New Developments in Disorders of Intracellular Vitamin B_{12} (Cobalamin) Metabolism

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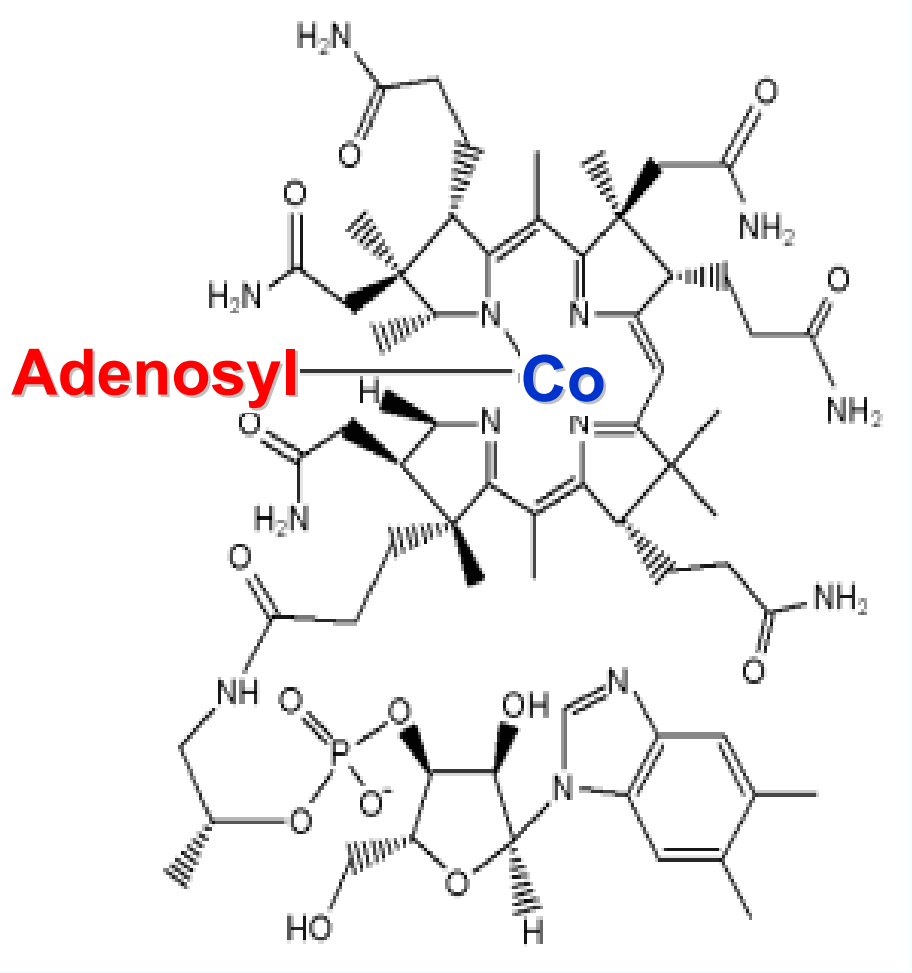
Structure of Vitamin B_{12} (Cobalamin, Cbl)

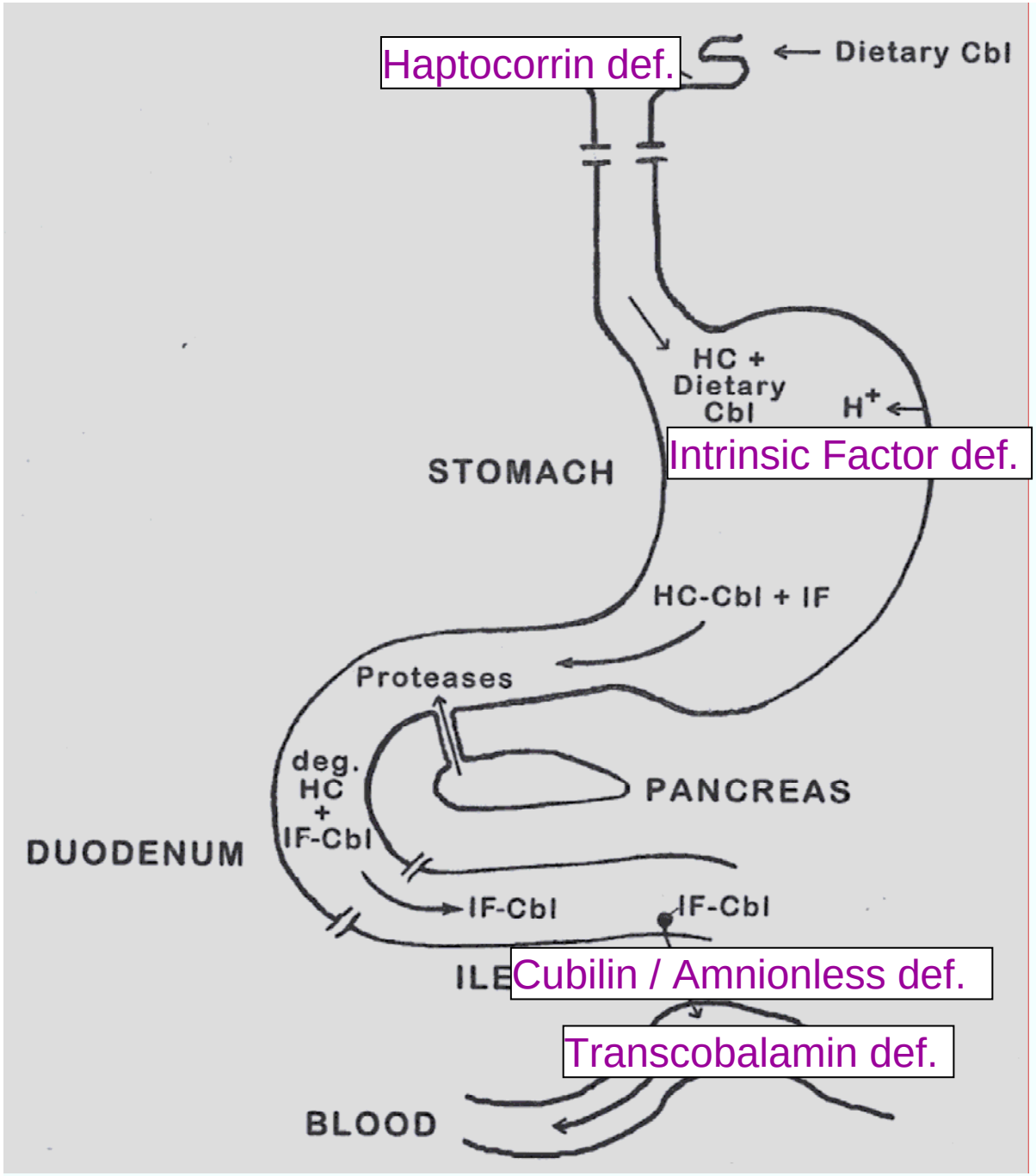
Essential cofactor for 2 Enzymes

Methionine Synthase
Methyl-Cbl

Methylmalonyl CoA-Mutase
Adenosyl-Cbl



Foods rich in Vitamin B_{12} (Cobalamin)



Gastrointestinal Absorption of dietary Vit-B_{12}

Cbl = Cobalamin

HC = Haptocorrin-bound Cbl

deg.HC = degraded HC

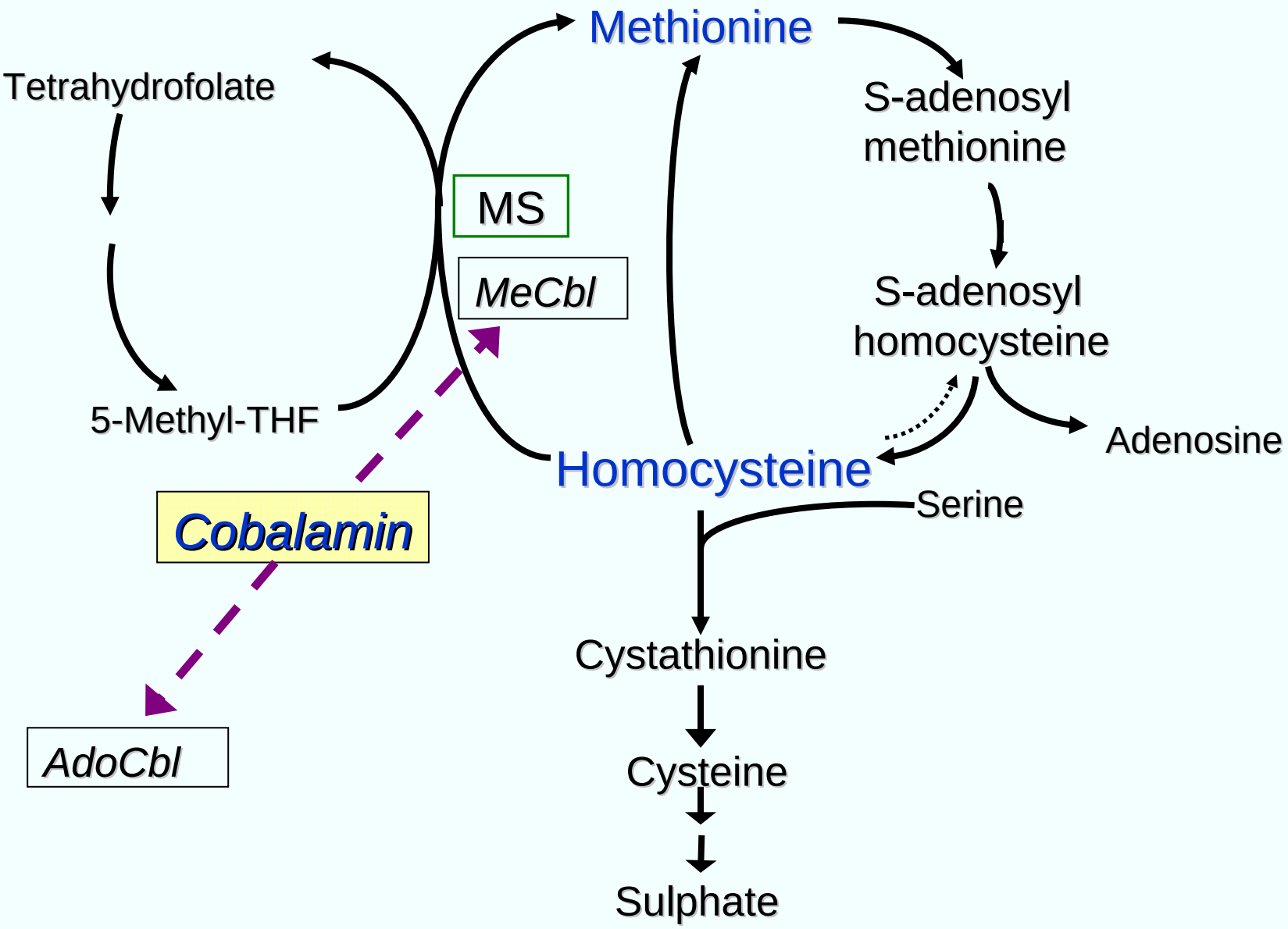
IF = Intrinsic Factor

IF-Cbl = IF-bound Cbl

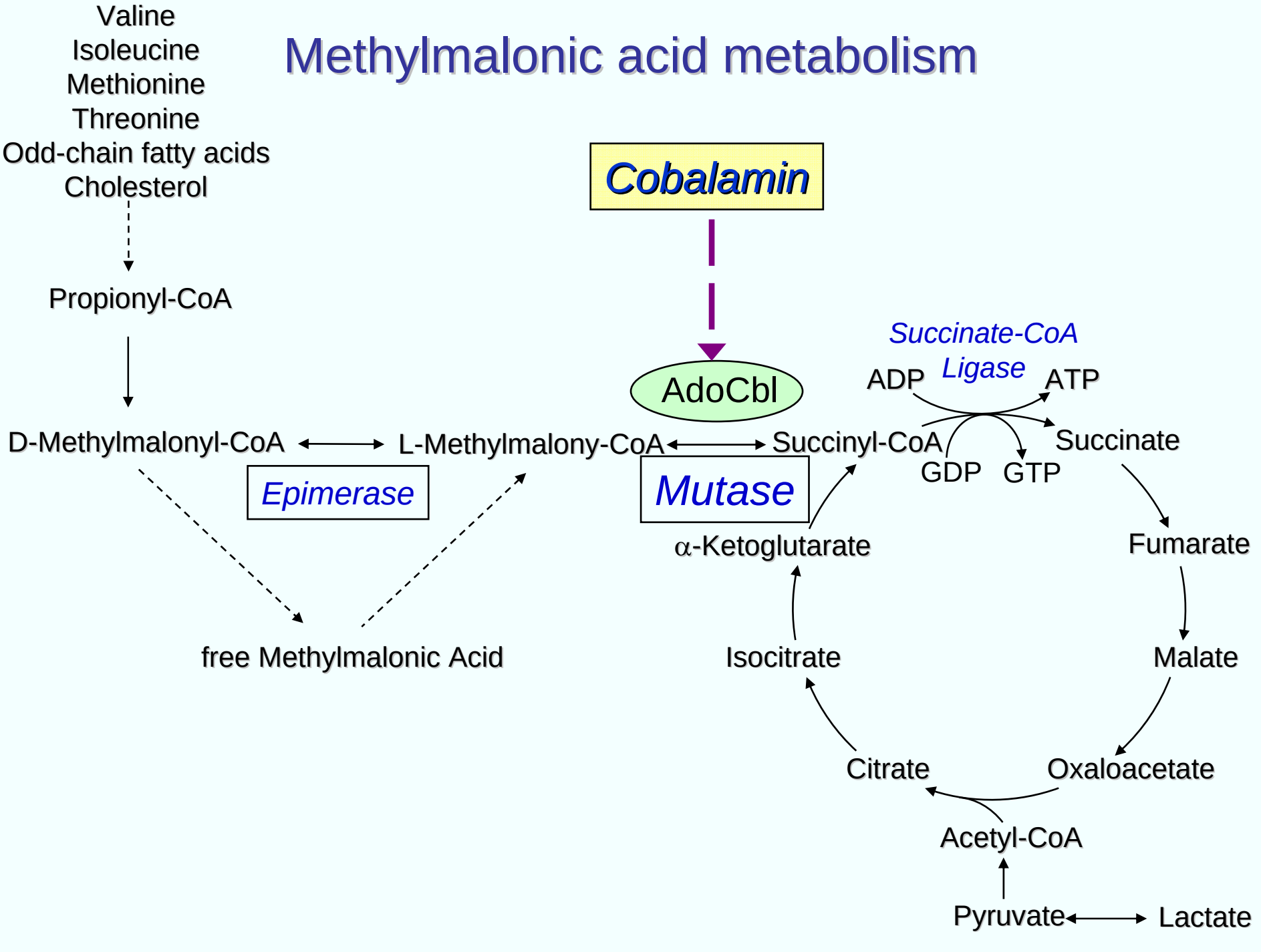
●-IF-Cbl = IF-Cbl attached to Cubilin (Ileal receptor)

TCII-Cbl = Transcobalamin bound Cbl.

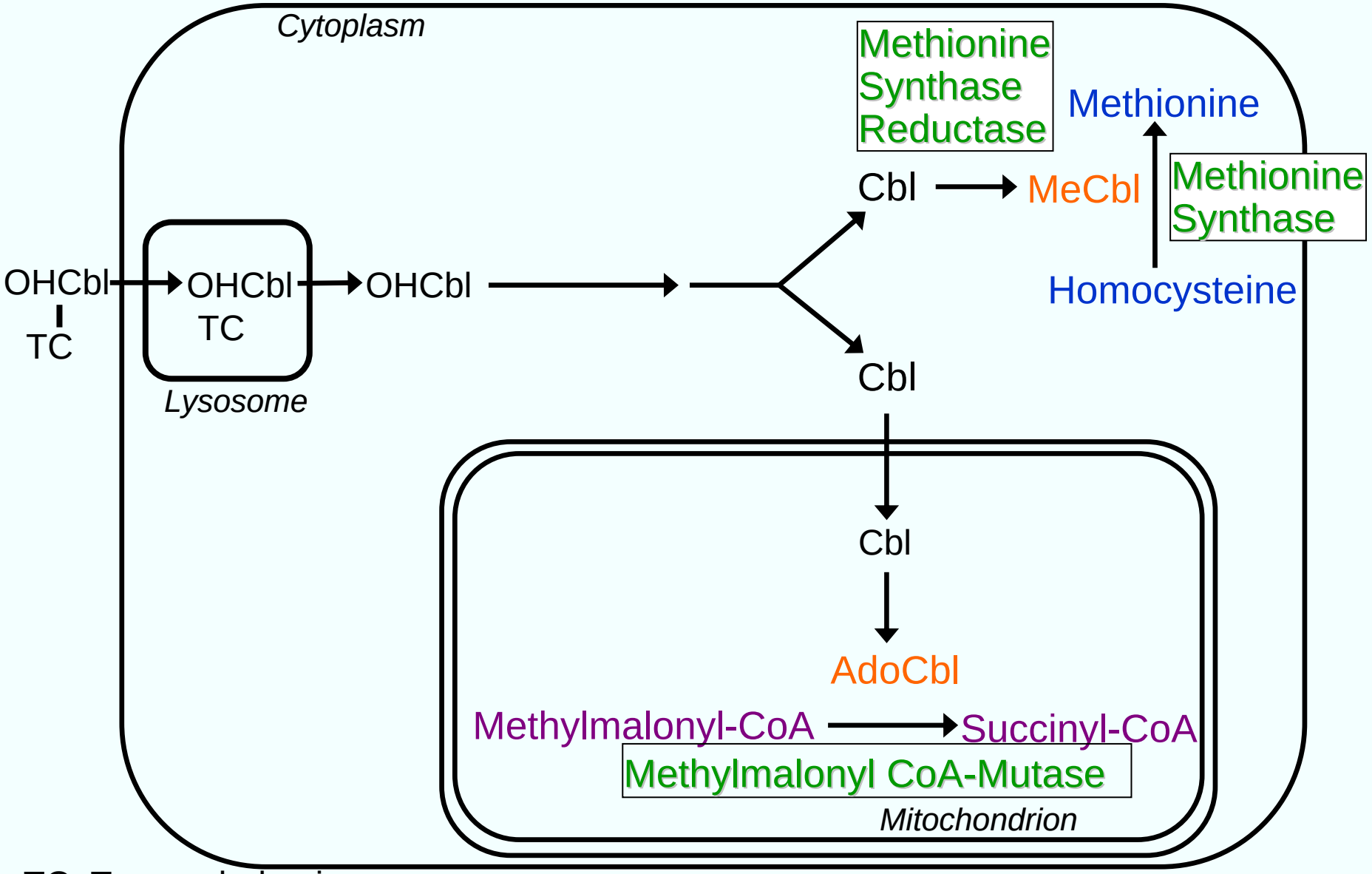
Vitamin B_{12} and Homocysteine Metabolism



Methylmalonic acid metabolism

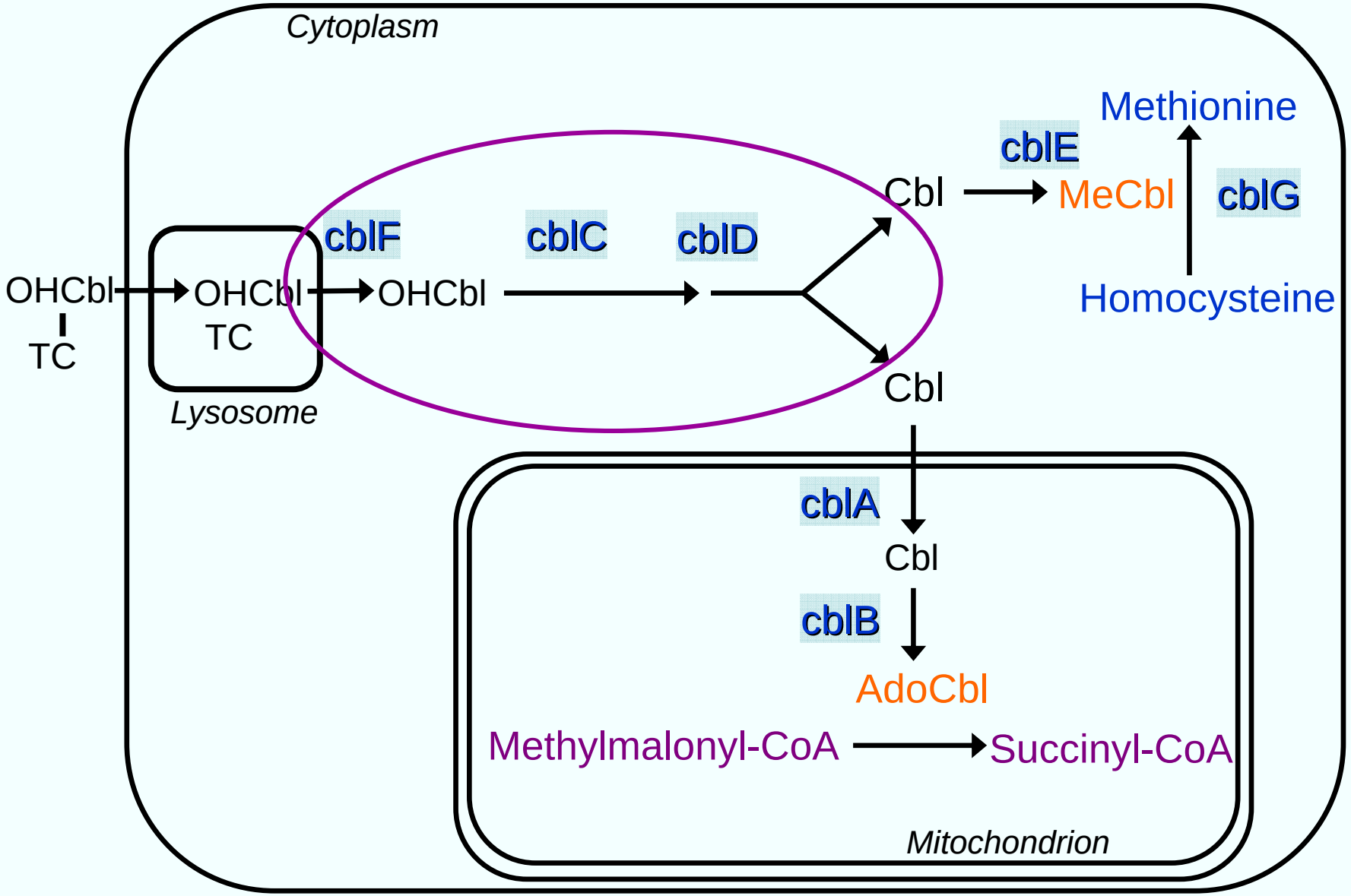


Intracellular Cobalamin Metabolism: 2002



TC, Transcobalamin

Intracellular Cobalamin Metabolism: 2002 complementation groups



CblC defect: early clinical presentation

3.5 w.	Feeding problems
	temperature dysregulation
	Pale, irritable, unconscious, dystrophic
	poor growth
	neurological abnormalities, tachycardia
	anaemia

Plasma	Hcy ↑↑↑	Methionine ↓
Urine	Methylmalonic acid ↑↑↑	
Treated	OH-Cbl i.m 1mg/d.	betaine, carnitine

4 months re-admitted to hospital
died one day later - multi-organ failure/hyperthermia

Prof. Sengers, Nijmegen

CblC defect, late Clinical presentation

Clinical

12y- 21y.

Unsteady gait, urinary incontinence

Spinal cord involvement, neuropathy

inability to walk

respiratory insufficiency (respirator)

Thought to have multiple sclerosis: Steroid treatment

Laboratory

Urine MMA ↑↑↑

Plasma total homocysteine ↑↑

Treated i.m. OH-Cbl 500µg / d. - 10mg /week

Gold et al. 1995

The CblC defect of cellular cobalamin metabolism

Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type

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NATURE GENETICS 38 I NUMBER1 I JANUARY 2006 93-100

- Discovered by homozygosity mapping
- Located on chromosome 1p
- Codes for ca. 30 kDa protein

The cblC gene

- In 204 individuals, 42 different mutations
- One mutation, 271dupA, accounted for 40% of all disease alleles.
- In further 118 individuals, 11 additional mutations

42% 271dupA *(Lerner-Ellis…Fowler:Hum Mutation 2009)*

cblC genotype / phenotype correlations

Early-onset disease

c.271dupA

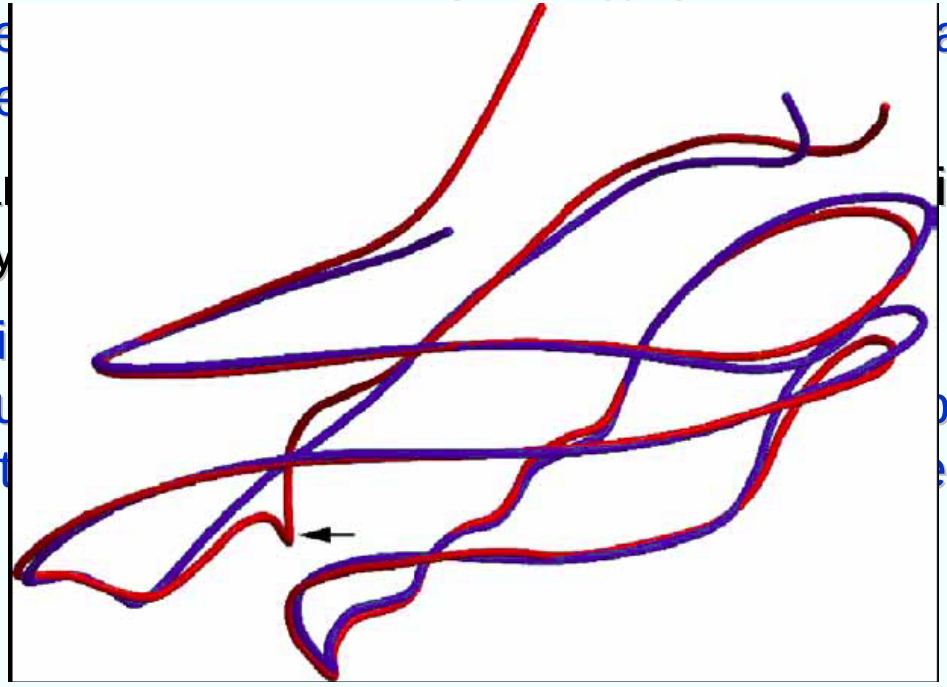
c.331C>T

Late-onset disease

c.394C>T (stop codon)

Function of the cblC protein

- Similar motifs to those seen in bacterial genes with cobalamin-related functions e.g. TonB
- TonB is involved in transducing energy generated from the proton motive force across the outer bacterial me
- Recombinan ions *in vitro* as a CNCbl decy
- Exact functi Evidence su balamin trafficking chaperone t r cobalamin related proteins.



MMACHC homology model (red) and the three-dimensional NMR structure of the monomeric C-terminal domain of TonB (blue).

The CblD Defect

The cblD complementation group

(Willard et al. 1978, Am. J. Hum. Genet.)

Originally Assigned to two siblings with homocystinuria / MMAuria *(Goodman et al. 1970, Biochem. Med.)*

Fibroblasts studies

↓ uptake of Cbl
↓ synthesis of AdoCbl and MeCbl
↓ MMCoA-mutase and Methionine synthase activity

BUT *less* severe than those in cblC defect

The CblD defect of cellular Cbl metabolism one gene – three phenotypes

- Original cblD

  2 siblings with combined defect methylmalonic-aciduria (MMA) and homocystinuria (Hcy)

- Our study (2004, Suormala, Coelho, Fowler et al. J B Chem:279: 42742)

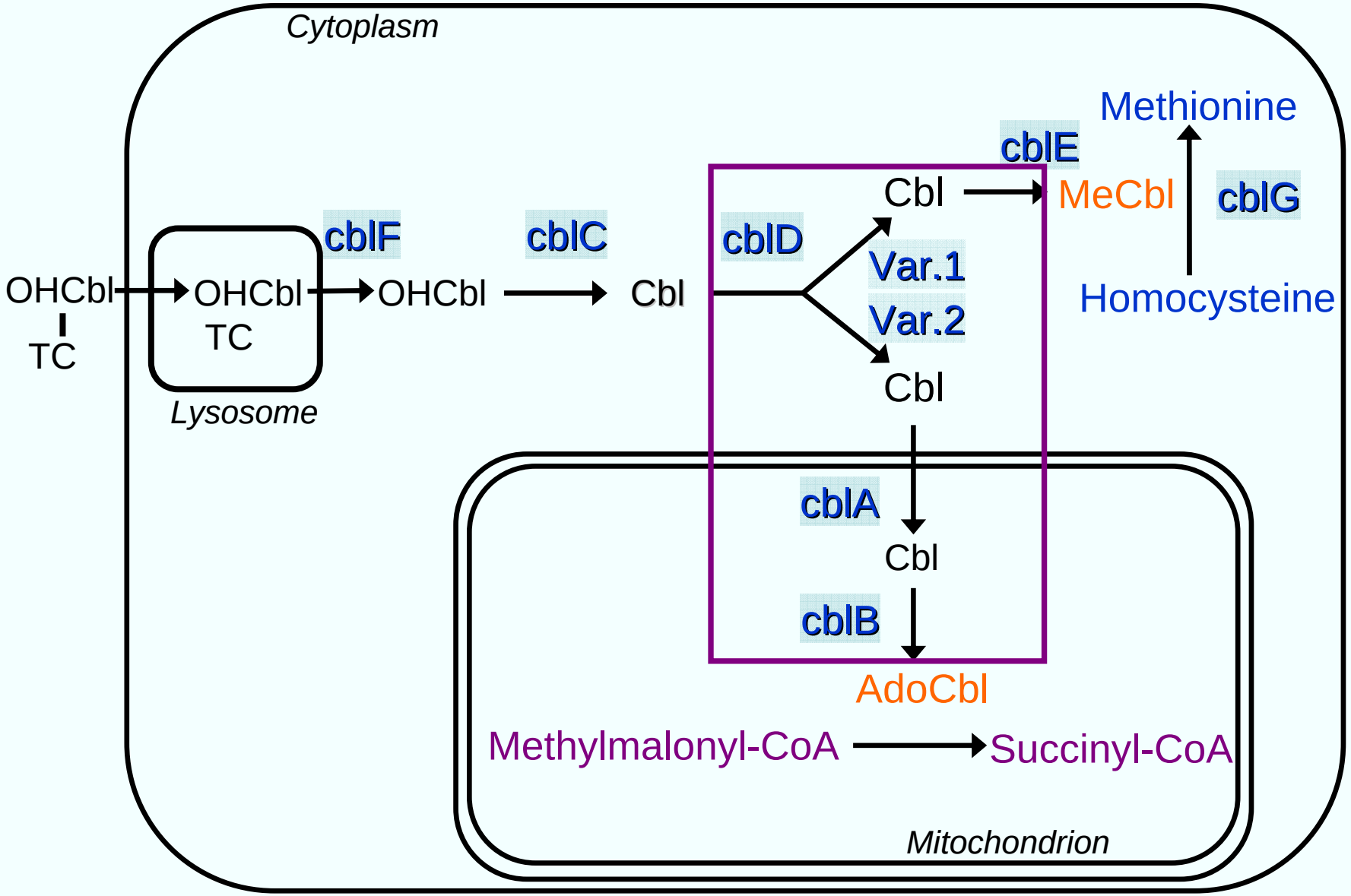
  - 2 patients with isolated Hcy - normal MMA
  - 1 patient with isolated MMA –normal Hcy

  Complementation studies proved that these patients belong to the cblD complementation group = gene

- Now 13 patients known

  4 combined defect (2 described 1980, 2 new ones)
  4 isolated homocystinuria,
  5 isolated Methylmalonic acidaemia

Intracellular Cobalamin Metabolism



Classification of cblD patients

	Urine	Plasma	Clinical findings
CblD-MMA/HC 4 patients	MMA ↑↑↑	Hcy ↑↑↑ Methionine ↓	Development delay Seizures Hypotonia Lethargy Feeding difficulties Megaloblastic anemia
CblD-HC 4 patients	MMA normal	Hcy ↑↑↑ Methionine ↓	Development delay Ataxia Absent ankle reflex Megaloblastic anemia
CblD-MMA 5 patients	MMA ↑↑↑	Hcy normal Methionine normal	Respiratory distress Cranial haemorrhage Seizures EEG abnormal

Search for the cblD gene

- Identification of the chromosome

Microcell Mediated Chromosome Transfer (MMCT)

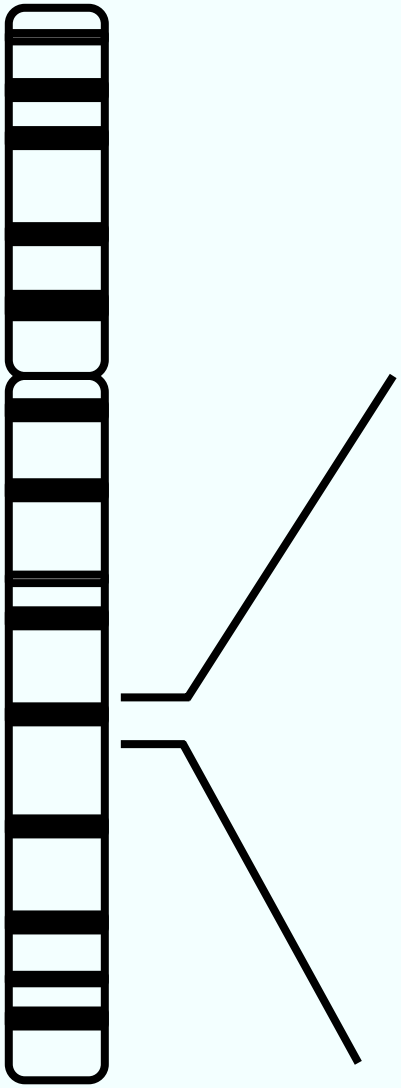
- Chromosomal region

Polymorphic markers analysis

- Candidate gene

Homology search with bacterial proteins

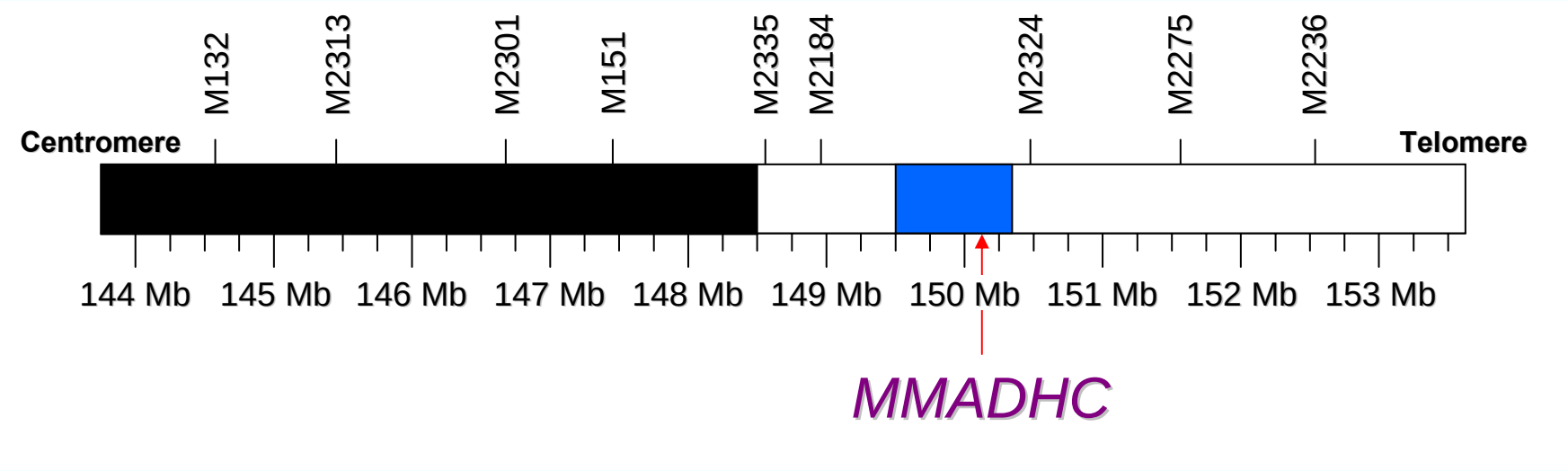
Mapping analysis of chromosome 2



- 10.2 Mb chromosomal interval (2006 29 Mb)
- containing 28 genes (2006 168, 2 candidates studied)
- 8 uncharacterized genes

cM	Markers	Size	HET (%)
154,4	**150**	218-242	83
154,4	**2286**	218-264	70
154,4	**2334**	259-273	77
154,6	**349**	129-135	47
154,8	**129**	162-180	77
155,9	**132**	189-213	76
156,4	**381**	298-312	60
156,4	**2270**	209-221	56
156,4	**151**	211-229	80
156,9	**2335**	153-173	76
157	**2301**	108-135	73
158,6	**2277**	245-259	58
158,6	**2324**	130-156	71

Chromosomal location of the candidate gene

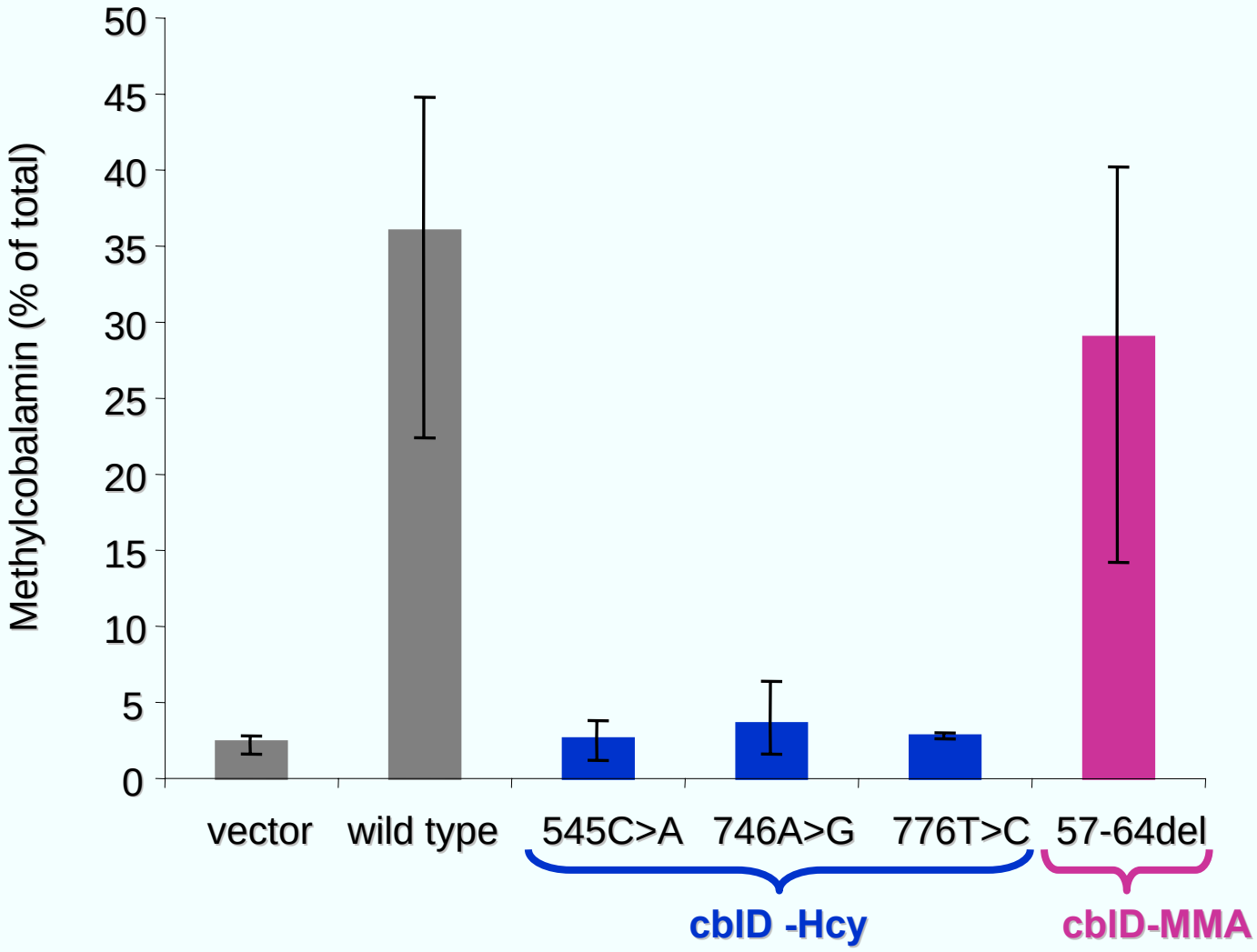


- Significant homology with bacterial genes related to ABC transporters
- Encodes a polypeptide of 296 aminoacids (32.8 kDa)
- Mutations were found in all cblD patients

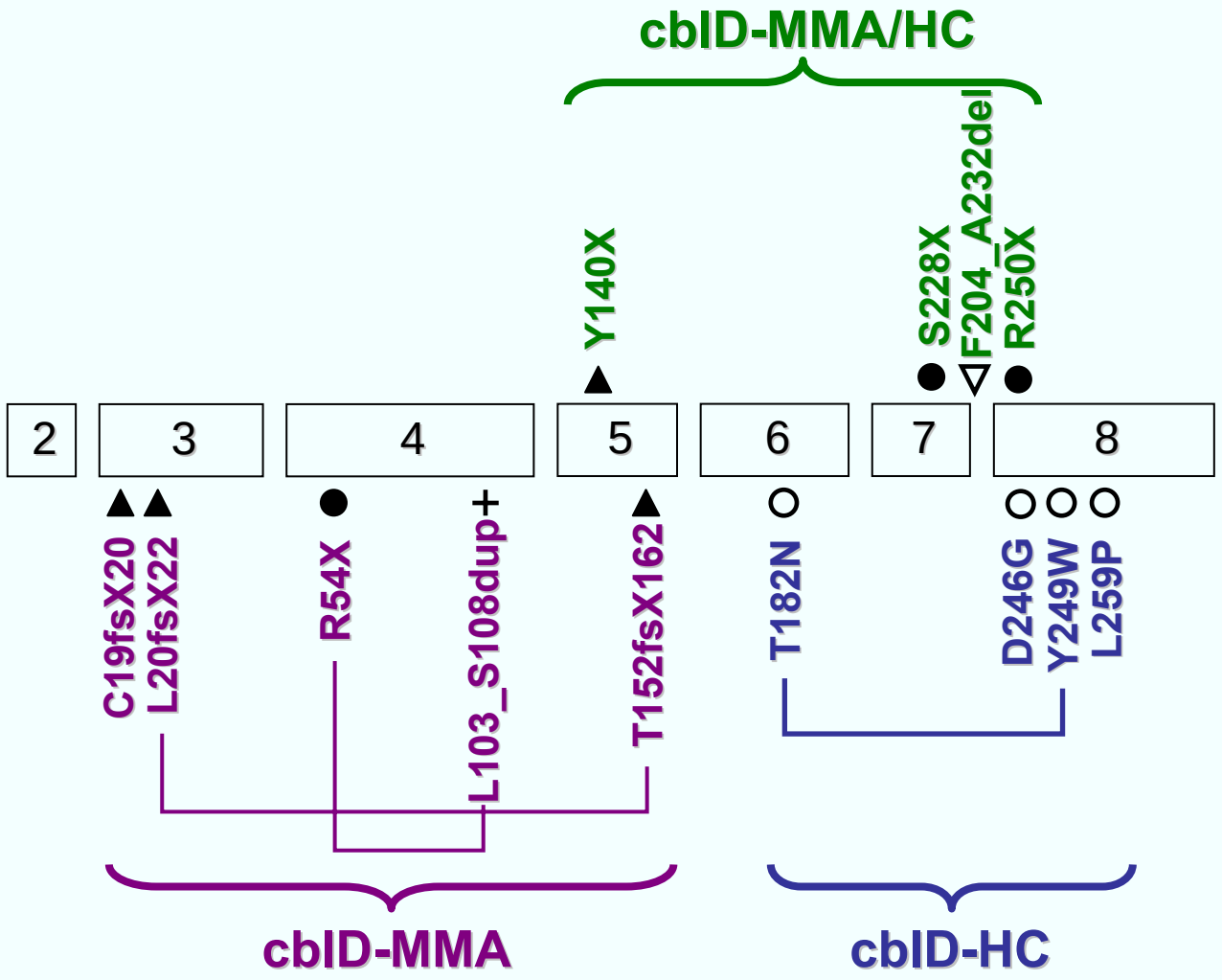
→ **M**ethyl**M**alonic **A**ciduria and **H**omo**C**ystinuria cbl**D** type (*MMADHC*)

- Maps to chromosome 2q23.2

Transfection of *combined defect* fibroblasts with wild type and mutant *MMADHC*



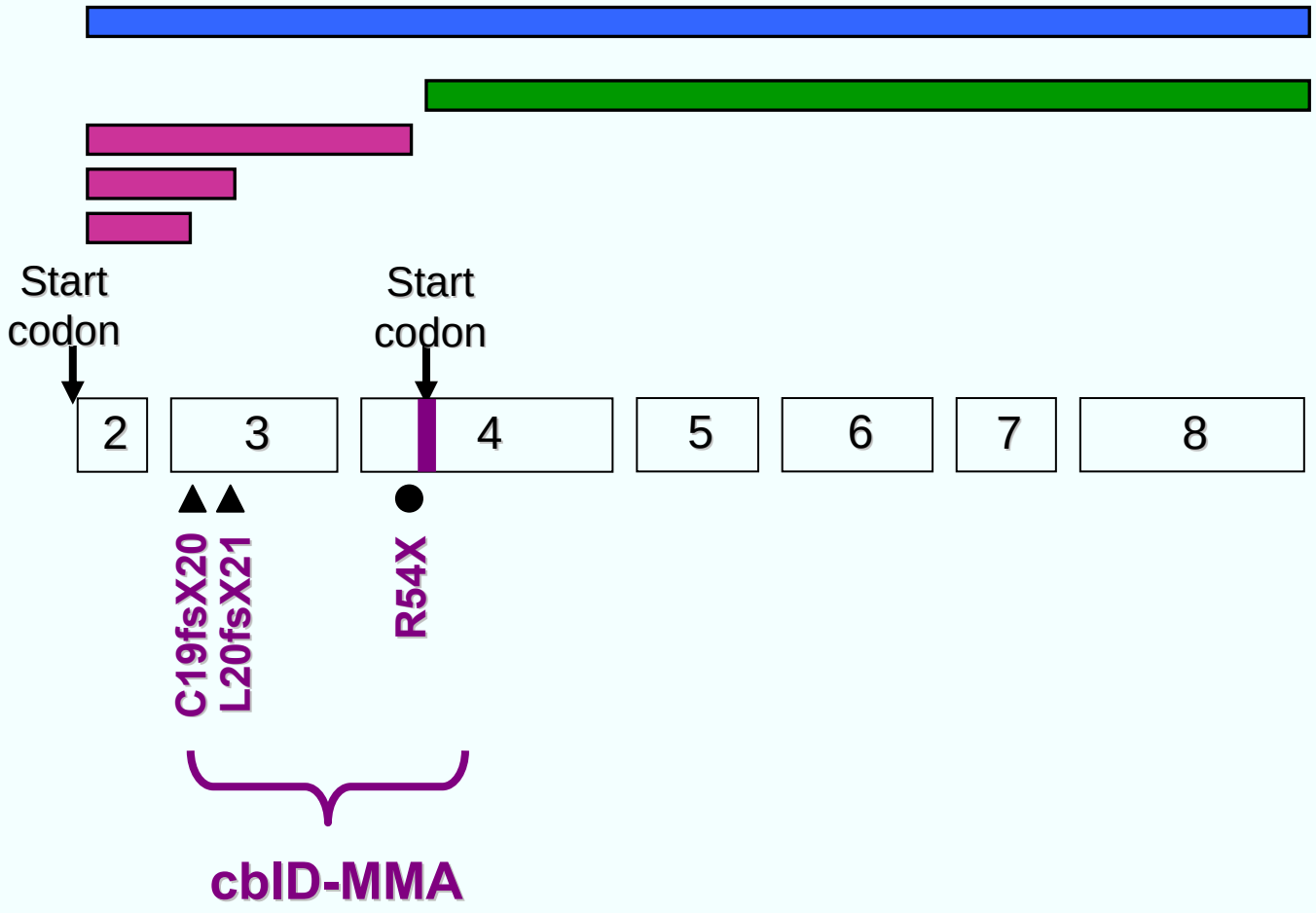
MMADHC mutations



▲ Frameshift/Stop codon ● Nonsense

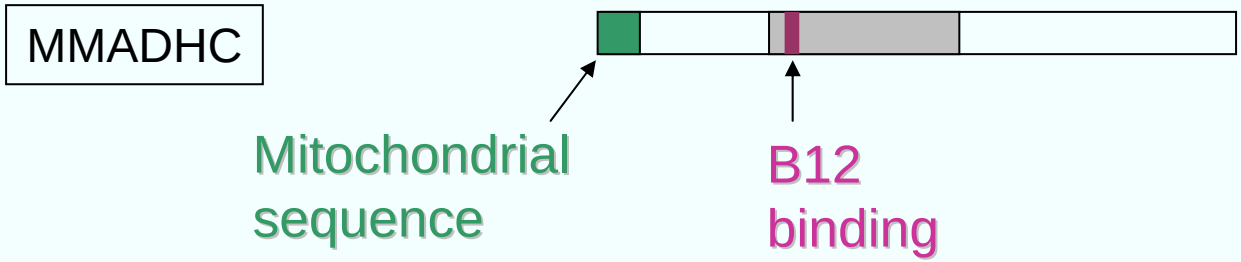
▽ Splice site deletion ○ Missense + Inframe duplication

MMADHC mutations in cblD-MMA

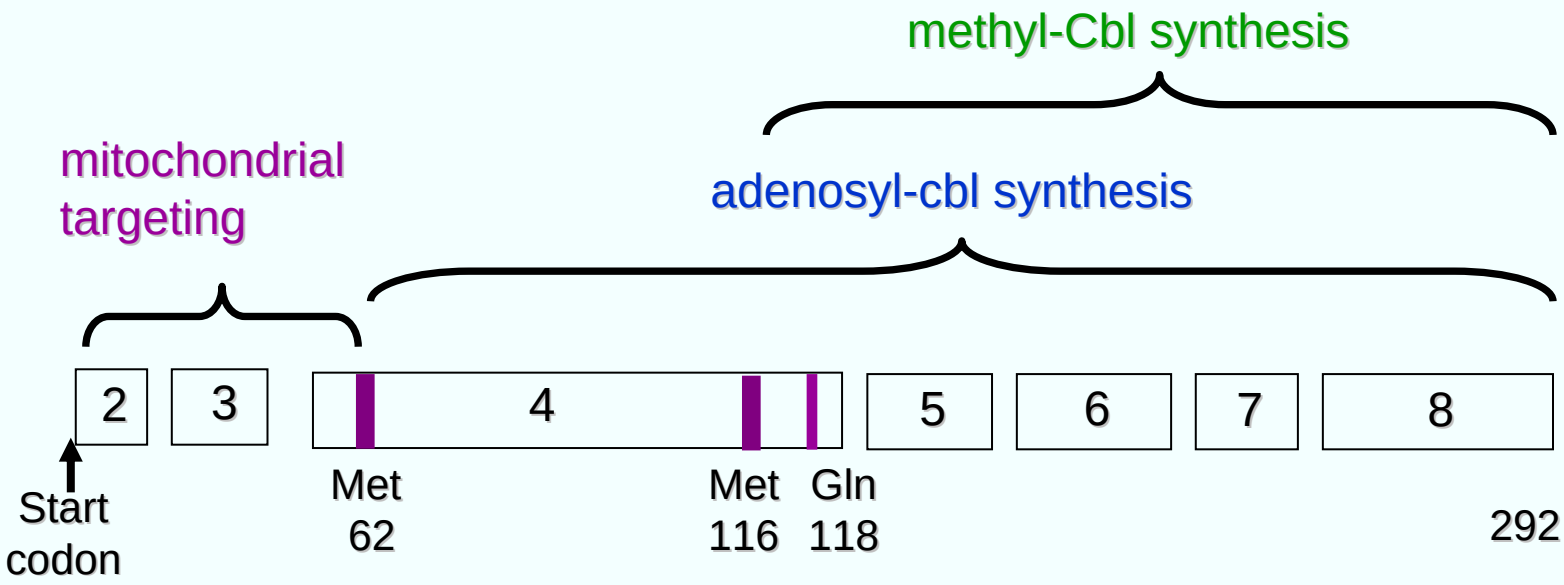


Possible function of the cblD Protein

- Protein sequence highly conserved in mammalian species
- *MMADHC* not member of previously identified gene family
- Shares similarity with putative ATPase component of bacterial ABC transporter
- Lacks critical domains of most ABC transporters
- Possible mitochondrial leader sequence
- Vitamin B_{12} binding motif DxHxxG



Identification of AdoCbl and MeCbl functional domains



cblF defect

Clinical Presentation (Fernandes book, 2006)

- Seven of eight presented in the first year of life. (now 14 patients known)
- Megaloblastic anemia, neutropenia and thrombocytopenia.
- Failure to thrive, recurrent infections, developmental delay, lethargy, hypotonia, aspiration pneumonia, hepatomegaly and encephalopathy, pancytopenia, and heart anomalies.
- Original infant girl had glossitis and stomatitis in first week of life.
  - severe feeding difficulties requiring tube feeding.
  - Tooth abnormalities and dextrocardia were present.
- One infant died suddenly at home in the first year of life.
- One boy developed juvenile rheumatoid arthritis at the age of 4 years and a pigmented skin abnormality at 10 years.

cblF defect

Metabolic Derangement

- Failure of Cbl transport across the lysosomal membrane following degradation of TC in the lysosome.
- No conversion of Cbl to either AdoCbl or MeCbl.
- Abnormal Schilling test suggests IF-Cbl has to pass through a lysosomal stage in the enterocyte before Cbl is released into the portal circulation.

Genetics

- Autosomal recessive.
- The gene responsible for cblF has not been identified

Treatment

Parenteral OHCbl, 1mg/day, (first daily and then biweekly, or even less frequently) seems to be effective in correcting the metabolic and clinical findings.

cblF defect: Diagnostic Tests

Plasma Hcy ↑↑↑

cobalamin ↓ / Normal

Urine

Schilling test abnormal

Methionine ↓

Methylmalonic acid ↑↑↑

Precise diagnosis requires tests in cultured fibroblasts.

- Incorporation of [14C] propionate into macromolecules - screen for integrity of methylmalonyl-CoA mutase activity
- Conversion of [14C] labeled formate to methionine reliably measures methionine synthase function
- Total incorporation of [57Co] CNCbl and conversion to both MeCbl and AdoCbl, can differentiate Cbl disorders.

In *cblF* patients, total incorporation of labeled CNCbl elevated, but CNCbl not converted to AdoCbl or MeCbl.

nature genetics

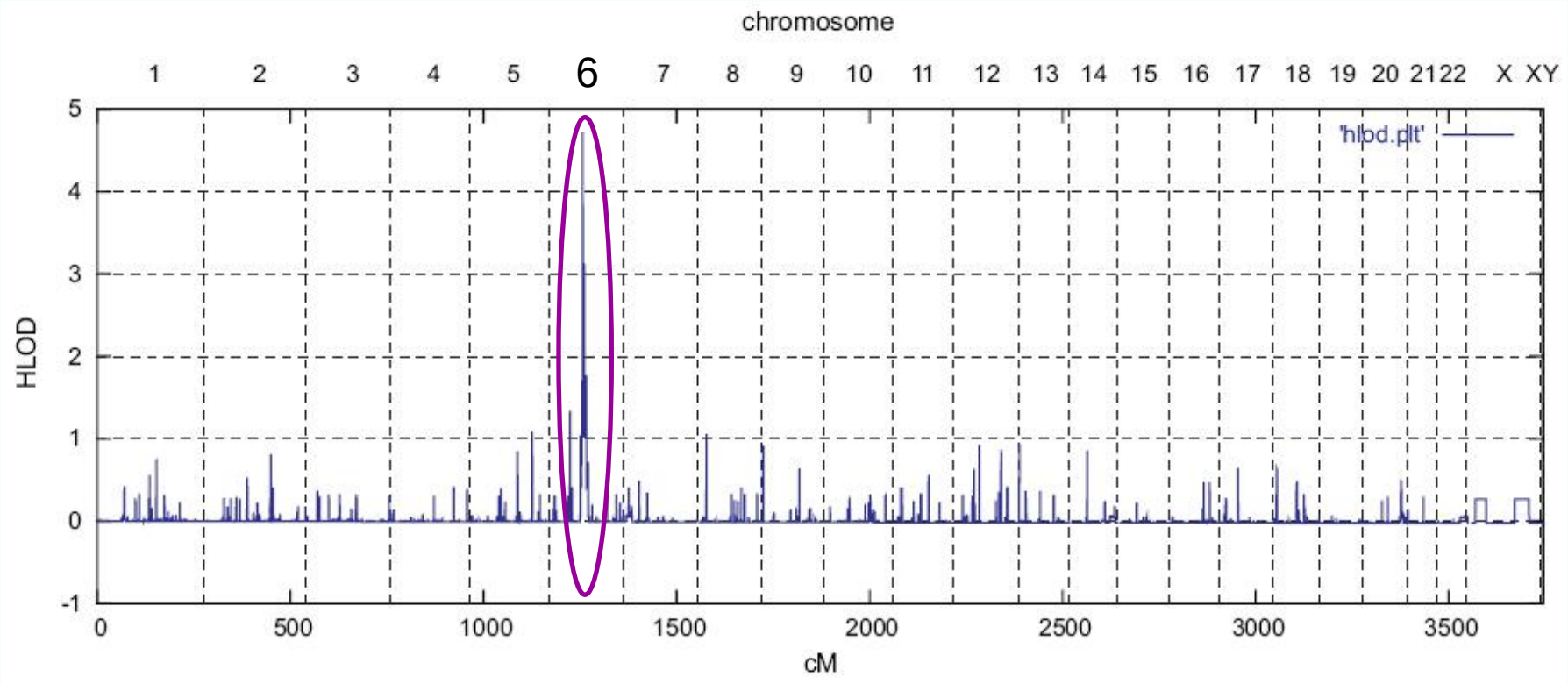
2009: 41;234

Identification of a putative lysosomal cobalamin exporter altered in the cblF defect of vitamin B_{12} metabolism

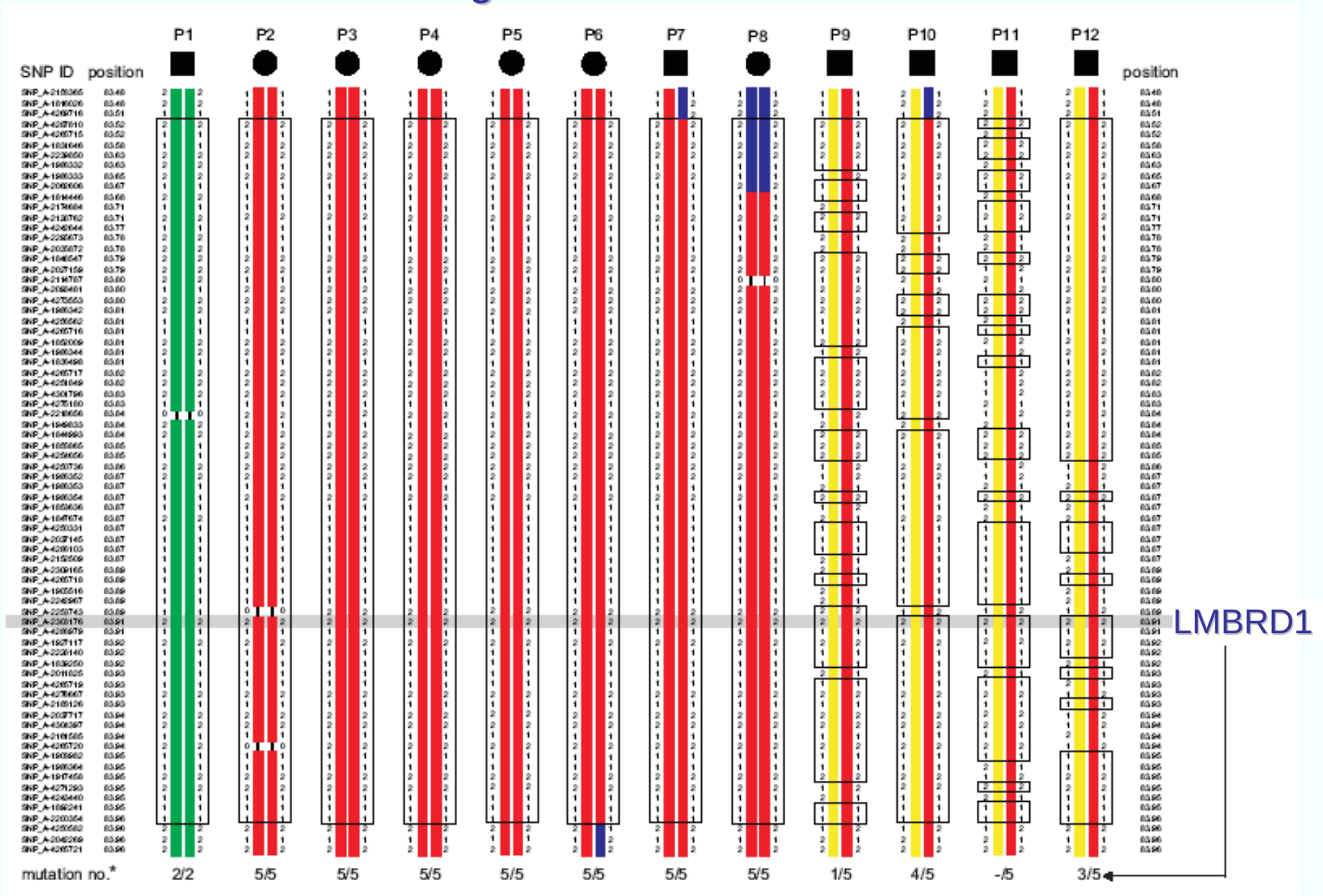
Frank Rutsch[1], Susann Gailus[1], Isabelle R Miousse[2], Terttu Suormala[3], Corinne Sagné[4], Mohammad Reza Toliat[5], Gudrun Nürnberg[5], Tanja Wittkampf[1], Insa Buers[6], Azita Sharifi[4], Martin Stucki[7,8], Christian Becker[5], Matthias Baumgartner[7], Horst Robenek[6], Thorsten Marquardt[1], Wolfgang Höhne[9], Bruno Gasnier[4], David S Rosenblatt[2], Brian Fowler[3] & Peter Nürnberg[5,10]

Homozygosity mapping

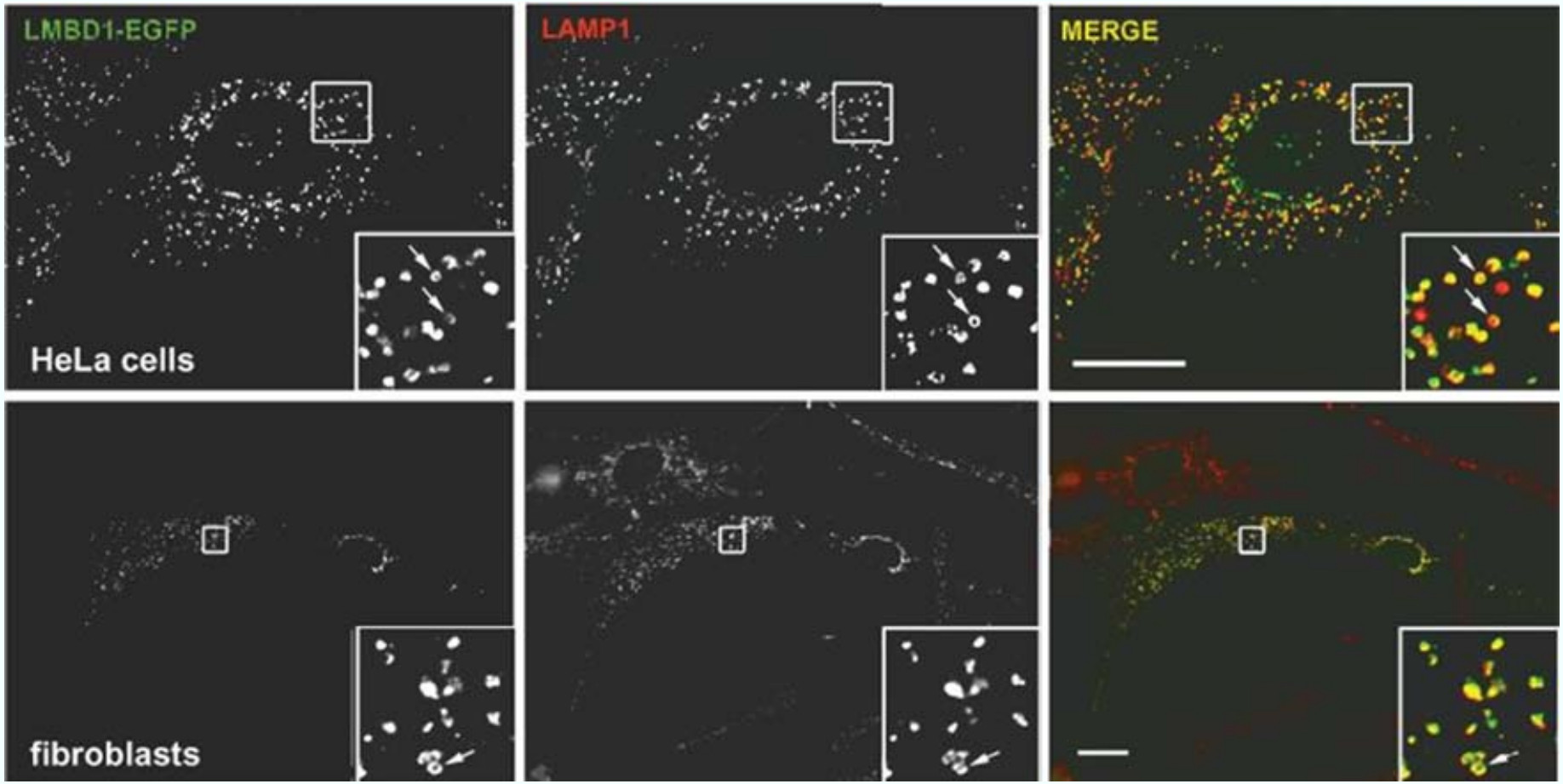
Multi-point linkage analysis of the genome-wide scan using the Affymetrix GeneChip® Human Mapping 250K Sty Array.



Fine polymorphic mapping and haplotype analysis reveals LMBRD1 as candidate gene



Localisation of transiently expressed LMBD1-EGFP with the lysosomal marker LAMP1



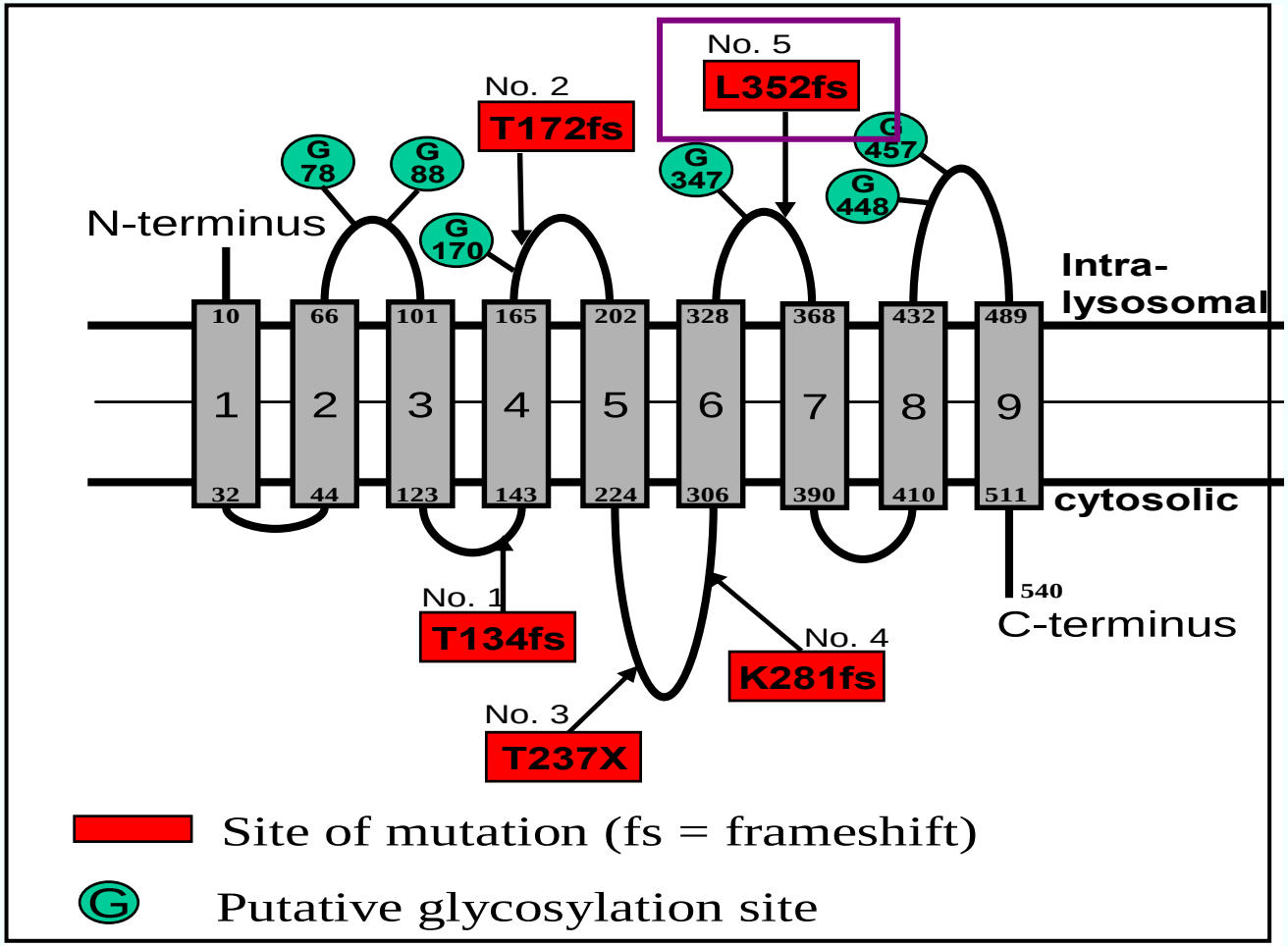
- Mutations in the LMBRD1 gene (encoding LMBD1, a lysosomal membrane protein) found in cblF patients

- Transfection of cblF fibroblasts with wild-type LMBD1 rescued cobalamin coenzyme synthesis

- LMBRD1 identifed as cblF gene and suggests that LMBD1 is a lysosomal membrane exporter for cobalamin

Mutations in the LMBRD1 gene (encoding LMBD1, a lysosomal membrane protein) found in cblF patients

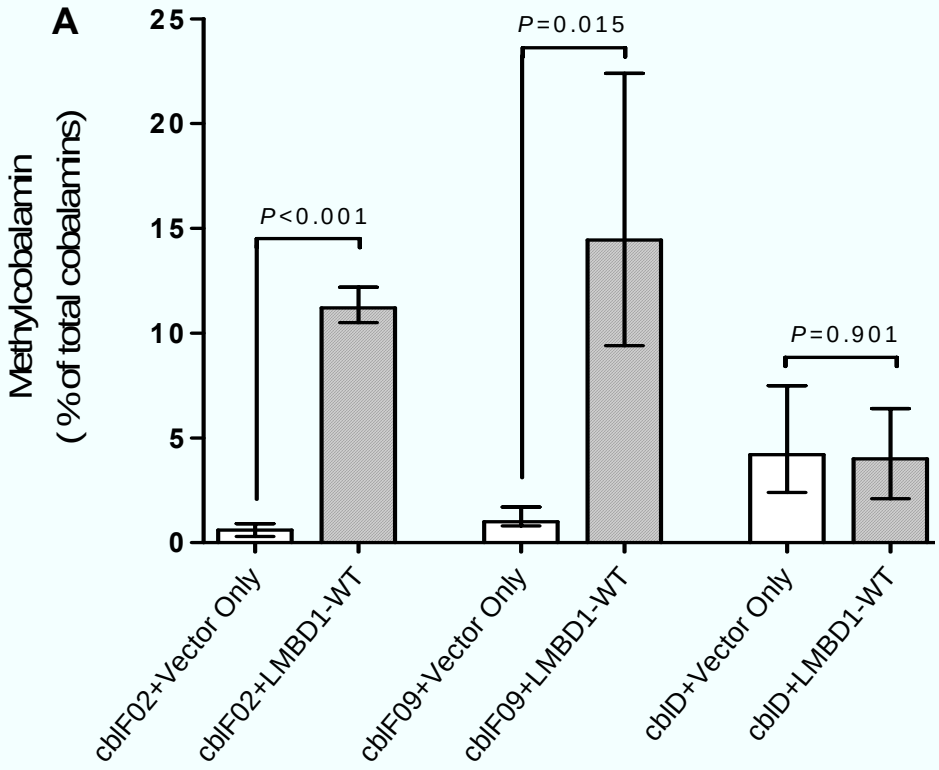
- 6q13
- 18 exons
- 61.4 kDa protein
- 540 amino acids



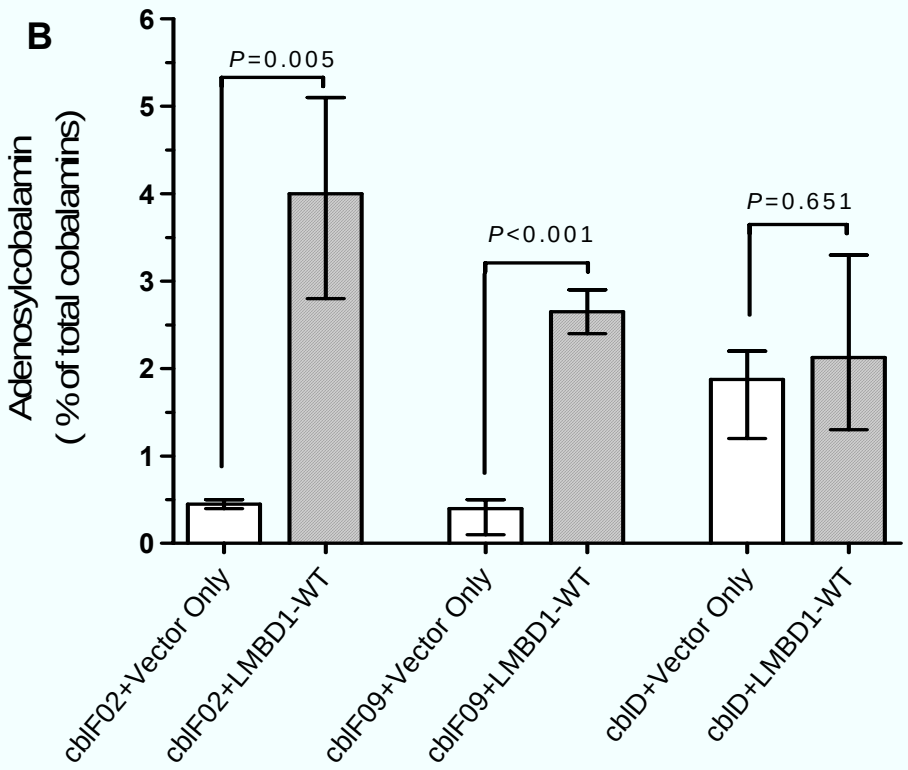
The 1056delG allele (No. 5) leading to a frameshift and premature termination codon in exon 11 was present on 18 of the 24 disease chromosomes (common 1.34 Mb haplotype).

Transfection of cblF fibroblasts wild-type LMBD1 rescued cobalamin coenzyme synthesis and function.

Methylcobalamin synthesis

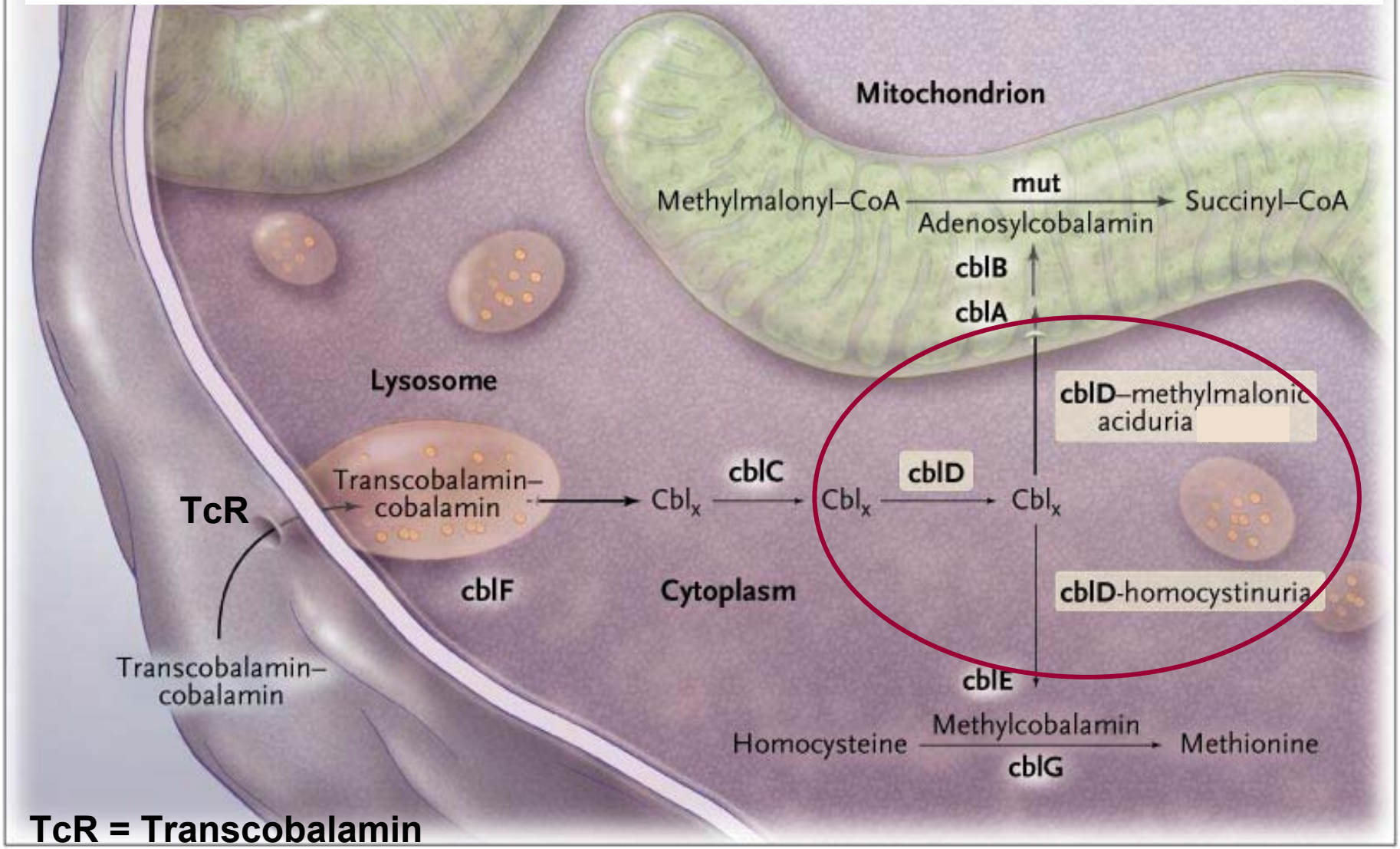


Adenosylcobalamin synthesis



Coelho D, Suormal T, Stucki M, Lerner-Ellis JP, Rosenblatt DS, Newbold R, Baumgartner M, Fowler B.

N ENGL J MED 358;14 WWW.NEJM.ORG APRIL 3, 2008



Acknowledgements

- Terttu Suormala, David Coelho (Basel),
- Matthias Baumgartner, Martin Stucki (Zürich)
- Jordan Lerner-Ellis, David Rosenblatt (Montreal)
- Petra Zavadakova, Viktor Kožich (Prague)
- Hans Georg Koch, Martin Berghäuser (Münster)
- James E. Wraith (Manchester) , Alberto Burlina (Padua)
- Adrian Sewell, Jürgen Herwig (Frankfurt)
- Many colleagues who sent us fibroblasts

- Swiss National Science Foundation grant No 3200-066878