DMP):

c) fenylketonurie (PKU) a hyperfenylalaninemie (HPA)
d) leucinóza (nemoc javorového sirupu, MSUD)
e) deficit acyl-CoA dehydrogenázy mastných kyselin se středně dlouhým řetězcem (MCAD)
f) deficit 3-hydroxyacyl-CoA dehydrogenázy mastných kyselin s dlouhým řetězcem (LCHAD)
g) deficit acyl-CoA dehydrogenázy mastných kyselin s velmi dlouhým řetězcem (VLCAD)
h) deficit karnitinpalmitoyltransferázy I (CPT I)
i) deficit karnitinpalmitoyltransferázy II (CPT II)
j) deficit karnitinacylkarnitintranslokázy (CACT)
k) glutarová acidurie typ I (GA I)
l) izovalerová acidurie (IVA)

Jiná onemocnění:

m) cystická fibróza (CF)

Expanded screening 2009-2014

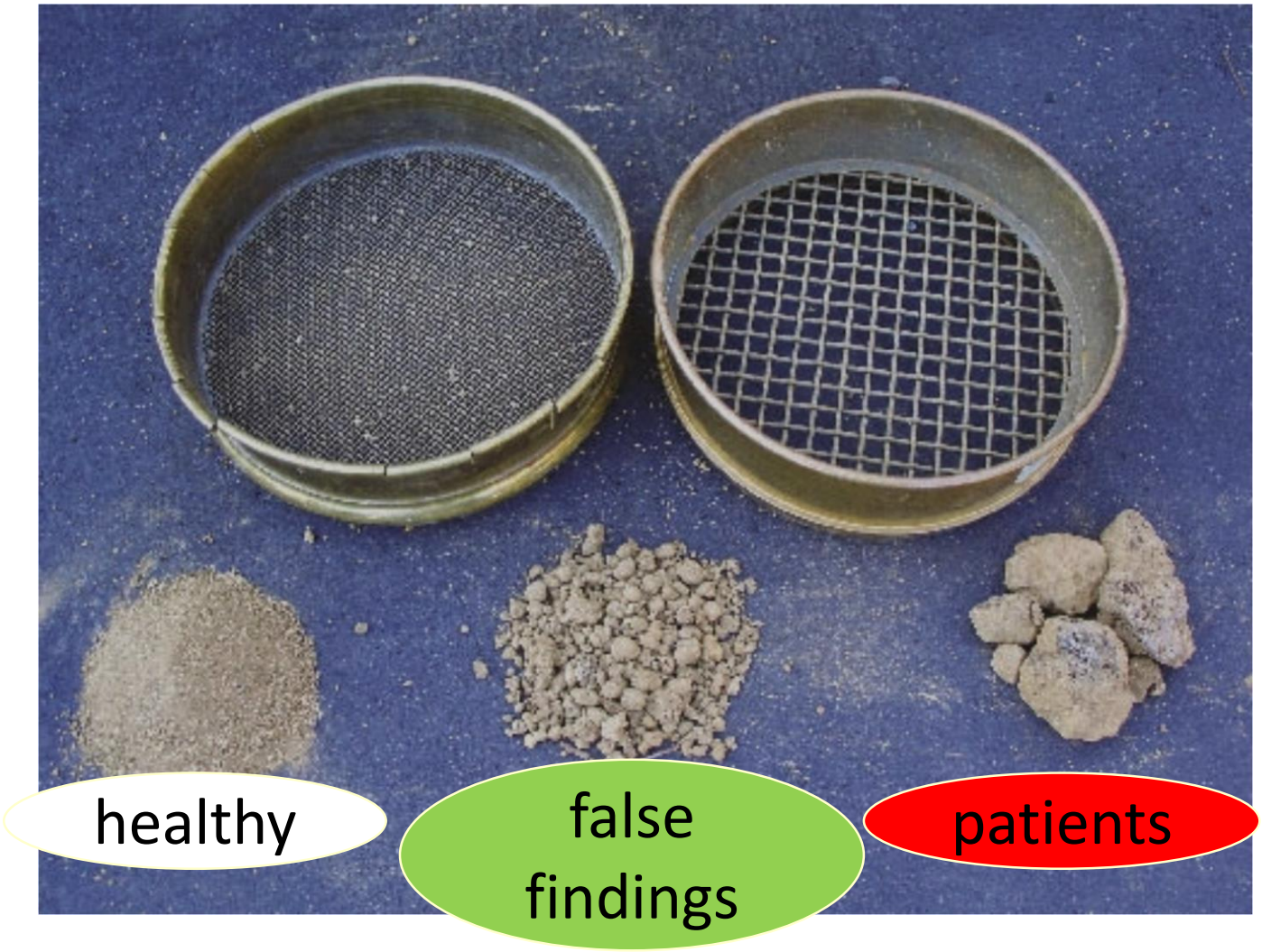
Detection rate 2009-2014

DISEASE	INTRODUCED	DETECTION RATE
PKU/HPA	1975 GUTHRIE, PC	1:8,000
	2009 MS/MS	1:5,200
CH	1975	1:2,600
CAH	2002	1:13,800
CF	2009	1: 6,500
NINE IEMs	2009	1:10,100
TOTAL	**AS OF 2014**	**1:1,100**

Detection rate IEMs

576,000 newborns (IX/2009-XII/2014)

IEM	Number of patients	Detection rate
PKU/HPA	110	1:5 200
MCAD deficiency	29	1:19 900
LCHAD/MTP deficiency	10	1:57 800
VLCAD deficiency	4	1:144 400
Hydroxyprolinemia	3	1:192 600
MSUD	3	1:192 600
IVA	3	1:192 600
GA I	3	1:192 600
CPT I deficiency	2	1:289 000
Total	**167**	**1:3 460**

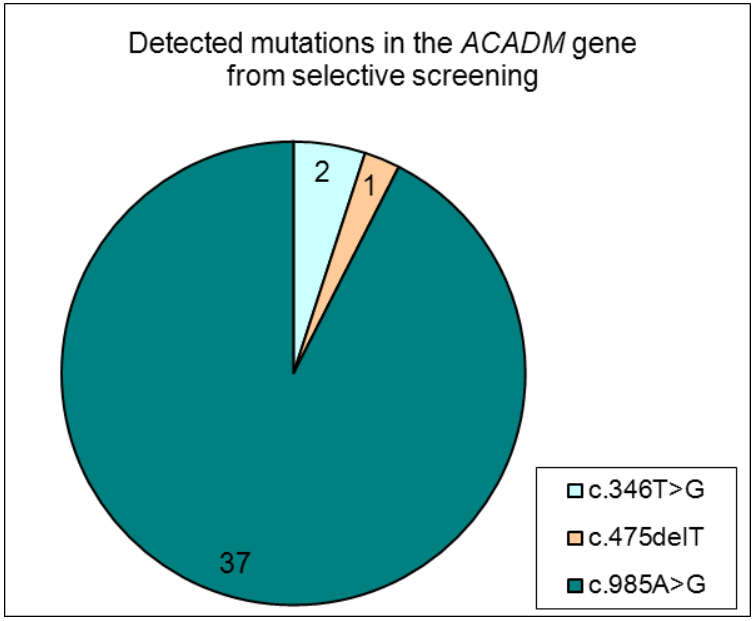

healthy
false findings
patients

Performance 2009-2014

	overall	**<2,500g**	**>2,500g**
DR	1:1,140	---	---
FPR	0.69%	4.4%	0.29%
IEM-DR	1:3,460	---	---
IEM-FPR	0.08%	0,58%	0,04%
IEM-PPV	26%	42%	7%

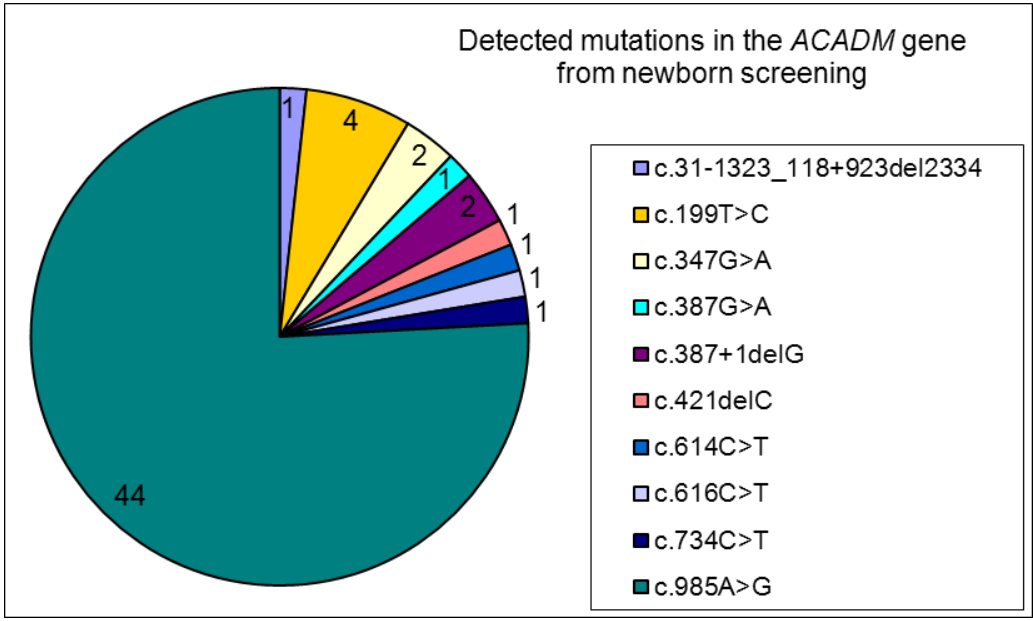
MCAD deficiency

Pre-NBS



1:211,000

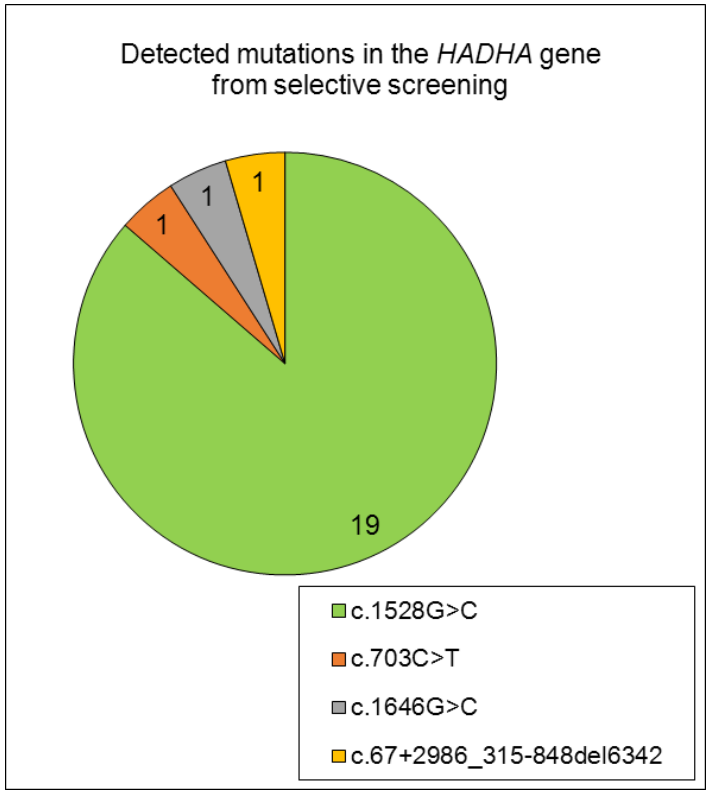
NBS



1:22,800

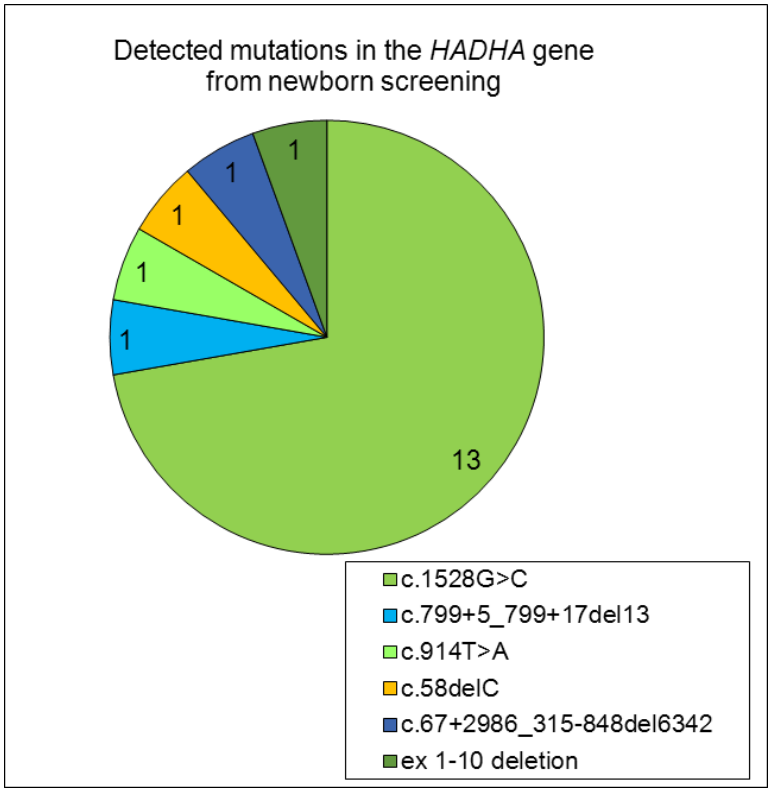
LCHAD deficiency

Pre-NBS



1:141,300

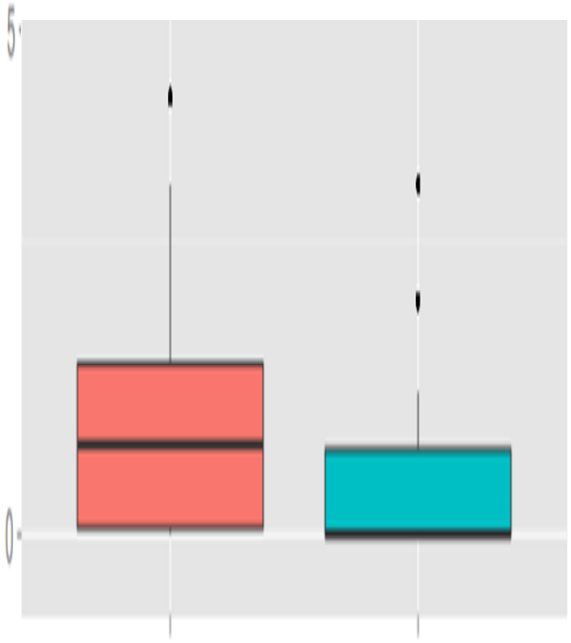
NBS



1:66,100

Fatty acid oxidation defects: clinical outcome/severity score

MCAD

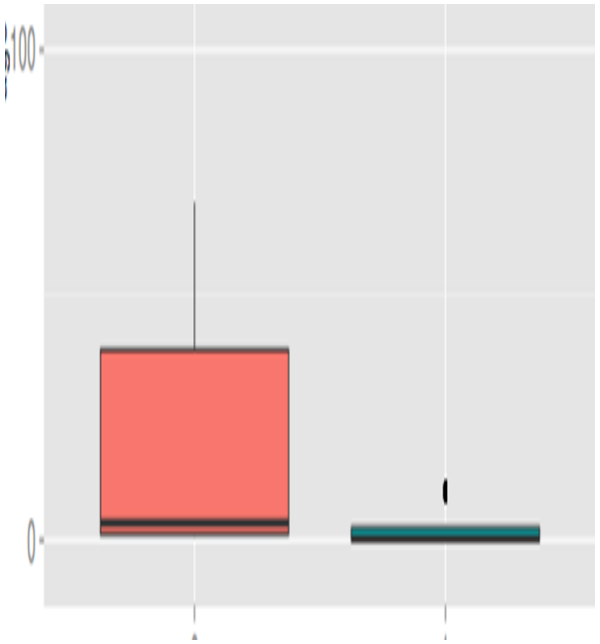


preNBS 0.8

NBS 0.0

p=0.01

LCHAD



pre-NBS 3.5

NBS 0.4

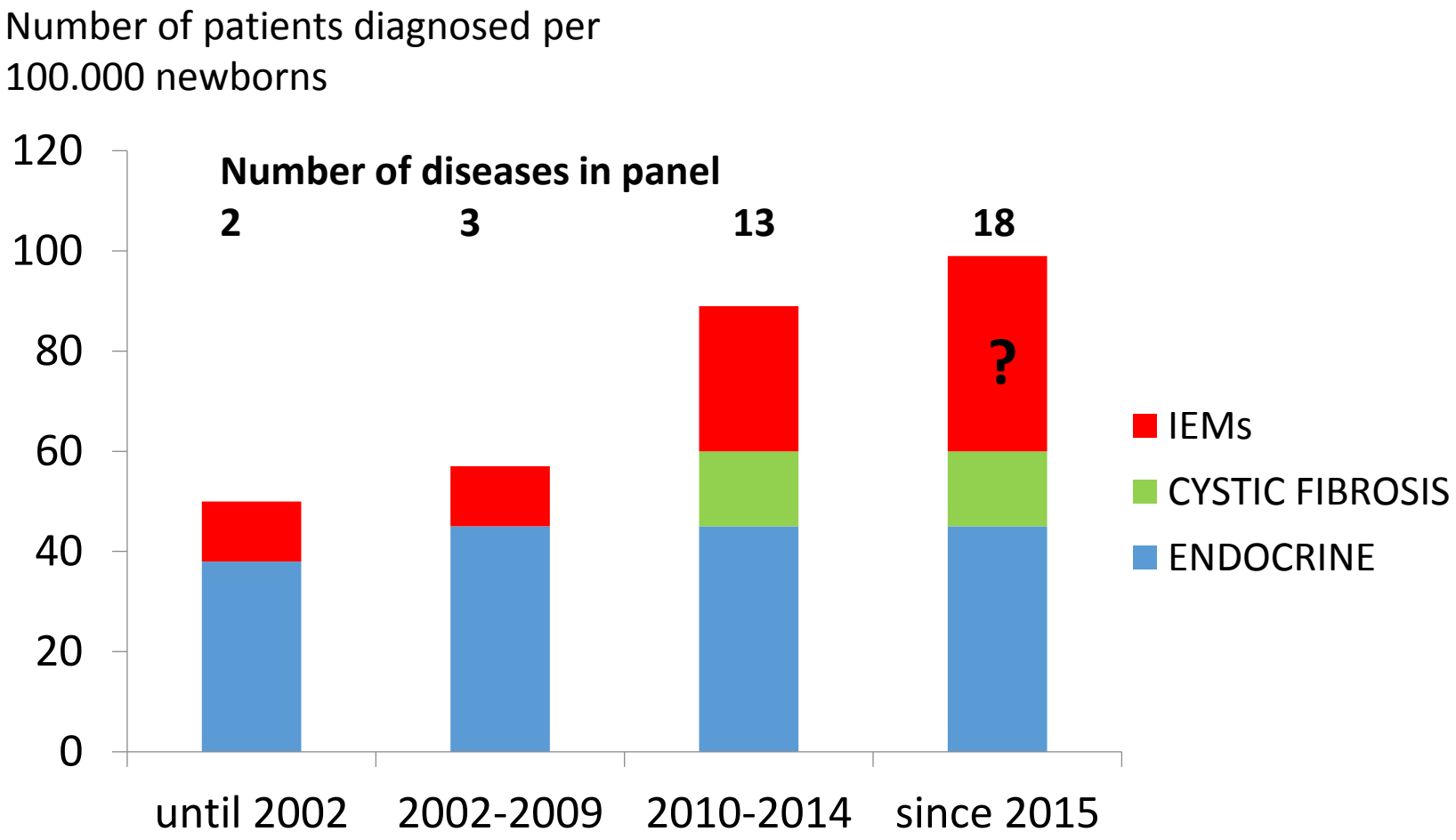
p=0.01


Future
NEXT EXIT

Further expansion in 2015

Disease		Markers	
Primary target	**Secondary targets**	**Primary**	**2nd tier**
CIT I	ASA, CIT II, def.PC, CIT I (mat)	Cit, Cit/Phe Cit/Arg	–
ARG	–	Arg/Orn	
CBS	MATI/III, GNMT, AHCY	high Met, Met/Phe	tHcy
MTHFR	cblG/E/Dvar1, cblC/D/F/H, vitamin B12 deficiency (mat)	low Met, Met/Phe	tHcy MMA
BTD	–	biotinidase activity	–

Efficacy of neonatal screening program in the Czech Republic



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- Collaborating partners
- Co-authors, especially Martina Huemer, Henk Blom and Piero Rinaldo

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Ačiū už dėmesį