Vitamin $\text{B}_{12}$: Can you teach an old vitamin, new tricks?

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McGill University
Learning Objectives

• 1) To distinguish the complementation groups involved in the inherited disorders of cobalamin metabolism;

• 2) To understand that knowledge of a metabolic pathway is constantly evolving.
Megaloblastic Anemia
Cobalamin Deficiency-Acquired

Cobalamin Levels

Holo TC
Tests of Cobalamin Absorption-Schilling test, Food Cobalamin Malabsorption, CobaSorb
Single-Sample Fecal Excretion Test
Methylmalonic aciduria and total homocysteine (tHcy)
Deoxyuridine Suppression Test

Is MCV useful in era of folic acid fortification?
Infants of vegan mothers or mothers with subclinical pernicious anemia
Cobalamin Absorption

I-F-Cobalamin Receptor Deficiency
(Imerslund –Gräsbeck Syndrome) (MGA1)

Example of One Phenotype, 2 Genes
I-F-Cobalamin Receptor Deficiency (Imerslund-Gräsbeck) (MGA1)

• Early onset megaloblastic anemia, low serum cobalamin levels, and proteinuria
• Homocystinuria and methylmalonic aciduria may be found but are not prominent
• Decreased absorption of cobalamin in the presence of normal synthesis of intrinsic factor
Transcobalamin Receptor

• TC Receptor-First Inborn Error
  – Infant with methylmalonic aciduria detected on newborn screening.
  – Response to treatment with cobalamin but low level MMA persisted.
Transcobalamin Receptor

- Total Cobalamin Uptake
Transcobalamin Receptor

Amino acid sequence of TCblR
(Deletion is shown in red and the polymorphisms in green)
Transcobalamin TCII (TC)

- TC deficiency
- Early onset - Can be mistaken for LEUKEMIA
- Unsaturated B$_{12}$ Binding Capacity (UBBC) – needs to be done BEFORE treatment
- Commercial antibodies to TC to measure TC levels
INTRACELLULAR METABOLISM
OF VITAMIN B₁₂

Homocysteine → Methionine
  ↓
MeCbl
  ↓
MTRR

Homocysteine

MTR

Cbl

MMAB

MMAA

D-Methylmalonyl CoA
  ↓
MCEE
L-Methylmalonyl CoA
  ↓
AdoCbl
  ↓
MUT
Succinyl CoA

MMACHC → MMADHC

CD320 → LMRBD1 → ABCD4

TC-Cbl

LYSOSOMES

MITOCHONDRIA

Familial ~ 25%
INTRACELLULAR METABOLISM
OF VITAMIN B_{12}

**AdoCbl**
**L-Methylmalonyl CoA**
**Succinyl CoA**

**MUT**
**MMACHC**
**MMADHC**

**Homocysteine**
**Methionine**

**MeCbl**
**MTR**
**cblE**

**MMAB**
**cblB**

**MMAA**
**cblA**

**D-Methylmalonyl CoA**
**MCEE**

**L-Methylmalonyl CoA**

**AdoCbl**
**Succinyl CoA**

**MTRR**
**cblG**

**TC-Cbl**
**CD320**

**LMRBD1**
**ABCD4**

**cblF**
**cblC**
**cblD**

**cblJ**

**mitochondria**

**MITOCHONDRIA**
cbIF

- 15 known patients
  - Developmental delay (9), failure to thrive (6), feeding difficulties (6), SGA (5), hypotonia (4)
  - Hematological disturbances
    - Megaloblastic anemia (5)
    - Neutropenia (2), pancytopenia (1) and thrombocytopenia (1)
  - Inflammation
    - Stomatitis (4), glossitis (2), rashes (2), gastritis (1), arthritis (1)
  - Cardiac malformations
    - VSD (2), ASD (2)
  - Tooth abnormalities (3)
  - Seizures (2)
Low serum B$_{12}$ in 5 out of 7 with data available
• 2 completely asymptomatic (NBS)
• 2 completely symptom-free on B$_{12}$ therapy
• 3 died (2 after cardiac surgery, 1 SIDS)
• Remaining have mild growth and/or developmental delay.
• Short stature was reported in 6 of 12 surviving patients.
Cobalamin is trapped in the lysosomes

- Silver grains were used to trace $^{57}$Co cobalamin to lysosomes in the patients
- Vassiliadis et al.
Mutations in *ABCD4* cause a new inborn error of vitamin B\textsubscript{12} metabolism

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# Two Patients

<table>
<thead>
<tr>
<th>Clinical Findings</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotonia</td>
<td></td>
<td>Heart defects</td>
</tr>
<tr>
<td>Lethargy</td>
<td></td>
<td>Pancytopenia</td>
</tr>
<tr>
<td>Bone marrow suppression</td>
<td></td>
<td>Mild mental retardation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microcephaly</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical Phenotypes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocystinuria &amp; methylmalonic aciduria</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cultured fibroblasts**
- Decreased synthesis of MeCbl and AdoCbl
- Increased uptake of CNCbl (exogenous form)

**cblF-phenocopy**

**BUT**, no mutations found in the *LMBRD1* gene (*cblF*)
# Identification of *cblJ* Gene

<table>
<thead>
<tr>
<th>Searching for the Gene</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-Exome Capture and Sequencing</td>
<td>Microcell-Mediated Chromosome Transfer</td>
<td></td>
</tr>
<tr>
<td>Exome Capture and Sequencing of Chr.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Candidate Verification</th>
<th>Sanger Re-sequencing &amp; Segregation Analysis</th>
</tr>
</thead>
</table>

| Common Candidate | ABCD4 |

**ATP-Binding Cassette, subfamily D, member 4**

- ABCD1/2/3 are peroxisomal half-transporters
- Function of ABCD4 protein is unknown
### Mutations in *ABCD4*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutant Alleles</th>
<th>Predicted Amino Acid Mutation</th>
<th>Ethnic Group</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.955A&gt;G</td>
<td>p.Tyr319Cys</td>
<td>Northern European</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>c.1746_1747insCT</td>
<td>p.Glu583LeufsX9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>c.542+1G&gt;T</td>
<td>p.Asp143_Ser181del</td>
<td>German</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>(splice donor site)</td>
<td>(in frame exon skipping)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1456G&gt;T</td>
<td>p.Gly486Cys</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **39-328**: ABC transmembrane domain
- **389-603**: ABC transporter domain
Clinical features-\textit{cbl/C}

Based on the age of onset

I. Early-onset form
   – Symptoms in the first year of life
   – feeding difficulties, failure to thrive, somnolence/lethargy and hypotonia

II. Late onset form-rarer
    – childhood to adulthood
    – milder/no hematological abnormalities
    – purely neurological (extrapyramidal symptoms)
    – rapid mental deterioration: confusion, dementia, delirium, psychosis
    – renal damage: chronic thrombotic microangiopathic glomerulo-nephropathy
Genotype-Phenotype Correlations in cblC Disease

cblC

Early Onset
- Present within the first year of life
- Systemic, neurologic, hematologic, ophthalmologic pathologies

Late Onset
- Present after the age of 4 years
- Predominantly neurologic symptoms: ataxia, dementia and psychosis
**MMACHC gene**

- > 500 cases
- Gene location -1p34.1
- 5 exons
- >60 mutations

Froese, 2010
Table 1  **MMACHC** mutations identified in 204 cbiC individuals

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Predicted effect on protein or mRNA</th>
<th>No. of alleles detected in 204 cbiC individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A→T</td>
<td>M17</td>
<td>1</td>
</tr>
<tr>
<td>3G→A</td>
<td>M17</td>
<td>10</td>
</tr>
<tr>
<td>80A→G</td>
<td>G27R [r.spl?]</td>
<td>2</td>
</tr>
<tr>
<td>81→G</td>
<td>r.spl?</td>
<td>1</td>
</tr>
<tr>
<td>82_9_12deTTC</td>
<td>r.spl?</td>
<td>2</td>
</tr>
<tr>
<td>217C→T</td>
<td>R73X</td>
<td>9</td>
</tr>
<tr>
<td>271dupA</td>
<td>R91KfsX14</td>
<td>165</td>
</tr>
<tr>
<td>32R_63G_64AUA</td>
<td>N100G/100G</td>
<td>8</td>
</tr>
<tr>
<td>331C→T</td>
<td>R111X</td>
<td>36</td>
</tr>
<tr>
<td>347T→C</td>
<td>L116P</td>
<td>2</td>
</tr>
<tr>
<td>3592T[C]</td>
<td>Q118RfsX6</td>
<td>4</td>
</tr>
<tr>
<td>360A→G</td>
<td>H122R</td>
<td>1</td>
</tr>
<tr>
<td>388T→C</td>
<td>Y130H</td>
<td>2</td>
</tr>
<tr>
<td>388_390delAA</td>
<td>Y130del/130del</td>
<td>6</td>
</tr>
<tr>
<td>391C→T</td>
<td>Q131X</td>
<td>1</td>
</tr>
<tr>
<td>394C→T</td>
<td>R132X</td>
<td>34</td>
</tr>
<tr>
<td>398_399delAA</td>
<td>Q133fsX14</td>
<td>1</td>
</tr>
<tr>
<td>420G→A</td>
<td>W140X</td>
<td>2</td>
</tr>
<tr>
<td>435_436delAT</td>
<td>S146fsX33</td>
<td>2</td>
</tr>
<tr>
<td>440G→A</td>
<td>G147D</td>
<td>9</td>
</tr>
<tr>
<td>440G→C</td>
<td>G147A</td>
<td>3</td>
</tr>
<tr>
<td>450_479dup30</td>
<td>l1500_A1590dup</td>
<td>1</td>
</tr>
<tr>
<td>457C→T</td>
<td>R153X</td>
<td>4</td>
</tr>
<tr>
<td>467G→A</td>
<td>G156D</td>
<td>1</td>
</tr>
<tr>
<td>468_469delCT</td>
<td>W157fsX24</td>
<td>2</td>
</tr>
<tr>
<td>471G→C</td>
<td>W157C</td>
<td>1</td>
</tr>
<tr>
<td>481C→T</td>
<td>R161X</td>
<td>4</td>
</tr>
<tr>
<td>481C→G</td>
<td>R161G</td>
<td>1</td>
</tr>
<tr>
<td>482G→A</td>
<td>R161Q</td>
<td>8</td>
</tr>
<tr>
<td>500delC</td>
<td>P167QfsX3</td>
<td>1</td>
</tr>
<tr>
<td>547_548delGT</td>
<td>V183fsX5</td>
<td>1</td>
</tr>
<tr>
<td>569C→A</td>
<td>R189S</td>
<td>1</td>
</tr>
<tr>
<td>596C→C</td>
<td>R189fsX21</td>
<td>1</td>
</tr>
<tr>
<td>578T→C</td>
<td>L193P</td>
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<tr>
<td>600A→A</td>
<td>W200X</td>
<td>1</td>
</tr>
<tr>
<td>609G→A</td>
<td>W200X</td>
<td>12</td>
</tr>
<tr>
<td>615C→G</td>
<td>Y200X</td>
<td>11</td>
</tr>
<tr>
<td>616C→T</td>
<td>R206W</td>
<td>2</td>
</tr>
<tr>
<td>617G→C</td>
<td>R206P</td>
<td>2</td>
</tr>
<tr>
<td>658_659delAA</td>
<td>K220fsX24</td>
<td>1</td>
</tr>
<tr>
<td>666C→A</td>
<td>Y222X</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mutation</th>
<th>No. of alleles detected in 204 cbiC individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not identified</td>
<td>33</td>
</tr>
</tbody>
</table>

- 204 patients
- 42 mutations
  - c.271dupA (p.R91KfsX14): 40%
  - c.331C>T (p.R11X): 9%
  - c.394C>T (p.R132X): 8%
Genotype-Phenotype Correlations in cblC Disease

- 57 different mutations are found in cblC patients (Lerner-Ellis, JP et al.)
- Of common mutations, c.271dupA and c331C>T are associated with early onset disease, whereas c.394C>T is associated with later onset disease.
Homocysteine and Heart Disease

COMBINED HOMOCYSTINURIA AND METHYLMALONIC ACIDURIA

cblC
Cardiopulmonary:
- congenital heart disease: VSD, pulmonary stenosis, dysplastic pulmonary valve, atrial defects, mitral valve prolapse
- cardiomyopathy and left ventricular non compaction (4 cases)
- bronchiolitis-like symptoms ad cor pulmonale
Neurological signs

Table 1 Clinical and neuroradiological signs in Cbl-C defect according to the age of disease onset

<table>
<thead>
<tr>
<th>Neurological signs</th>
<th>Early onset</th>
<th>Late onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotonia</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Developmental delay/mental retardation</td>
<td>++</td>
<td>+/-</td>
</tr>
<tr>
<td>Seizures</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Psychiatric signs</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Spasticity</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Myelopathy</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Gait abnormalities</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Brain MRI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>White matter alterations</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Brain atrophy</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Basal ganglia lesions</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Martinelli, 2010
# Neurological signs

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at MRI</strong></td>
<td>1 m</td>
<td>4 m</td>
<td>2 m</td>
<td>1 m</td>
<td>24 m</td>
<td>13 m</td>
<td>11 m</td>
</tr>
<tr>
<td>Ventricular system</td>
<td>tetraventricular hydrocephalus</td>
<td>tetraventricular hydrocephalus</td>
<td>tetraventricular hydrocephalus</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Supratentorial WM</td>
<td>diffuse swelling/ hyperintensity “U” fibres involved</td>
<td>diffuse swelling/ hyperintensity “U” fibres involved</td>
<td>diffuse swelling/ hyperintensity “U” fibres involved</td>
<td>–</td>
<td>–</td>
<td>mild periventricular hyperintensity</td>
<td>–</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>markedly thin</td>
<td>markedly thin</td>
<td>markedly thin</td>
<td>–</td>
<td>–</td>
<td>mild splenium thinning</td>
<td>–</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>–</td>
<td>–</td>
<td>multiple patchy cavitating lesions</td>
<td>small lesion in left caudate</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Other finding</td>
<td>subependymal haemorrhage</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>pons myelinolysis</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$^1$H-MRS (age)</td>
<td>(17 m) lactate in periventricular WM</td>
<td>n.p.</td>
<td>(7 m) lactate in BG</td>
<td>(28 m) lactate in BG</td>
<td>n.p.</td>
<td>(4.3 y) normal</td>
<td>(4.4 y) normal</td>
</tr>
<tr>
<td>MRI follow-up (age)</td>
<td>(17 m) ↓ hydrocephalus, severe WM volume loss</td>
<td>(24 m) ↓ hydrocephalus, mild WM volume loss, mild periventricular WM hyperintensity, (5 y) periventricular leukodystrophic pattern</td>
<td>(7 m) ↓ hydrocephalus, mild WM volume loss, mild periventricular WM hyperintensity, ↓ BG lesions</td>
<td>(28 m) new cavitating lesion in right putamen</td>
<td>(10 y) normal; (13 y) normal</td>
<td>(4.3 y) stable</td>
<td>(4.4 y) normal</td>
</tr>
</tbody>
</table>

WM = white matter; BG = basal ganglia; n.p. = not performed

Longo et al., 2005
Ocular signs

Early onset:
- nystagmus and visual impairment (++);
- optic atrophy and pigmentary retinopathy (+)

Late onset:
- visual impairment and pigmentary retinopathy (+/-)
- ERG: early--lower limits for scotopic and photopic responses; progress to attenuated or nonrecordable

Schimmel et al., 2006
Ocular signs

• Progression of maculopathy despite treatment

Etiology:
• ?RPE is protected against oxidative injury by reduced glutathione (GSH)
• GSH would need “S “chain from methionine (Schimmel, 2006)
• GSH- receiver of alkyl group from MMACHC (Hannibal, 2009)

Fulton, 2005
Atypical Hemolytic –Uremic syndrome

- Triad: azotemia, thrombocytopenia and microangiopathic hemolytic anemia
- 11 cases reported
  Baumgartner, 1979; Geraghty, 1992; Russo, 1992; Chenel, 1993; Kind, 2002; Van Hove, 2002; Andres, 2006; Sharma, 2007
    → 7 with the infantile form;
    5 deceased
    → 4 with the late onset form – no renal sequelae
- No dialysis necessary; high dose OH-Cbl
$cbID$

- 20 patients

- Three variants:
  - Combined, classical: HCY + MMA
  - Variant 1: HCY
  - Variant 2: MMA
cblD

- Variable presentations
- Clinical Findings Include:
  - Developmental delay, seizures, poor feeding, megaloblastic anemia, respiratory infections
  - 1 marfanoid appearance, 1 autistic features, 1 progressive 4-ventricle hydrocephalus, 1 ketoacidotic coma
- 1 patient died, 1 patients never developed symptoms, most mild mental retardation
Hyperhomocysteinemia

With high methionine:
  *CBS* – very high tHcy levels

With low or methionine within reference range:
  *MTHFR*: No megaloblastic anemia
  *MTR*
  *MTRR*
  *MMADHC-cblD-variant 1*

With low or methionine within reference range and MMA:
  *MMACHC*
  *MMADHC-cblD-classical*
  *LMBRD1*
  *cblF*-like (new disease)

Defects in cobalamin intake or absorption (Genetic and acquired)
Homocysteine Diagnostic Flowchart

- **Severe**
  - tHcy > 100 µmol/L
- **Moderate - high**
  - tHcy 50 - 100
- **Moderate - low**
  - tHcy 16 - 50
- **Normal range**
  - tHcy < 15

**Plasma Methionine**

- **Low-Normal**
- **High**
  - Most probably: CBS deficiency
  - Test urinary MMA excretion for further differentiation

- **Grey area**
  - Most probably: Remethylation defects
  - Most probably: Variants of MTHFR, iatrogenic causes

*tHcy = plasma total homocysteine µmol/L*
Methylmalonic Acidemia

- Is the MMA isolated? Is tHcy elevated?
- Low serum cobalamin levels should lead one to expect a disorder of intake or transport: Breast-fed infant of vegan mother or mother with subclinical PA
- Imersund-Grasbeck (MGA1)-mutations in cublin or amnionless (Stephan Tanner-Ohio)
- Combined MMA and Homocystinuria (cblC, cblD, cblF)
Genes Associated with Isolated MMA

- MUT
- MMAA
- MMAB
- MMADHC
- MCEE-may not be related to clinical
- SUCLA2-developmental delay
- SUCLG1-fatal infantile lactic acidosis
MUT
mut MMA

• At least 178 different mutations
• Difficult to make genotype/phenotype correlations. Many patients are compound heterozygotes and different patients homozygous for the same mutation may have different phenotypes
• There are a number of mutations that are more common in specific ethnic groups and a number of common mutations.
Missense Mutations

Nonsense Mutations

Deletions and insertions

Splice Mutations

• seen in more than one patient
• seen in only one family
Cobalamin-responsive MMA

• Two genes cloned on the basis of homology:

• MMAA: \textit{cblA} complementation group

• MMAB: \textit{cblB} complementation group
<table>
<thead>
<tr>
<th>Finding at Clinical Onset</th>
<th>Mutant Class</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$cblA$</td>
<td>$cblB$</td>
</tr>
<tr>
<td>Normal serum cobalamin</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>Ketonemia and/or ketonuria</td>
<td>78</td>
<td>67</td>
</tr>
<tr>
<td>Hyperammonemia</td>
<td>50</td>
<td>83</td>
</tr>
<tr>
<td>Hyperglycinemia and/or glycinuria</td>
<td>70</td>
<td>83</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>70</td>
<td>45</td>
</tr>
<tr>
<td>Anemia</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>75</td>
<td>45</td>
</tr>
</tbody>
</table>

*Numerical values are the percentages of the patients in each group in whom the particular finding was made.

Source: From Matsui et al.⁴⁵¹
MMAA
MMAA

- At least 29 mutations known

- C.433C>T accounts for 43% alleles in one North American Study

- c503delC more frequent in Japan (8 of 14 mutant alleles)
MMAB
**MMAB Mutations**

- 25 mutations Identified
- Most predicted to affect the active site of the enzyme, identified from the crystal structure of its bacterial ortholog
- C.556C>T (p.R186W) represents 33% of affected alleles.
**MMADHC-cbID** variant = *cblH*

- Associated with isolated MMA
- Decreased propionate incorporation
- Decreased AdoCbl synthesis
- Identical to *cblH*
- Mutations in N-terminal regions associated with isolated MMA
Biochemical diagnostic-MMA
Lessons from Expanded Newborn Screening for MMA

- Identification of vitamin $\text{B}_{12}$ in the MOTHER on the basis of elevated MMA in the baby (subclinical pernicious anemia) (Marble et al. J. Pediatr. 152:731-733, 2008)

- Identification of an inborn error in the MOTHER on the basis of elevated MMA in the baby ($cblC$-ASYMPTOMATIC) (Lin et al. J.Pediatr. 155:924-927, 2009)

- Identification of a new inborn error of vitamin $\text{B}_{12}$ transport on the basis of a positive newborn screen. (Mutation in the gene for the TC Receptor: Quadros et al. Blood. 113:186-192, 2009)
INTRACELLULAR METABOLISM
OF VITAMIN B$_{12}$