Inborn errors of metabolism: Application of metabolomic tools in genetic diseases

Damayanti Rusli Sjarif
*Div Pediatric Nutrition and Metabolic Diseases - Dept of Pediatrics FKUI-RSCM
** Human Genetic Research Cluster IMERI
Jakarta - INDONESIA

What is metabolism?
From the Greek word metabole for "change". Sum of all the chemical reactions that convert nutrients (fats, carbohydrates and proteins we eat as food) into energy and complex molecules required for living systems, by enzymatic reactions helped by minerals and vitamins.

Omic based strategies in precision medicine (Int. J. Mol. Sci. 2016, 17, 1555)

What is metabolism?
From the Greek word metabolos for "change".
Sum of all the chemical reactions that convert nutrients (fats, carbohydrates and proteins we eat as food) into energy and complex molecules required for living systems, by enzymatic reactions helped by minerals and vitamins.
Inborn Errors of Metabolism

*a genetic disease also known as biochemical genetics*

**Gene-level**
- Gene mutation
  - Abnormal protein

**Protein-level**
- Abnormal protein
  - Enzyme
  - Transport protein
  - Other protein

**Metabolic-level**
- Abnormal metabolites

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**History**

1902: The concept of a single enzyme deficiency resulting in lifelong disease was recognized first by Sir Archibald Garrod, when he described alkaptonuria.

1908: Sir Archibald Garrod firstly coined the expression *inborn error of metabolism (IEM)* to describe a group of disorders (alkaptonuria, benign pentosuria, albinism, & cystinuria).

- Caused by point defect in metabolism
- Life long condition
- Not significantly affected by treatment
- Transmitted by recessive trait by Mendel’s law of inheritance
- Relatively benign

**Alkaptonuria**

- Urine dark brown
- Ocular signs
- Osteoarthritis
- arterial disease
History (con’t)

- **1934**: Felling discovered PKU
  - associated with a severe form of mental retardation
  - could be prevented by dietary treatment


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**THE DISCOVERY OF PKU
The First Family**

- A-D. Borgny and Harry Egeland, with their 2 children approximately 2 years before the diagnosis of PKU in 1934.
- These parents were intelligent and educated, and the children were attractive but severely retarded and irritable with destructive behavior

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**Fohling Test**

- FeCl₂ 10% + acidified urine of PKU patients
- dark green color appeared, faded within a few minutes
- This did not occur in the urine samples from normal persons
Metabolic pathway of phenylalanine

PKU \Rightarrow mental retardation

PKU \Rightarrow mental retardation (1953)

IQ untreated PKU
- IQ > 70 \Rightarrow 1%
- IQ < 40 \Rightarrow 85%
- IQ < 10 \Rightarrow 37%

Figure 1. Phenylalanine competes with other L-amino acids for the same carrier to pass the blood-brain barrier. High phenylalanine levels, as seen in PKU patients, reduce the brain uptake of other L-amino acids and their availability in the brain.

A-C, FLAIR (A), trace diffusion-weighted (B), and ADCav (C) images show extensive white matter abnormalities with restricted diffusion.
Low Phenylalanine Food Pattern

- 1953—Horst Bickel Publishes in the medical journal Lancet the results of dietary therapy and formula treatment developed by himself, Evelyn Hickmans, John Gerrad, and Louis Woolf

The diaper test to screen young infants under 2 months of age for PKU (designed by Willard R. Centerwall)

- A. Adding a drop of pale, clear-yellow 10% aqueous ferric chloride solution to a wet diaper within a few hours of voiding, the immediate appearance of a green spot, which begins to fade in less than a minute suggests possible PKU.
- B. The ferric salt impregnated Phenistix dipstick pressed against a recently wet diaper (or dipped in fresh urine) was infrequently used if the ferric chloride solution was not available.

The heel-prick test (Guthrie 1961)

Contrast untreated and treated phenylketonurics

The 11-year-old boy is severely retarded. His 21/2-year-old sister, diagnosed in early infancy and promptly treated with the mind-saving diet, is normal.
### Integration of Low Protein Local Food Products

<table>
<thead>
<tr>
<th></th>
<th>Breakfast</th>
<th>Dinner</th>
<th>Lunch</th>
<th>Snack</th>
<th>Phe-free milk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uduk rice</strong></td>
<td>45g, sweet potato, lettuce, pumpkin, carrot, tomato</td>
<td>Rice 45 g, sago porridge, carrot, tomato</td>
<td>Rice 45 g, vegetables, bakwan, pak, lettuce</td>
<td>Getuk, honey</td>
<td>600 mL/day</td>
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<tr>
<td><strong>Kuning rice</strong></td>
<td>45g, carrot, lettuce</td>
<td>Low protein spaghetti 40g, tomato, mushroom, carrot, onion</td>
<td>Rice 45 g, sweet potato, lettuce</td>
<td>Cassava &amp; furikake, nesquick</td>
<td>600 mL/hari</td>
</tr>
</tbody>
</table>

First PKU patient in Indonesia

Substrates reduction ⇒
Free Phenylalanine Foods for PKU
Co-factor supplementation

Aromatic Amino Acid
Hydroxylases and Function
of Tetrahydrobipterin

Orphan Drugs

Gene therapy

Indonesian PTPS Patients
History (con’t)

- 1934: Følling discovered PKU – associated with a severe form of mental retardation – could be prevented by dietary treatment

Incidence of IEM

- IEM are individually rare (less than 1 per 100,000 births). However, when considered collectively, the incidence may approach 1 in 1000 births
- ±20% of infants presenting with a "sepsis" picture in the absence of risk factors (such as prematurity, chorioamnionitis, etc.) have an IEM.
Pathogenesis of clinical manifestations

- **Intoxication**
  - Fatty acids
    - Ammonia, lactate
    - Uric acid
  - Energy deficiency
    - Hypoglycemia
    - Less reducing equivalent to OXPHOS
    - No ketone bodies to extrahepatic tissues

**Clinical manifestations**

<table>
<thead>
<tr>
<th>Normal</th>
<th>Intoxication</th>
<th>Energy deficiency</th>
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<tbody>
<tr>
<td>A</td>
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**Initial laboratory investigation**

- **Blood**
  - Hematology
  - Blood gases & electrolytes
  - Glucose, lactate, pyruvate, ketone bodies
  - Ammonia, AST, ALT, CK, ALP, LDH, TG, Cholesterol
  - Creatinin, urea, uric acid
  - Ferritin
- **Urine**
  - Color and odor
  - Reducing substance
  - FeCl3 tests
  - DNPH and Acetest
  - Nitroprusside test
- **LCS**
- **BMP**
- **X-ray, USG, Echocardiography, ECG**

**TABLE 1. Clinical Symptomatology of Inborn Errors of Metabolism (IEM) in the Neonate or Infant**

- Symptoms indicating possibility of an IEM (one or all)
  - Infant becomes acutely ill after period of normal behavior and feeding; this may occur within hours or weeks
  - Neonate or infant with seizures and/or hypotonia, especially if seizures are intractable
  - Neonate or infant with an unusual odor
  - Symptoms indicating strong probability of an IEM, particularly when coupled with the above symptoms
  - Persistent or recurrent vomiting
  - Failure to thrive (failure to gain weight or weight los)
  - Apnea or respiratory distress (tachypnea)
  - Jaundice or hepatomegaly
  - Lethargy
  - Coma (particularly intermittent)
  - Unexplained hemorrhage
  - Family history of neonatal deaths, or of similar illness, especially in siblings
  - Parental consanguinity
  - Sepsis (particularly Escherichia coli)

**Inborn Errors of Metabolism and Associated Laboratory Findings**

- Abnormal liver function tests (e.g., elevated transaminase or hyperbilirubinemia (jaundice))
- Hemochromatosis (1:300)
- Inherited fructose intolerance (1:20,000 to 1:50,000)
- Mitochondrial disorders (1:30,000), e.g., mitochondrial EMG (spidled waves)
- Galactosemia (1:40,000)
- Wilson's disease (1:50,000)
- Gaucher's disease (1:60,000, type I and IV in Ashkenazi Jews)
- Hypophosphatemia (1:80,000)
- Chondrodystrophy (atrophy disease (new disease)
- Glycogen storage disease, type IV (new)
- Marfan-Weill disease, types A and B (both new)
- Type 1 tyrosinaemia (new)
- Winkle's disease (new)
- Hyperuricemia
- Carbohydrate metabolism disorders (1:10,000)
- Fatigue and weakness (1:10,000)
- Hereditary fructose intolerance (1:20,000 to 1:50,000)
- Glycogen storage diseases (1:25,000)
- Galactosemia (1:40,000)
- Organic acidemia (1:50,000)
- Phosphoenolpyruvate carboxykinase deficiency (new)
- Primary hypercholesterolaemia (new)
- Hyperphosphataemia
- Lesch-Nyhan syndrome (1:1000, e.g., neurotOSP)
- X-linked hypophosphatemic rickets (1:20,000)
- Hypophosphatemia
- Lesch-Nyhan syndrome (1:1000, e.g., neurotOSP)
- X-linked disease (1:4,000)
- Malignant hyperthermia deficiency (new)
- Paroxysmal nocturnal hemoglobinuria (new)
- Increased CSF protein
- Mitochondrial disorders (1:20,000, e.g., MELAS syndrome (new), NUBD syndrome, X-linked hypophosphatemic rickets (new)
- Peroxisomal disorders (1:50,000), e.g., Zellweger syndrome, neonatal adrenoleukodystrophy, Refsum's disease
- Leukodystrophy (e.g., Krabbe's disease, metachromatic leukodystrophy (1:20,000), multiple sulfatase deficiency (new)
- Z-synuclein (new)
- Congenital disorders of glycosylation (new)
- Kollins
- Ammonia-ureas (1:40,000)
- Organic acidemia (1:75,000)
- Mitochondrial acidosis
- Ammonia-ureas (1:40,000)
- Organic acidemia (1:75,000)
- Primary hyperparathyroidism (new)
- Renal tubular acidosis (new)
Metabolic investigation

- Screening
  - Amino acid analysis
  - Organic acid analysis
  - Urinary mucopolysaccharide & oligosaccharide
  - Plasma VLCFA & phytanic acid, plasmalagens, pipericolic acids
  - Urine Purines & Pyrimidines

- Specific
  - Function tests
  - Enzyme measurements
    - Blood
    - Fibroblasts (skin biopsy)
    - Liver, muscle
  - Mutation analysis

Paradigm shift in Inborn Errors of Metabolism (IEM) diagnosis workflow.

Metabolomics: study of small molecules

Metabolomic Tools
An illustration of the relationship between sensitivity (or lower detection limit in concentration units) and the number of metabolites detected (using a Log10 scale) via different metabolomics technologies.

Chromatogram of PTC Amino Acid 18 Components

Table 6: Significant Metabolites in Samples from Patients with Inborn Errors of Metabolism, As Candidate Biomarkers For Diagnosis After Biochemical Analysis

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Genetic counselling in IEM

- IEM are hereditary in nature → the family should have formal genetic counseling
- Education for patient & family to
  - understand the disease → Prognosis
  - Mode of inheritance → Recurrence risk
  - Early diagnosis & prompt treatment → newborn screening
  - Recent development of treatment
  - Family planning → Prenatal diagnosis

Principles of treatment

- Symptomatic and supportive therapy
- Provision of C
- Limit intake and production of B (and A)
- Enhance Enzyme activity
  - Replacement
  - Stabilization
  - Gene transfer
- Activation of alternative metabolic pathways (D)

Principle of newborn screening

- Specific reason for genetic screening should be clearly define, for medical intervention, reproductive planning, or research
- Screening for medical intervention should be carried out as a part of an integrated program with the facilities, resource and personnel to provide
- High risk individuals should be detectable by a simple, inexpensive test with high sensitivity, specificity, and predictive efficiency

Table 1. History of the neonatal screening

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>1933</td>
<td>First successful treatment of phenylketonuria (H. Bickel)</td>
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<tr>
<td>1961</td>
<td>Introduction of &quot;Fölling/Windeltest&quot; in Germany (H. Bickel)</td>
</tr>
<tr>
<td>1961</td>
<td>Development of microbiological inhibition assay (&quot;Guthrie Test&quot;) for screening for phenylketonuria (R. Guthrie)</td>
</tr>
<tr>
<td>Early 1970's</td>
<td>Extension of neonatal screening for galactosemia, maple syrup urine disease, homocystinuria</td>
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<tr>
<td>1978</td>
<td>Congenital hypothyroidism</td>
</tr>
<tr>
<td>1987</td>
<td>Biotinidase deficiency</td>
</tr>
<tr>
<td>1994</td>
<td>Switch from &quot;Guthrie Test&quot; to enzymatic methods (Heidelberg)</td>
</tr>
<tr>
<td>1997</td>
<td>Congenital adrenal hypoplasia (CAH)</td>
</tr>
<tr>
<td>1998</td>
<td>Introduction of ESI-MS/MS → beginning of Extended Neonatal Screening (Heidelberg)</td>
</tr>
</tbody>
</table>

*ESI = electrospray ionization; MS/MS = tandem mass spectrometry*
Newborn screening

Tandem mass spectrometry

• Tandem mass spectrometer ⇒ computer-controlled device that separates & quantities compounds based on their mass to charge ratio.
• From a single blood spot sample on filter paper ⇒ can detect genetic disorders of amino acids, organic acids, and fatty acids

Is Inborn Error of Metabolism exists in Indonesia?

GSD, Homocystinuria, MPS II, MPS IV, FH, Gaucher, etc

PTPS deficiency, GSD, X-ALD, etc.

PTPS deficiency, GSD, X-ALD, etc.

LSDs: MPS IUIV, VUX, NCL II, MPS IIIP, Gaucher, CPT 1 deficiency, Primary Orotate deficiencies PKU, PTPS def, MSUD, MMA, PCD, PDH, MELAS, IVA, FH, X-ALD, Crigler-Najjar, Rotor, Neonatal Hemachromatosis, UCD, etc
IEMs diagnosed by clinical judgment & metabolomic approach

- 3 days old newborn girl, 3\textsuperscript{rd} child of consanguineous parents, uneventful pregnancy, born full term SC, BW 3000 g, Apgar Score 9/10 \rightarrow \geq 24 hours: seizures & coma
- History:
  - 1\textsuperscript{st} baby boy \rightarrow \dagger 3 days
  - 2\textsuperscript{nd} baby boy \rightarrow recurrent coma \rightarrow \dagger 8 month
- 1\textsuperscript{st} level metabolic screening
  - metabolic acidosis & anion gap ↑,
  - hyperammonemia,
  - Ketonuria (+)

Errors of Metabolism with Hyperammonemia in Newborns

<table>
<thead>
<tr>
<th>Metaboloma</th>
<th><a href="http://www.metaboloma.com">www.metaboloma.com</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase (AL)</td>
<td>Argininosuccinic acid lyase deficiency (ASL)</td>
</tr>
<tr>
<td>Argininosuccinic acid synthetase deficiency (AS)</td>
<td>Carboxylase phosphatase synthetase deficiency (CPS)</td>
</tr>
<tr>
<td>Hyperargininemia-hyperammonemia-homocitrullinuria syndrome (HHG)</td>
<td>Lysine urea cycle defects (LUD)</td>
</tr>
<tr>
<td>Ornithine transcarbamylase deficiency (OTC)</td>
<td>N-Acetylglutamate synthase deficiency (NAGS)</td>
</tr>
<tr>
<td>Fatty acid oxidation defects</td>
<td>Carnitine palmitoyltransferase II deficiency (CPT-II)</td>
</tr>
<tr>
<td>Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCADD)</td>
<td>Very long chain acyl-CoA dehydrogenase deficiency (VLCADD)</td>
</tr>
</tbody>
</table>

Organic acidemia defects
- 3-Hydroxy-3-methylglutaryl-CoA (HMG) lyase deficiency
- Beta-oxalylacetic aciduria (BOA)
- Isocitrate lyase deficiency (IC)
- Multiple acyl-CoA dehydrogenase deficiency (MADD)
- Propionic acidemia (PA)

Clinical diagnosis: organic acidemia (MMA ?)
Management MMA

- **Special formula**
  - Free isoleucine, valine, methionine, threonine
- **Genetic counselling**
  - Prenatal diagnosis or Immediate Newborn screening for the Next Pregnancy!

Prenatal diagnosis

The First Enzyme Replacement Therapy in Indonesia
January 2015
Diagnosed MPS IV at 2.5 years - ERT at 13.5 years
Conclusions

- Inborn errors of metabolism (IEMs) individually are rare but collectively are common.
- Presentation can occur at any time, even in adulthood.
- Extensive knowledge of biochemical pathways help to understand the pathogenesis of clinical manifestations and provides the basis for knowing when to consider the diagnosis.
- A high index of suspicion is most important in making the diagnosis.
- Metabolomic approach help to diagnose and follow up treatment adequacy.
- Successful emergency treatment depends on prompt institution of therapy aimed at metabolic stabilization.