2nd ACIMD/12th AEWIEM/12th KCIMD

JOINT MEETING

The 2nd Asian Congress for Inherited Metabolic Diseases &
The 12th Asian-European Workshop on Inborn Errors of Metabolism &
The 12th Korean Congress of Inherited Metabolic Disease

Lotte Hotel, Seoul, Korea
April 1st-4th 2012
Post Congress Tour
April 4th–6th
Gyeongju

“Healthier People, Healthier Asia, Healthier World
Through The Development of IEM”

Hosted by
Korean Society of Inherited Metabolic Disease
Supported by
Korean Pediatric Society
Korean Academy of Medical Science
Korean Academy of Medical Genetics
Korean Tourism Organization/Seoul Convention & Visitors Bureau

ISSN
Journal of The Korean Society of Inherited Metabolic Disease
Vol. 12 Suppl. 1
The 2nd Asian Congress for Inherited Metabolic Diseases &
The 12th Asian-European Workshop on Inborn Errors of Metabolism
& The 12th Korean Congress of Inherited Metabolic Disease

PROGRAM AND ABSTRACTS

Date:
April 1(Sun)-4(Wed), 2012

Venue:
Lotte Hotel Seoul
1 Sogong-Dong, Jung-Gu, Seoul, 100-070, Korea
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Invitation

Dear Colleagues and Friends,

We are pleased to invite you to the joint meeting of the 2nd Asian congress for Inherited Metabolic disease and the 12th Asian-European Workshop on Inborn Errors of Metabolism which will be held from April 1st-4th 2012 at Lotte Hotel in Seoul, Korea. Scientific sessions will be followed by a three day tour to Gyeongju, the capital of the Silla Kingdom (BC 57-AD 935). The post congress tour is the most amiable characteristic of AEWIEM. In addition to excellent academic programs, the tour will promote intellectual interactions and renewal of friendships, further encouraging the development of collaborative projects for our region. Seoul is a wonderful city where tradition and modernity co-exist in perfect harmony and spring is the most beautiful season in Korea. The exciting 2nd Asian Congress for Inherited Metabolic Diseases and the 12th Asian-European Workshop on Inborn Errors of Metabolism will offer you a beautiful experience long to be remembered.

We hope to welcome all of you in April this year in Seoul.

Dong Hwan Lee
Chairperson of ACIMD 2012, Seoul

Yoon Sook Shin
President of AEWIEM
Welcome Message

Fumio Endo (ASIMD President)

Dear Colleagues,

On behalf of the Asian Society for Inherited Metabolic Disorders (ASIMD) may we extend a warm invitation to the second ACIMD 2012 (Asian Congress for Inborn Error of Metabolism), which will be held in Lotte Hotel, Seoul, Korea, from April 1st to 4th, 2012. This meeting will be held as a joint meeting of the 2nd Asian Congress for Inherited Metabolic Diseases & the 12th Asian-European Workshop on Inborn Errors of Metabolism.

I am sure it will be a very fruitful meeting and we also expect many participants from European countries in addition to the Asia area. I would like to congratulate Prof. Dong Hwan Lee, Chairman of ACIMD 2012 Seoul. Prof. Lee and I had worked together to establish the Asian Society for Inherited Metabolic Disorders and had a wonderful meeting in Fukuoka, Japan in 2010. This year Prof. Lee together with Prof. Yoon Sook Shin, President of AEWIEM, has prepared a very exciting meeting. The scientific program looks stimulating, offering: opening sessions and plenary sessions dedicated both to the main topic of this year, meeting interactions and regulation in IEM and to other latest topics.

We are delighted to support the ACIMD 2012 and very much look forward to welcoming colleagues to Seoul.

Fumio Endo
President of ASIMD
President of JSIMD
General Information

Date
April 1st-4th, 2012
Post Congress Tour April 4th-6th Gyeongju

Venue
Lotte Hotel Seoul
1 Sogong-Dong, Jung-Gu, Seoul, 100-070, Korea
Phone: +82-2-771-1000 / Fax: +82-2-752-8602
reservation@hotellotte.co.kr
http://www.lottehotel.com

Registration/Information Desk:
The registration/information desk is located on the 3th floor of Lotte Hotel Seoul

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
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<tr>
<td>April 1st (Sun)</td>
<td>15:00-19:00</td>
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<tr>
<td>April 2nd (Mon)</td>
<td>08:00-18:00</td>
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<tr>
<td>April 3rd (Tue)</td>
<td>08:00-18:00</td>
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<td>April 4th (Wed)</td>
<td>08:00-12:00</td>
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Registration Fee:
Member: USD 300
Non- Member: USD 320
Trainee/Resident/Nurse: USD 200
Accompanying Person: USD 200

Registration fees entitle the participants to: All Scientific Sessions, welcome reception on April 1st, lunch in 2nd - 4th and dinner in 3rd - 4th, Coffee Breaks and Abstract Book.

Abstract Book: USD 20
Official Language: English

Cell Phone/Camera/Video Policy:

Attendees are asked to be respectful of their colleagues by turning off all cell phones and PDA devices before entering meeting rooms. Cameras and all other recording devices are STRICTLY PROHIBITED in all session rooms, on the exhibit floor, and in all poster/oral presentations unless pre-approved as an authorized vendor by organizing committee. Thank you for your cooperation.

Exhibition:

Industrial exhibition will be held during the meeting period in the Lobby on the 3th floor. Anyone will be welcome to visit and see the exciting exhibition at anytime.

Smoking:

Smoking is NOT allowed inside the building except smoking rooms in hotel.

Parking:

You can park on parking spaces in basement floor of Lotte Hotel Seoul. You should receive free ticket in registration desk.

Instructions for Oral Presentation:

The computers supplied at the Congress are Window (OS: Windows XP or Vista).

Applications for presentation can be run on Windows only, so speakers are requested to prepare their presentations by Microsoft PowerPoint XP, 2003 or 2007. Macintosh will not be available. (Macintosh data may not be converted correctly.) Please ask for further instructions at the PC Preview Section.

Instructions for Poster Presentation:
Venue: Lotte Hotel Seoul

3rd floor

Access and Transportation
Accommodations

A. Lotte Hotel Seoul
B. President Hotel
C. IBIS Myeong Dong
D. Best Western, New Seoul Hotel
E. Ulji-Ro, Co-Op Residence
F. Western, Co-Op Residence

Day 1
Sunday, April 1st 2012

Day 2
Monday, April 2nd 2012

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>9:10 AM</td>
<td>Opening Ceremony</td>
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<tr>
<td>9:30 AM</td>
<td>Plenary Lecture I Sapphire Ballroom (3rd fl)</td>
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<tr>
<td>10:30 AM</td>
<td>Coffee Break</td>
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<tr>
<th>Location</th>
<th>Travel Options</th>
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<tr>
<td>Incheon International Airport</td>
<td>Bus, Metro: 30min, Taxi: 30min</td>
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<td>Kimpo International Airport</td>
<td>Bus(6001), Metro: 50min, Taxi: 40min</td>
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<td>Lotte Hotel, Seoul</td>
<td>Bus(6701): 60min</td>
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<tr>
<td>Time</td>
<td>Event</td>
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<tr>
<td>11:00 AM</td>
<td>Symposium I “IMD in Asia”</td>
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<tr>
<td>12:30 PM</td>
<td>Luncheon Symposium I</td>
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<tr>
<td>13:30 PM</td>
<td>Oral Session I</td>
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<tr>
<td>14:30 PM</td>
<td>Coffee Break</td>
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<tr>
<td>15:00 PM</td>
<td>Symposium II “Expanded Screening”</td>
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<tr>
<td>18:30 PM</td>
<td>Welcome reception</td>
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<td>21:00 PM</td>
<td>17:00 PM</td>
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**Day 3**
Tuesday, April 3rd 2012

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<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Details</th>
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<tbody>
<tr>
<td>8:30 AM</td>
<td>Oral Session II (3rd fl)</td>
<td>Chairperson:</td>
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<tr>
<td></td>
<td>Sapphire Ballroom</td>
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<td>O7-O12</td>
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</tbody>
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**Day 4**
Wednesday, April 4th 2012

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<tr>
<th>Time</th>
<th>Event</th>
<th>Details</th>
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<tbody>
<tr>
<td>8:30 AM</td>
<td>Oral Session IV (3rd fl)</td>
<td>Chairpersons: Theodor Podskarby (Germany) &amp; Xiaoping Luo</td>
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<td></td>
<td>Sapphire Ballroom</td>
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<td>O19-O24</td>
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<tr>
<td>Time</td>
<td>Event</td>
<td>Chairpersons/Speakers</td>
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<tr>
<td>9:30 AM</td>
<td>Plenary Lecture II</td>
<td>Chairperson: Fumio Endo (Japan) Speaker: Yoshikatsu Eto (Japan)</td>
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<tr>
<td>10:30 AM</td>
<td>Coffee Break</td>
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</tr>
<tr>
<td>11:00 AM</td>
<td>Symposium III <em>New Treatment of IMD</em></td>
<td>Chairpersons: Hiroyuki Ida (Japan) K. Ohno (Japan) T. Saheki (Japan) Yasuyuki Suzuki (Japan)</td>
</tr>
<tr>
<td>11:00 AM</td>
<td>Symposium V <em>Basics of IMD</em></td>
<td>Chairpersons: Yoon S. Shin (Germany) &amp; ? Speakers: J. Sykut-Cegielska (Poland) H. Bohles (Germany) R. Steinfeld (Germany) Hyun Taek Lim (Korea)</td>
</tr>
<tr>
<td>12:30 PM</td>
<td>Luncheon Symposium II</td>
<td>Chairperson: Han-Wook Yoo (Korea) Speaker: Dong-Kyu Jin (Korea)</td>
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<tr>
<td>13:30 PM</td>
<td>Oral Session III</td>
<td>Chairpersons: Damayanti Rusli Sjarif (Indonesia) &amp; Akemi Tanaka (Japan) O13-O18</td>
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<tr>
<td>14:30 PM</td>
<td>Coffee Break</td>
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<tr>
<td>15:00 PM</td>
<td>Symposium IV <em>Amino acid &amp; Energy Metabolism</em></td>
<td>Chairpersons: Seiji Yamaguchi (Japan) &amp; A.M. Das (Germany) Speakers: Yangling Yang (China) Kwang Jen Hsiao (Taiwan) A.M. Das (Germany) Seiji Yamaguchi (Japan)</td>
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<td>17:00 PM</td>
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**Post Congress Tour**

Gyeongju was the capital of the Silla Kingdom for 1,000 years and the valley in which it is situated has a great concentration of historical buildings, temples and artifacts. After Silla unified the peninsula in 676, the city developed into one of the world’s major cultural centers. The Gyeongju Historical District has been designated as a World Heritage by UNESCO. The area preserves a vast amount of significant and fascinating historical
heritages and is truly a museum without walls.

April 4  14:00  Depart from Lotte Hotel
    15:30  Depart from Seoul by KTX143 express train
    17:38  Arrive at the city of Gyeongju

April 5  Sightseeing of Gyeongju
    Morning: Seokguram Grotto- Bulguksa Temple- Folk Craft Museum (SillaYo: An atelier that reenacts Silla earthenware being made)
    Afternoon: Daereungwon (Grand Tumuli Park)- Cheomseongdae Observatory-Anapji Pond

April 6  Sightseeing of Gyeongju
    Morning: Yangdong Village- Gyeongju National Museum
    15:58  Depart from Gyeongju by KTX144 express train
    18:14  Arrive at Seoul
    19:00  Arrive at Lotte Hotel
DAY1 April 1st 2012 (Sunday)

Welcome Reception

April 1st 2012

18:30-21:00 P.M. Emerald room (2nd fl)

Chairperson: Hong Jin Lee (Chunchon, Korea)
DAY2 April 2nd 2012 (Monday)

Plenary Lecture I

9:30-10:30 A.M. Sapphire Ballroom (3rd fl)
Chairperson: Dong Hwan Lee (Seoul, Korea)

2-PL I. Redefining the diagnostic spectrum of mitochondrial disorders by the clinical application of next generation sequencing technology

Sihoun Hahn (Seattle, USA)

Symposium I “IMD in Asia”

11:00 A.M.-12:30 P.M. Sapphire Ballroom
2-SI-1  **Inborn Errors of Metabolism in Asia**  
Pornswan Wasant (Bangkok, Thailand)

2-SI-2  **IEM in the Philippines : 10 years review**  
Sylvia C Estrada (Philippines)

2-SI-3  **Wilson Disease : from proteome & gene to clinical manifestations**  
Han-Wook Yoo (Seoul, Korea)

2-SI-4  **Leukodystrophies – The Indian Scenerio**  
I.C. Verma (New Delhi, India)

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**Luncheon Symposium I**  
April 2nd 2012

12:30 P.M.-13:00 P.M. Sapphire Ballroom

Chairperson: Sihoun Hahn (Seattle, U.S.A.)

**2-L1.  Research into rare diseases as a new paradigm for drug discovery**

Seng Cheng (USA)

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**Oral Session I**  
April 2nd 2012

13:30 P.M.-14:30 P.M. Sapphire Ballroom

Chairpersons: T Saheki (Kumamoto, Japan)  
Makoto Yshino (Kurume, Japan)
O-1 Classical and Nonclassical Treatment of Nonketotic Hyperglycinemia
Vladimir Bzduch, Behulova D, Kolnikova M, Skoknova M, Skodova J, Fabriciova K, Payerova J (Bratislava, Slovakia)

O-2 Mutations in Genes Encoding the Glycine Cleavage System Predispose to Neural Tube Defects in Mice and Humans
Shigeo Kure, Ayumi Narisawa, Atsuo Kikuchi, Yoko Aoki, Tetsuya Niihori, Teiji Tominaga, Nicholas DE Greene, Andrew J Copp, Yoich Matsubara (Sendai, Japan)

O-3 Long-term Outcome and Intervention of Urea Cycle Disorders in Japan
Kimitoshi Nakamura, Jun Kido, Hiroshi Mitsubuchi, Toshihiro Ohura, Masaki Takayanagi, Masafumi Matsuo, Makoto Yoshino, Yosuke Shigematsu, Tohru Yorifuji, Mureo Kasahara, Reiko Horikawa, Fumio Endo (Kumamoto, Japan)

O-4 Application of Haplotype Analysis Employing SNPs in the Human OTC Locus to the Study of Origin of Mutant Allele
Makoto Yoshino, Naomi Harada, Sanae Numata, Yoriko Watanabe, Jun-ichiro Okada, Yoshiro Koda (Kurume, Japan)

O-5 SLC25A13 Gene Analysis in Citrin-deficient Patients: Experience on an Eighty-nine-case Cohort in a Chinese Pediatric Center
Yuan-Zong Song, Mei Deng, Xin-Jing Zhao, Wei-Xia Lin, Zhan-Hui Zhang (Guangzhou, China)

O-6 Simple and Rapid Genetic Testing for Citrin Deficiency by Screening 11 Prevalent Mutations in SLC25A13
Atsuo Kikuchi, Natsuko Arai-Ichinoi, Osamu Sakamoto, Yoichi Matsubara, Takeyori Saheki, Keiko Kobayashi, Toshihiro Ohura, Shigeo Kure (Sendai, Japan)

Symposium II “Expanded Screening”
April 2nd 2012
15:00 P.M.-17:00 P.M. Sapphire Ballroom
Chairpersons: Teruo Kitagawa (Tokyo, Japan)
Carmencita Padilla (Manila, Philippines)
2-SII-1 Newborn Screening in the Asia Pacific Region
   Carmencita Padilla (Manila, Philippines)

2-SII-2 Expanded Neonatal Screening and Prenatal Diagnosis for IEM in Shanghai
   Xeo Fan Gu (Shanghai, China)

2-SII-3 Newborn Screening of Severe Combined Immunodeficiency Syndrome in Taiwan
   Yin-Hsiu Chien (Taipei, Taiwan)

2-SII-4 Screening for Fabry Disease in Patients Referred to Metabolic or Nephrologic Clinics and Patients Referred to Dialysis Centers
   Teruo Kitagawa (Tokyo, Japan)

2-SII-5 Screening of LSDs & Galactosemia
   Jung Han Song (Seoul, Korea)

2-SII-6 Current Issues Regarding Storage and Use of Residual Dried Blood Spots
   Brad Therrel (USA)

DAY3 April 3rd 2012 (Tuesday)

Oral Session II
   April 3rd 2012
   08:30 A.M.-09:30 A.M. Sapphire Ballroom
   Chairpersons: ()

O-7 High Risk Screening on Inborn Error Metabolism Disorders in National Hospital of Paediatrics in Vietnam
   Hoan Nguyen Thi (Hanoi, Vietnam)

O-8 Phenotype and Genotype of Vietnamese Patients with Maple Syrup Urine Disease

O-9 The Clinical and Molecular Genetic Characteristics of Korean Patients with Fabry Disease
Han-Wook Yoo, Beom Hee Lee, Yoo-mi Kim, Jin Lee, Chang-Woo Jung, Sun Hee Heo, Gu-Hwan Kim, Jin-Ho Choi (Seoul, Korea)

O-10 Plasma Globotriaosylsphingosine (LysoGb3) is a Reliable Biomarker for Cardiac Variant Fabry Disease Causing by Chinese Hotspot Mutation (IVS4 + 919G→A)

Hsuan-Chieh Liao, Yu-Hsiu Huang, Shu-Min Kao, Shao-Tzu Li, Chun-Kai Huang, Chuan-Chi Chiang, Dau-Ming Niu (Taipei, Taiwan)

O-11 Mechanism Of Endoplasmic Reticulum Stress-Independent Autophagic Activation In Pompe Disease Fibroblasts

Yurika Nishiyama, Yohta Shimada, Takayuki Yokoi, Hiroshi Kobayashi, Yoshikatsu Eto, Hiroyuki Ida, Toya Ohashi (Tokyo, Japan)

O-12 Differentiation into Skeletal Muscle Cells from Mouse Pompe-iPS cells and Possible Application for Cell Therapy

Shiho Kawagoe, Takashi Higuchi, MengXing-Li, Yohta Shimada, Hiromi Shimizu, Takahiro Fukuda, Hsi Chang, Tatsutoshi Nakahata (Tokyo, Japan)

Plenary Lecture II

April 3rd 2012
9:30-10:30 A.M. Sapphire Ballroom
Chairperson: Fumio Endo (Kumamoto, Japan)

3-PL-II Novel Treatment Strategies for Genetic Diseases

Yoshikatsu Eto (Tokyo, Japan)

Symposium III “New Treatment of IMD”

April 3rd 2012
11:00 A.M.-12:30 P.M. Sapphire Ballroom
Chairpersons: ()

3-SIII-1 Gaucher disease in Japan

Hiroyuki Ida (Tokyo, Japan)

3-SIII-2 Chemical chaperone therapy for Gaucher disease

K. Ohno (Yonago, Japan)

2-SIII-3 Treatment of Citrin Deficiency: Assessment by Using an Animal Model
T. Saheki (Kumamoto, Japan)

3-SIII-4  Hematopoietic Stem Cell Transplantation for X-linked Adrenoleukodystrophy: Outcome in Japan
Yasuyuki Suzuki (Gifu, Japan)

Luncheon Symposium II
April 3rd 2012
12:30 P.M.-13:00 P.M. Sapphire Ballroom
Chairperson: Han-Wook Yoo (Seoul, Korea)

3-L-II  Phase I/II Clinical Trial of Enzyme Replacement Therapy with GC1111 in Hunter Syndrome
Dong-Kyu Jin (Seoul, Korea)

Oral Session III
April 3rd 2012
13:30 P.M.-14:30 P.M. Sapphire Ballroom
Chairpersons: Damayanti Rusli Sjarif (Jakarta, Indonesia)
Akemi Tanaka (Osaka, Japan)

O-13  Prenatal Counseling and Diagnosis of Gaucher Disease In Egypt
Ahmed Aboulnasr, Ekram Fateen (Cairo Egypt)

O-14  Pseudodeficiency Alleles of Iduronate 2-sulfatase Gene and the Structural Modeling of the Enzyme Protein
Tomo Sawada, Akemi Tanaka, Ken Suzuki, Hitoshi Sakuraba, Seiji Saitou, Tomoko Sakaguchi, Teruo Kitagawa (Osaka, Japan)

O-15  Intraventricular Enzyme Replacement Therapy for MPS II Murine Model
Takashi Higuchi, Hiromi Shimizu, Takahiro Fukuda, Shiho Kawagoe, Juri Matsumoto, Reimi Hirayama, Yohta Shimada, Hiroshi Kobayashi, Hiroyuki Ida, Toya Ohashi, Yoshikatsu Eto (Tokyo, Japan)

O-16  Optimal Clinical Outcome in Early Initiation of Enzyme Replacement Therapy for a Pre-symptomatic Newborn Patient with Mucopolysaccharidosis VI
Torayuki Okuyama, Mahoko Furujo, Toshihide Kubo, Motomichi Kosuga, Kazuhiro Kida (Tokyo, Japan)

O-17  Pulmonary Function Assessment in Patients with Mucopolysaccharidoses: Experience in Taiwan
O-18 Lentiviral Vector Mediated Neonatal Gene Therapy of Krabbe Disease Model Mice
Hiroshi Kobayashi, Yota Shimada, Sayoko Izuka, Takashi Higuchi, Masamichi Ariga, Takeo Iwamoto, Takahiro Fukuda, Hiroyuki Ida, Yoshikatsu Eto, Toya Ohashi (Tokyo, Japan)

Symposium IV “Amino Acid & Energy Metabolism”
April 3rd
15:00 P.M.-17:00 P.M. Sapphire Ballroom
Chairpersons: ()

3-SIV-2 Phenylketonuria
M. Demirkol (Istanbul, Turkey)

3-SIV-3 The Treatment and Outcome of Organic Aciduria
Yangling Yang (China)

3-SIV-4 Mutation Profile of MMA in Chinese Populations
Kwang Jen Hsiao (Taiwan)

3-SIV-5 Inborn Errors of Metabolism and the Heart
A.M. Das (Hannover, Germany)

3-SIV-6 Current Topics in Diagnosis and Treatment of Mitochondrial Fatty Acid Oxidation Disorders
Seiji Yamaguchi (Shimane, Japan)
Oral Session IV

April 4th 2012

08:30 A.M.-09:30 A.M. Sapphire Ballroom

Chairpersons: Theodor Podskarby (Munich, Germany)

O-19 Laboratory Diagnosis of Glycogen Storage Disease in Egypt: Ten Years Experience
Ekram Fateen, Amr Gouda, Mona Mahmoud, Theodor Podskarby, Yoon Shin
(Cairo, Egypt)

O-20 Clinical and Molecular Aspects of Japanese Children with Medium Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency
Jamiyan Purevsuren, Yuichi Mushimoto, Hironori Kobayashi, Yuki Hasegawa, Kenji Yamada, Tomoo Takahashi, Seiji Yamaguchi (Izumo, Japan)

O-21 Clinical and Molecular Analysis of Chinese Patients with Primary Carnitine Deficiency
Lianshu Han (Shanghai, China)

O-22 Diagnosis and Molecular Basis of Mitochondrial Respiratory Chain Disorders in Japan: the Experiment of Systematic Analysis for Causative Genes
Kei Murayama, Emi Kawachi, Tomoko Tsuruoka, Masato Mori, Taro Yamazaki, Yasushi Okazaki, Masaki Takayanagi, Akira Ohtake (Chiba, Japan)

O-23 Smith-Lemli-Opitz Syndrome: Complex Analysis of 15 Patients from Slovakia
Martina Skoknova, Vladimir Bzduch1, Darina Behulova (Bratislava, Slovakia)

Plenary Lecture III

4-PL-III  Current Aspects on Glycogen Storage Disorders
Yoon S. Shin (Munich, Germany)

Symposium V  “Basics of IMD”

4-SV-1  Intoxication Type Inborn Errors of Metabolism
J. Sykut-Cegielska (Warsaw, Poland)

4-SV-2  The Interaction of Urea Synthesis and Acid Base Metabolism
H. Böhles (Frankfurt, Germany)

4-SV-3  Disorders of Folate Transport
R. Steinfeld (Goettingen, Germany)

4-SV-4  Ocular Manifestations of IMDs
Hyun Taek Lim (Seoul, Korea)
POSTER SESSION

P-01 Application of Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in Screening of Newborn in the NICU and High Risk Children with Inherited Metabolic Disease in and around Beijing
Wanqiao Zhang, Haihong Liu, Hongqing Chen, Lei Yan, Xiyu He (Beijing, China)

P-02 Outcome of Newborn Screening for Inherited Metabolic Disorders – Report of 11-years by Korea Genetics Research Center
Sook Za Kim, Wung Ju Song (Cheong Ju, Korea)

P-03 Quality Assurance Program for Neonatal Screening of Glucose-6-Phosphate Dehydrogenase Deficiency
Szu-Hui Chiang, Mei-Ling Fan, Yu-Shih Shiau, Kwang-Jen Hsiao (Taipei, Taiwan)

P-04 Newborn Screening for Infantile Pompe Disease in National Center for Child Health and Development Hospital; A Pilot Study
MOTOMICHI KOSUGA, Kazuhiro Kida, Eri Oda, Naoko Fuji, Torayuki Okuyama (Tokyo, Japan)

P-05 Newborn Screening for Fabry Diseases in Japan
Kimitoshi Nakamura, Kiyoko Hattori, Shiro Matsumoto, Hiroshi Mitsubuchi, Fumio Endo (Kumamoto, Japan)

P-06 The First Korean Case of Lysinuric Protein Intolerance; Presented with Short Stature and Increased Somnolence
Jung Min Ko, Choong Ho Shin, Sei Won Yang, Moon Woo Seong, Sung Sup Park, Jung-Han Song (Seoul, Korea)

P-07 Amino Acid Disorders Detected by Quantitative Amino Acid HPLC Analysis in Thailand: An Eight-Year Experience
P-08 Glutathione Synthetase Deficiency (5 Oxoprolinuria) in Vietnamese Patients: Clinical Manifestations and Outcome
Ngoc Khanh Nguyen, Dung Vu Chi, Hoan Nguyen Thi, Thao Bui Phuong, Ngoc Can Thi Bich, Dung Khu Thi Khanh, Liem Nguyen Thanh, Seiji Yamaguchi (Hanoi, Vietnam)

P-09 Clinical Features of Propionic Acidemia in Vietnamese Patients: a Report of 7 Cases
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Aidah Juliaty Wahyudin1, Damayanti Rusli Sjarif2 (Makassar, Indonesia)
Day2 April 2\textsuperscript{nd}

Abstracts
Redefining the Diagnostic Spectrum of Mitochondrial Disorders by the Clinical Application of Next-Generation Sequencing Technology

Sihoun Hahn

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Objective: Variability in clinical presentation and lack of reliable diagnostic screening makes the diagnosis of mitochondrial diseases challenging. Currently, the diagnosis of mitochondrial disorders relies largely on the enzymatic analysis of the respiratory chain complexes (RCC) in muscle tissues and extensive biochemical analyses; however, the results are often inconclusive. Mutations can occur in mitochondrial DNA (mtDNA) or in nuclear genes encoding mitochondrial proteins. Since approximately 1500 proteins are likely involved in mitochondrial structure and function, many disease causing gene mutations remain unidentified.

Methods: We recently developed and validated targeted next-generation sequencing on 26 patients with known or suspected mitochondrial disorders for the exons of 908 known and candidate nuclear genes using Illumina genome analyzer. In this study, none of the 17 patients with various abnormal respiratory chain complex (RCC) activities showed molecular defects in either subunits or assembly factors of mitochondrial RCC enzymes. Instead, several variants in other known pathogenic genes and a few potentially pathogenic variants in candidate genes were identified. Given this promising outcome, we further developed and implemented a clinical test (Nuclear Mitome test®, Transgenomic Lab) to diagnose mitochondrial disorders by an exon capture method that targets 450 known nuclear genes encoding entire subunits of the mitochondrial respiratory chain complex (RCC), assembly factors, transcription/translation factors, enzymes, carrier proteins and genes causing secondary mitochondrial defects or presenting with similar phenotypes.

Results: Up to date, 53 patient samples were submitted by physicians experienced with mitochondrial disorders. Sequencing was carried out on an Illumina GAIIx, and the data were analyzed through a laboratory-validated pipeline using publically available software (BWA, Samtools, Picard and GATK) with modification. On average, ~600 variants were found in the targeted genes including exons and 20bp at each intronic end. After removing variants with a relatively high minor allele frequency in our cohort or public SNP databases, we found an average of 7 variants per patient that could be pathogenic. Most of these were unpublished, single heterozygous variants of unknown significance (VUS) in genes with autosomal recessive (AR) disease inheritance, resulting in possible carrier status for the corresponding disease. The overall clinical yield of this test was estimated to be ~40%, though further parental testing and clinical evaluation is necessary to determine how many of the cases are truly positive. Of note, we did not find any pathogenic variants in RCC subunits or assembly factors.

Conclusions: Despite the limitations in finding mutations in all patients, our findings underscore the considerable clinical benefits of targeted next-generation sequencing. The diagnostic spectrum of mitochondrial disorders appears much broader than previously thought which could potentially lead to misdiagnosis and/or inappropriate treatment. While NGS shows promise for diagnosing these patients, the challenges remain high as the underlying genetic heterogeneity may be greater than suspected.

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April 2nd 2012
2-SI-1
Symposium I “IMD in Asia”

Inborn Errors of Metabolism in Asia

Pornswan Wasant
The study of inherited metabolic disorders (IMD) in Asia first started in Japan in 1960’s. The Japanese Society for Inborn Errors of Metabolism was established in 1965, and currently has about 700 members. Research and treatment of IMD e.g. enzyme replacement therapy for lysosomal storage disorders (LSD) was started in 1996, liver transplantation in 1990’s. Development of IMD in Japan is considered the most advanced in Asia. As for other developed countries in Asia e.g. Korea and Taiwan - the facilities for biochemical and molecular diagnosis have been available in Korea since early 1990’s: treatment of IMD included special formula for diet therapy, organ transplantation and enzyme replacement therapy for LSD also are available. Genetic Health Act in 1985 and Prevention and treatment of rare disease Act in 2010 play important role in improving quality of life of IEM patients in Taiwan. As for developing countries - Malaysia, Philippines and Thailand where small number of clinical geneticists and metabolic specialists are available; however, numerous cases of IMD have been identified. Diagnostic laboratory facilities are also being developed at the tertiary care centers. Collaborations with more advanced countries in Asia (Japan, Korea, Taiwan) and United States, Europe and Australia have been very active.

Newborn screening (NBS) by dried blood spot (DBS) was started in Japan in 1977, followed by Korea and Taiwan in 1985. Japan screen for PKU, MSUD, CH, CAH homocystinuria and galactosemia. Taiwan screen for CH, PKU, homocystinuria, galactosemia and G6PD, where Korea screen for CH and PKU. Currently tandem mass spectrometry is available in Japan, Korea and Taiwan. Development of NBS in developing countries - Malaysia currently screen for G6PD deficiency and CH since 1975, and started a pilot study using tandem mass spectrometry in 2006. NBS in the Philippines was established in 1996, and currently screen for 5 disorders: PKU, CH, CAH, galactosemia and homocystinuria however the data on the coverage is unavailable. Thailand started nationwide program in 1996, screen for 2 disorders CH and PKU (like www.acimd2012.org many other countries in Asia), where the coverage is 90–95 percent. India and Indonesia are in developing stage of development.

Common problems in developing countries in Asia are: (1) IMD are not on the priority list due to competing health care priorities e.g. infectious diseases, malnutrition etc. leading to lack of governmental support (2) difficulties in providing special formulas for patients with small molecule disease (3) unable to afford expensive enzyme replacement therapy for treatment of patients with large molecule disease (4) inadequate educational program and lack of awareness among health care professionals.
Inborn Errors of Metabolism in the Philippines: a 10 Year Review

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Institute of Human Genetics, National Institutes of Health UP Manila

The field of biochemical genetics in the Philippines is young and still growing. Knowledge and awareness of IEM was sparked by the introduction of newborn screening for PKU and galactosemia in 1996.

IEM are progressive and cause permanent, chronic neurologic impairment. Among the more common presenting manifestations of IEM (neurologic, hepatic, cardiac, myopathy or renal tubular defect), the “neurologic syndrome” is reported to be the most frequently occurring. In the Philippines, Maple syrup urine disease and mucopolysaccharidoses are the top two neurometabolic conditions diagnosed through the Institute of Human Genetics, NIH in the last 10 years.

A major challenge is the accessibility and affordability of more specific diagnostic tests. With the establishment of a biochemical genetics unit at the Institute of Human Genetics in 2001, basic IEM screening through quantitative plasma amino acid analysis (HPLC), urine metabolic profile (high voltage electrophoresis) and urine organic acid analysis (GCMS) became available. Screening for fatty acid oxidation defects, lysosomal storage disorders, organic and amino acidopathies as well as more specific tests such as VLCFA, carnitine profile, DNA mutation studies and enzyme assays are currently arranged as “send outs” to service or research facilities overseas. Because of the expansion of the diagnostic capabilities, more patients with IEM are now identified and treated. Furthermore, collaboration with international experts and researchers has made the diagnosis and management of metabolic conditions more current and comprehensive in the local setting.

April 2nd 2012

2-SI-3

Symposium I “IMD in Asia”

Wilson disease: from proteome & gene to clinical manifestations

Han-Wook Yoo1,2,3, Beom Hee Lee1,2,3, Joo Hyun Kim2, Kwi-Joo Kim2, Jung-Young Park2, Gu-Hwan Kim2,3
Wilson’s disease (WD) is an autosomal recessive disorder of copper metabolism. Wide phenotypic and genotypic heterogeneities have been reported, hampering the study on their correlations. This study was performed to identify factors related to genotypic–phenotypic diversities of Wilson disease (WD) in 237 unrelated Korean WD families. Presenting phenotypes were classified as hepatic (H1, H2), neurological (N1, N2, NX), and asymptomatic (ASx), modifying the guidelines by Ferenci et al. (Liver Int 2003;23:139–42). Presenting phenotypes in our cohort were H1 (12.2%), H2 (42.4%), N1 (21.6%), N2 (0.4%), NX (0.4%), ASx (22.4%), and other (0.4%). Onset age was youngest in patients with ASx presentation, but similar among H1, H2 and N1 patients. Liver cirrhosis was associated in 63% of N1 patients, similar as in H1+H2 patients (49%). On follow-up (average, 8.2±5.8 years), liver cirrhosis was rarest in ASx patients (4%). Decompensated cirrhosis was highest in H1 (48%) but similar between N1 (2%) and H2 (5%) patients. Favorable outcome was rarest in N1 patients (8%). Forty-seven ATP7B mutations including 11 novel ATP7B mutations were identified in 85% of the 474 alleles. MLPA assay in ATP7B and analyses of ATOX1 and COMMD1 identified no additional mutations. Yeast complementation and growth curve assays demonstrated functional perturbation of the seven novel missense mutants. Five major mutations, p.Arg778Leu (36.5%), p.Ala874Val (9.9%), p.Asn1270Ser (8.0%), p.Lys838SerfsX35 (4.2%), and p.Leu1083Phe (4.0%), accounted for 63% of the alleles. H1 was more common (26.8% vs 8.5%, P = 0.006), onset age was younger (12 vs 15 years, P = 0.009), and N1+N2+NX tended to be less common (12.2% vs 27.0%, P = 0.06), in patients with nonsense, frame-shifting or splicing mutations than in those with missense mutations alone. No other genotype–phenotype correlations were identified among common mutations or genotypes. Widely heterogeneous presenting phenotypes were noted in patients with both mutations in a single domain of ATP7B. In conclusion, the presenting phenotype strongly affects the clinical outcome of WD, and is related, in part, to mutation type. Several common mutations unique to those of particular ethnicity should be identified to allow rapid and easy diagnosis of WD without the need for invasive intervention. More effort is needed to identify genetic defects in those without ATP7B mutation and the factors related to genotypic–phenotypic heterogeneity. The proteins, C3, FB and α2M, identified by using comparative proteome analysis are related to oxidative stress and inflammation, and could be useful clue to accelerate the discovery of novel diagnostic biomarkers for early stages of WD.

April 2012
2-SI-4

Symposium I “IMD in Asia”

2-SI-4 Leukodystrophies – The Indian Scenerio

Leukodystrophies – The Indian Scenerio

Ishwar Verma, Renu Saxena, Ratna Puri, Sunita Bijarnia, Sudha Kohli
Objective: Leukodystrophies occur worldwide, but the types, and the mutations in the causative genes vary in different countries. In India leukodystrophies are frequent, due to the high rate of consanguinity among Hindus in South India, and among Muslims all over. The commonest leukodystrophy in India is cystic megalencephaly (CM) which runs a mild course. This disorder was initially recognized by Singhal in India in 1991, but became established as an entity after description by van der Knaap in 1995. It occurs predominantly among the Aggarwal community, which has origins in North India but has spread all over the world because of their astute business acumen. Most patients present at 8–12 months of age with enlarged head, developing seizures at one year, with slow and steady progress of the disease. Some children are able to complete schooling and even college education and become professionals albeit with large head and MRI changes in the brain. Among the Aggarwals c.135insC is a founder mutation in MLC1 gene. We have analyzed 105 patients with CM, and provided carrier screening and prenatal diagnosis to 25 families. The disorder also occurs in other communities in India but they carry different mutations (we detected four mutations of which one was novel). We are investigating the cause of variable clinical picture by examining the interaction of MLC1 and glial CAM genes. Adrenoleukodystrophy is also common in India. Mutation studies by us and others showed that about 40 % of 34 patients had novel mutations in ABCD1 gene. No founder mutation was identified. The benefit of molecular testing was demonstrated when a cousin of an affected child was detected to have the same mutation with no symptoms but having very early changes in the MRI, thus qualifying for bone marrow transplant. Sixteen of 23 patients with metachromatic leukodystrophy showed mutations in the ARSA gene. The common European mutation (c.459+G>A) was found in 2 patients, while the rest had different mutations. There were 8 reported and five novel mutations. Molecular diagnosis in 26 Krabbe’s disease patients, showed large deletion encompassing exons 11 through 17 in GALC gene in 24, while 2 had point mutations. Study of ASPA gene in three patients of Canavan disease revealed one known Ashkenazi Jewish mutation while two mutations were novel. A network of ten centers in India has embarked on molecular studies in different leukodystrophies, and hopefully founder mutations will be identified. This would make testing easier and cheaper, which is important in a resource poor country. Hopefully progress will also be made in therapy of leukodystrophies.

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Research into Rare Diseases as a New Paradigm for Drug Discovery

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It is evident that over the past few years there have been increasing interest by the scientific and medical communities to research and develop therapies for rare diseases. This is largely borne of the realization that as our understanding of the molecular basis of these ailments increased, innovative therapies that transformed patients’ lives could be developed. Examples include protein augmentation therapies for the hemophilias, lysosomal storage disorders, cystic fibrosis and α₁-antitrypsin deficiency, to name a few. However, clear evidence of effectiveness and value to the patient is becoming essential worldwide for acceptance of novel drugs. Gaucher disease, a rare lysosomal storage disorder, represents a case study in the new global approach to drug development. Initial efforts with unmodified β-glucocerebrosidase were unsuccessful until the realization for the need of a carbohydrate-modified version that targeted the enzyme to macrophages. Appreciation for the desire for improved therapies to address disease manifestations not well met by enzyme infusions subsequently prompted investigation into alternate strategies, using the same science-based approach. This ultimately led to the development of a new therapeutic concept referred to as substrate reduction therapy. As the drug is an orally available small molecule compound, it offered the potential to treat organs that were not well served by enzyme (e.g. brain and bone diseases) as well as improved the quality of care for the patients. Gene therapy represents yet another emerging technology platform that is being developed to address the limitations and challenges associated with both neuronopathic and non-neuronopathic Gaucher disease. A further improvement in our understanding of the disease through registries also revealed associated conditions such as Gaucher-related Parkinsonism. It is now generally recognized through this analysis that mutations in GBA1 represents a significant risk factor for developing Parkinson disease and dementia with Lewy bodies. This finding obviously lends to the possibility of deploying some of the therapies developed initially for Gaucher disease for this more common ailment. Perhaps more interestingly is the finding that as our understanding of the metabolic pathways associated with Gaucher disease increased, yet other diseases were found to be amenable to therapeutic intervention using these same treatments. These included diseases as divergent as polycystic kidney disease and type 2 diabetes. Clearly, this experience suggests that the benefits associated with the study and development of therapies for rare diseases can be far-reaching and extending to include significantly less rare ailments. In our opinion, it is not unreasonable to speculate that this experience will be shared by research on other rare diseases.

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Symposium II “Expanded Screening”

2-SII-1 Newborn Screening in the Asia Pacific region
Carmencita Padilla (Manila, Philippines)

Neonatal Screening in Asia Pacific Region
Carmencita Padilla MD, FPPS, MAHPS

Professor and Chair, Pediatrics, College of Medicine and Director, Newborn Screening Reference Center, University of the Philippines Manila

Newborn Screening is a well recognized public health prevention program aimed at the early identification of infants who are affected by certain genetic/metabolic/infectious conditions. Early identification of these conditions is particularly crucial, as timely intervention can lead to a significant reduction of morbidity, mortality, and associated disabilities in affected infants.

Many of the countries in the Asia Pacific Region, particularly those with depressed and developing economies are just initiating newborn screening programs for selected metabolic and other congenital disorders. The establishment of newborn screening programs in developing countries poses major challenges as it competes with other health priorities like control of infectious diseases, immunization, malnutrition, etc. Despite this, it is imperative that developing countries recognize the importance of newborn screening as it has been proven through decades of experience, that it can save thousands of babies from mental retardation, death and other complications. The cultural, geographic, language, and economic differences that exist throughout the region add to the challenges of developing sustainable newborn screening systems.

There are currently more developing programs than developed programs within the region. Newborn screening activities in the Asia Pacific Region are particularly important since births there account for approximately half of the world’s births. Experience has shown that certain critical factors are necessary for the ultimate success of a national newborn screening program. These factors include: 1) inclusion of newborn screening among the government priorities; 2) funding of the newborn screening program either through fees or inclusion in the national insurance system; 3) general public acceptance; 4) cooperation of the health practitioners; and 5) government participation in institutionalizing the newborn screening system. We have just completed the 3rd workshop facilitating the Newborn Screening Efforts in the Asia Pacific Region which caters primarily to countries with less than 50% coverage in the region. This session will present the highlights of the newborn screening programs (statistics, disorders, problems, strategies etc) in the following countries: Australia, Bangladesh, Cambodia, China, India, Indonesia Japan, Korea, Malaysia, Mongolia, Nepal, New Zealand, Pakistan, Philippines, Singapore, Sri Langka, Taiwan, Thailand, Vietnam.
Expanded Neonatal Screening and Prenatal Diagnosis for Inborn Errors of Metabolism in Shanghai

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Objective: Inborn errors of metabolism (IEM) are a large group of rare but often serious metabolic disorders, prompt recognition, diagnosis is important and the patient could benefit from the early, presymptomatic diagnosis and treatment, the patient’s family could benefit from prenatal diagnosis when they want to have a healthy child.

Neonatal screening in China began in Shanghai from 1981. Newborn screening programmes based on tandem-mass spectrometry (MS/MS) technology show a substantial variation in number and types of disorders included in the screening panel. The neonatal screening using tandem mass spectrometry was started from 2003 in Shanghai, until the end of Nov. 2011, about 410,462 neonatal samples were analyzed according to the no selective abnormal metabolites analysis and confirmed partially with genetic testing. 127 cases were confirmed including 15 kinds diseases: hyperphenylalaninemia, 3-methylcrotonyl-CoA carboxylase deficiency, methylmalonic acidemia, carnitine uptake defect, short chain Acyl CoA Dehydrogenase Deficiency, hypermethioninemia, Citrin Deficiency, Medium Chain Acyl CoA Dehydrogenase Deficiency, maple syrup urine disease, Propionic Acidemia, Propionic Acidemia, Homocystinuria, Arginemia, Arginemia, Glutaric Acidemia Type I, the prevalence was 1:3 232 in Shanghai area.

To prevent the risk of IEM in the same family to reappear, prenatal diagnosis to be a wise choice. The primary aim of a prenatal diagnosis is to provide an accurate diagnosis, including enzymes diagnosis and molecular diagnosis, that will allow the widest possible range of informed choice to those at increased risk of having children with IEM. Currently, more than 60 kinds molecular or enzymes tests are available in our center and more than 35 kind diseases have been applied in clinical prenatal diagnosis practice, including the diseases detected by MSMS, GCMS, by molecular or by enzymes
Symposium II “Expanded Screening”

Severe Combined Immunodeficiency Newborn Screening in Taiwan

Yin-Hsiu Chien , Wuh-Liang Hwu, Shu-Chuan Chiang, Kai-Ling Chang, Li-Wen Hsu, Min-Huei Hu, Li-Ping Tsai, Hsin-Hui Yu, Wen-I Lee

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Objective: In order to know the true incidence of severe combined immunodeficiency (SCID) in Chinese population, we conducted and implement SCID newborn screening in Taiwan.

Methods: Between May 1, 2010 and Dec. 31, 2011, the National Taiwan University Hospital Newborn Screening Center screened all newborns for T-cell lymphopenia by measuring the copy number of T-cell receptor excision circles (TRECs) and RNase P. Newborns with low TREC values were subjected to complete blood cell counts and flow cytometry.

Results: A total of 106 391 newborns were screened using the TREC assay during 19 months. Five newborns were immediately referred for confirmatory tests, and four of these were found to have persistent T-cell lymphopenia. We also identified five cases of 22q11.2 microdeletion syndrome. During this period, two SCID patients from among the unscreened newborns were reported.

Conclusions: Newborn screening to measure the number of TREC copies that successfully identifies newborns with T-cell lymphopenia, 22q11.2 microdeletion syndrome, and other high-risk conditions. Taking together, the incidence of T cell lymphopenia in apparently health newborns is more than 1 in 11 821, and further attention to their immune functions should be warranted.

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Screening for Fabry disease (FD) in patients referred to metabolic or nephrologic clinics and patients referred to dialysis centers.

Teruo Kitagawa

Chairman, Tokyo Health Service Association, Tokyo Health Service Association, Tokyo, Japan

Objective: 1) Screening for FD in patients referred to metabolic or nephrologic clinics. The objective of screening for FD in patients referred to metabolic or nephrologic clinics is to identify the disease before the onset of permanent tissue damage and to achieve a better long term outcome through enzyme replacement therapy (ERT). 2) Screening for FD in patients referred to dialysis centers. The objective of screening for FD in patients referred to dialysis centers is to identify the disease and to prevent fatal complications such as cardiac failure and stroke through an appropriate treatment such as ERT.

Methods: The most appropriate time for screening for FD is school age. For this reason, we have developed non-invasive methods measuring urinary α-galactosidase A (α-gal A) protein using ELISA, and globotriaosylceramide (GL-3) using tandem mass spectrometry (MS/MS). We measured these two biomarkers in the urine of the following: 432 healthy volunteers as controls, previously diagnosed FD 40 hemizygotes, 28 heterozygotes, and 112 patients at high risk of FD which had been referred to metabolic or nephrologic clinics. It has been reported that plasma GL-3 levels are not as good
indicators to identify FD when compared to urinary GL-3 levels. In patients on dialysis, however, measurement of biomarkers for FD in urine are not available. So, we measured both α-gal A activity using fluorogenic substrate and α-gal A protein using ELISA in plasma. We measured both α-gal A activity and α-gal A protein in the plasma of the following: 202 healthy adult volunteers as control, previously diagnosed FD 13 hemizygotes and 4 heterozygotes, and 8,520 patients referred to 104 dialysis centers.

Results: 1) Screening for FD in patients referred to metabolic or nephrologic clinics. All the classic FD hemizygotes were clearly distinguished from controls by either method alone and combining the two assays produced a 96% sensitivity for detecting heterozygotes. Among the above 112 high-risk patients, measurements exceeding the cut-off values for both biomarkers in male and female subjects were strong indicators for FD hemizygotes and heterozygotes. 2) Screening for FD in patients referred to dialysis centers. All the previously diagnosed classic FD hemizygotes were clearly distinguished from controls by either method alone and combining the two assays produced a 94% sensitivity for detecting previously diagnosed heterozygotes. Among the 8,520 patients (male 5,377: female 3,112 patients) on dialysis, the levels exceeded the cut-off values continuously in the first, second and third measurements for both α-gal A activity and enzyme protein levels were strong indicator for FD hemizygotes and heterozygotes, respectively.

Conclusions: We have developed the most reliable methods to screen for FD in patients who are referred to metabolic or nephrologic clinics and to dialysis centers.

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April 2nd 2012

Symposium II “Expanded Screening”

Screening of Lysosomal Storage Diseases & Galactosemia

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Objective: We proposed a second-tier multiplex enzyme assay for galactosemia that can be directly applied to dried blood spots (DBSs) and also evaluated the performance of multiplex tandem mass spectrometry (MS/MS) in newborn screening for detection of 6 lysosoamal storage diseases (LSDs), namely, Niemann-Pick A/B, Krabbe, Gaucher, Fabry, and Pompe diseases and Hurler syndrome.

Methods: In terms of galactosemia, supernatants from two rehydrated-punched 3.2-mm DBSs were incubated with a reaction mixture containing \( [^{13}C_6] \)galactose, \( [^{13}C_2] \)galactose-1-phosphate, and UDP-glucose as substrates for three galactose-metabolizing enzymes. After a 4-hour incubation, the end products from the combined reaction mixture, \( [^{13}C_6] \)galactose-1-phosphate, UDP-\( [^{13}C_2] \)galactose, and UDP-galactose, were simultaneously measured using UPLC-MS/MS. In terms of LSDs, we revised the conditions and procedures of multiplex enzyme assay for the MS/MS analysis. We evaluated the assay performances and measured enzyme activities in DBSs from normal individuals and the patients with galactosemia or LSDs.

Results: In terms of galactosemia, intra- and inter-assay imprecisions of the UPLC-MS/MS were 8.4-14.8% and 13.2-15.7% CV, respectively. DBSs from 10 galactosemic patients showed consistently lower enzyme activities as compared to those of 37 normal individuals. In terms of LSDs, the intra- and inter-assay precisions were 2.9-18.7% and 8.1-18.1%, respectively. The enzyme activities measured in the DBSs of 13 patients with LSDs were lower than those measured in the DBSs of 211 normal newborns.

Conclusions: Multiplex enzyme assays using UPLC-MS/MS can be successfully applied to DBS analysis. The performance of our revised techniques for MS/MS detection and enzyme assays was of the generally acceptable standard. This method allows a fast and effective newborn screening for galactosemia or LSDs.
Symposium II “Expanded Screening”

2-SII-6 Current Issues Regarding Storage and Use of Residual Dried Blood Spots

Issues Regarding the Retention and Use of Dried–Blood Spot Specimens after Newborn Screening

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Newborn screening (NBS) is a system characterized by highly successful public health programs that provide early identification of rare genetic, congenital and functional disorders, and assure early management and follow-up care for those affected. NBS programs are regulated and implemented differently in countries around the world and there is variation in their governing policies. Most NBS programs obtain and use blood specimens dried onto special filter paper for their laboratory screening tests. Unused portions of these specimens (residual specimens) are generally retained for some period of time after testing is complete. The primary justification for retaining residual specimens is to benefit the child and family by documenting that a specimen was collected, received, and properly analyzed by the screening program. Residual specimens may also be used for result verification and quality assurance activities for the program and laboratory (including new test validation). This collection of stored specimens is often referred to by some as a ‘biobank.’

NBS specimens are unique and valuable resources. They are usually the first blood specimen in a baby’s life and they are collected on essentially all newborns in many countries. They provide critical information about risk for certain congenital conditions. They also have the potential to generate population-based knowledge that can improve the health of children, support families, and provide information critical to understanding the antecedents of adult diseases. Therefore, residual specimen storage must assure that the culture, confidentiality and privacy of families are respected and that the specimens are protected. These specimens contain DNA and, therefore, have the potential for unintended uses and consequences to the individuals from whom they was collected. Many NBS programs have storage and usage policies that promote public trust, emphasize transparency of administrative practices, and create supporting information that encourages informed public participation, but many do not.

In some programs, issues have arisen when the public has learned that NBS residual specimens have been stored without their knowledge or approval. Some legal actions have been taken that have resulted in large numbers of specimens being destroyed, and, in some cases, new procedures legally and legislatively required. The experiences of programs who have encountered negative public and media responses should be reviewed and understood so that these same issues and outcomes will not occur in other programs. Moving forward, it will be important for all NBS programs to be aware of potential issues related to the storage and use of residual NBS bloodspots and to implement policies and practices that ensure their security and patients’ privacy. Education about NBS will need to include mention of the possible storage and use of specimens after screening in programs where storage exists.
Day3 April 3rd Abstracts
Objective: Recently, a number of treatment procedures for genetic diseases have been introduced: these are diet therapy, drugs (BH4, Penicillamine, etc), organ transplantation, hematopoietic stem cells therapy, enzyme replacement therapy (ERT), chaperon therapy, substrate reduction therapy, antilgonuclotide therapy (AON) and gene therapy/cell therapy. ERT is now golden standard therapy for lysosomal storage disease (LSD). However, there are some limitations for efficacy by ERT: These is an antibody formation against enzyme which influences to the efficacy. The treatment for antibody
formation is now essential, not only for Pompe disease, but also in other LSD such as Fabry disease, as well. Early diagnosis and treatment are also essential for better outcome by ERT. High risk/newborn screening are quite important to prevent from irreversible change/damage in LSD tissues, if treated earlier. Another remarkable development for the treatment of genetic diseases is recent advances of gene therapy, since ex vivo gene therapy by hematopoietic stem cells has been successfully carried out for patients with ADA deficiency, SCID, Wiscott–Aldrich syndrome, adrenoleukodystropy (ALD), metachromatic leukodystrophy and Sanfillipo syndrome either by retrovirus, lentivirus or AAV vector. Furthermore, iPS technology for the treatment of genetic disease will give us more intriguing prospect. Recently, we generated iPS cells from Pompe disease, Fabry disease, and Gaucher disease by Yamanaka’s method. We can generate skeletal muscle cells from Pompe iPS cells and also cardiomyocytes from Fabry iPS cells or neuronal cells from Gaucher disease iPS cells. The skeletal muscle cells from Pompe iPS cells accumulated massive glycogen in lysosomes and this technology can be used for the understanding of pathophysiology in Pompe disease. These cells could be treated by gene therapy with lentivirus/AAV vector. Furthermore, the editing gene therapy by Zinc finger nuclease (ZFN) method has been introduced and recently, targeted gene transfer into iPSCs, with subsequent selection and full characterization to ensure no off-target changes, holds promise for correction of monogenic diseases such as sickle cell anemia, chronic granulomatous disease and etc without the insertional mutagenesis caused by multisite integration of viral or plasmid vectors. In vivo editing gene therapy for hemophilia B was also successfully carried out in hemophilia B mouse using ZFN method. The level of gene targeting achieved was sufficient to correct the prolonged clotting times in a mouse model of hemophilia B, and remained persistent after induced liver regeneration. Thus, ZFN-driven gene correction can be achieved in vivo, raising the possibility of genome editing as a viable strategy for the treatment of genetic disease.

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Objective: Gaucher disease (GD) is caused by a deficiency of glucocerebrosidase. Clinical phenotypes are classified into three groups according to the absence or presence of neurological involvement and its progression: type 1, non-neuronopathic form; type 2, acute neuronopathic form; type 3, subacute neuronopathic form. All groups manifest anemia, thrombocytopenia and hepatosplenomegaly. The N370S mutation, protective mutation and prevalent mutation in western countries, is absent in Japan. Therefore phenotypic expression of Gaucher patients in Asian regions is distinct from that in Jewish and Caucasian. The incidence of type 1 is much lesser and the phenotypic expression of type 1 is more severe than that in other areas. And Gaucher patients with unique phenotype have been identified: neonatal GD is highly associated with null mutations, patients with hydrocephalus is tightly linked with the D409H mutation, the N188S mutation is found in patients manifesting progressive myoclonic epilepsy.

Enzyme replacement therapy (ERT) is one of treatment for GD. ERT is effective in hematological abnormalities and visceral involvement but not in neurological manifestations. We analyzed the survival rate of 82 GD patients treated with ERT. None of type 1 patient deceased. In contrast, that in type 2 and type 3 patients was 36% and 80%, respectively. And we investigated the clinical features and the efficacy of ERT in 42 patients with type 3 GD. The 42 patients fell into two groups: those diagnosed as having type 3 at diagnosis (group A; n=24) and those thought to have type 1 at diagnosis but who later developed neurological symptoms (group B, n=18). The genotype of group A patients varied widely: however, L444P/L444P and L444P/F213I genotypes accounted for 83% in group B. Nineteen patients received ERT in group A; however, 7 died despite the therapy. On the other hand, 14 patients received ERT in group B and 13 of them survived. In conclusion, some Japanese GD patients with characteristic genotypes who thought to have type 1 at diagnosis develop neurological symptoms during their clinical course. And ERT might not have a sufficient effect on the early onset neurological symptoms. A different treatment strategy is needed to improve the prognosis of these patients.

Contact Information: Hiroyuki Ida (hiroy@jikei.ac.jp)
Objective: The enzyme replacement therapy has been developed as an effective treatment for Gaucher disease (GD) but the effects on neurological symptoms are uncertain. We have reported N-octyl-b-valienamine (2004, 2007) and bicyclic nojirimycin (2009, 2010) enhance the enzyme activities and the protein levels of R120W, N188S, G202R, F213I and N370S mutant \( \beta \)-glucocerebrosidase (GBA) in cultured cells and in the tissues of normal mice (2010). On the other hand, Toronto’s group (2009) screened 1040 FDA approved drugs and found ambroxol hydrochloride (ABX) has chaperone activity on N370S and F213I mutant GBA. We have confirmed ABX also enhances R120W, N188S mutant GBA activities and enhances GBA activities significantly in the cerebellum of normal mice fed with drinking water containing various concentrations of ABX ad libitum for 7 days. Although the high doses (250mg-500mg b.d.) administrations have a uricosuric effect, the safety of high dose oral (990mg/day for 5 days, b.d.) or intravenous (1000mg/day for 3 days) administration are reported. We have initiated to study the effect and safety of ABX on neuronopathic Gaucher disease.

Results: A 30 years old female GD type3 patient presenting progressive myoclonic epilepsy by N188S/G193W mutations has been placed on oral ABX treatment since March 2010 at a starting dose of 3.3 mg/kg/day (t.d.). The dose was gradually up to 12 mg/kg over 6 months, maintained for 5 months and then increased to a maintenance dose of 15 mg/kg/day. The GBA activity in lymphocytes increased more than 9mg/kg/day and reached to 60-70% levels of controls at 15mg/kg/day. ABX was detected in CSF at a 17% level of serum concentration. Because she has severe motor and intellectual disability with tracheostomy, it is difficult to evaluate the clinical improvements. There were no clinical and biochemical adverse effects. Electrophysiological and radiological findings seem to be partially improved. According to the results of the first case we modified the protocol and started ABX treatment for neuronopathic GD patients in Japan. We will present preliminary effects of ABX on severe myoclonus of 15 years old and 20 years old sisters with progressive myoclonus epilepsy caused by N188S/ ? mutations.
Conclusions: There were no adverse effects of high-dose and long-term administration of ambroxol. Ambroxol may have therapeutic effects on neurological symptoms of Gaucher disease caused by specific mutations.

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April 3rd 2012

Symposium III “New Treatment of IMD”

Treatment of Citrin Deficiency: Assessment by Using an Animal Model

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Objective: Citrin deficiency is caused by mutations in SLC25A13 which encodes liver-type mitochondrial aspartate glutamate carrier, or citrin. Citrin plays a role not only in transport of mitochondrial aspartate to cytosol, but also in transporting cytosolic NADH reducing equivalent to mitochondria as a member of malate aspartate shuttle. It is important to note that treatment of citrin deficiency is completely different from the other urea cycle enzyme deficiency. Administrations of high-concentration-glucose solution or high-carbohydrate diets have deteriorated patients with citrin deficiency. It is important to understand the mechanism of the pathophysiological changes caused by administration of sugars and to establish novel treatment procedures for sugar toxicity.

Methods: We created an animal model for citrin deficiency, citrin (Ctn)/mitochondrial glycerol-3-phosphate dehydrogenase (mGPD) double-knockout (KO) mice, for analysis of pathophysiology and development of treatment. Food was changed from a laboratory chaw, CE-2, to a synthetic food proposed as a diet sufficient for mature rodents by American Institute for Nutrition (AIN-93M), which contains less amount of protein than CE-2, and food intake/day and body weight of Ctn/mGPD double-KO and control mice were assayed after a week of the dietary change. Effects of casein, amino acid, sodium pyruvate (Na-Pyr) or a various kinds of fat were assessed by adding to AIN-93M. Effects of the
substances were also assessed by assaying hepatic metabolites and blood ammonia 1h after simultaneous administration of sucrose solution.

Results: As reported previously, Ctrn/mGPD double-KO mice showed hyperammonemia by oral administration of sucrose accompanied by a large increase in hepatic glycerol-3-phosphate (G3P). Change of food from CE-2 to AIN-93M caused loss of body weight accompanied by a decrease in food intake in the double-KO mice. Addition of casein, certain amino acids, or Na-Pyr to AIN-93M normalized body weight and food intake to the CE-2 levels. Among fats, only medium-chain triglyceride (MCT) had effects similar to the above-listed substances. Addition of the amino acid or Na-Pyr decreased hepatic G3P levels when given with sucrose solution, while MCT did not show significant effect. Blood ammonia level of the double-KO mice was almost normalized by the amino acid or Na-Pyr.

Conclusions: Sugar toxicity in citrin deficiency, which is caused by a hepatic increase in cytosolic NADH, judged from a large increase in G3P and G3P/dihydroxyacetone phosphate ratio, leading to disturbances in glycolysis, urea cycle, amino acid metabolism and energy metabolism, can be ameliorated by oral administration of certain amino acids, sodium pyruvate and MCT, although mechanisms of those substances are apparently different.

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April 3rd 2012 3-SIII-4

Symposium III “New Treatment of IMD”

Hematopoietic Stem Cell Transplantation for X-linked Adrenoleukodystrophy: Outcome in Japan

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Objective: Adrenoleukodystrophy (ALD) is an X-linked recessive neurodegenerative disease characterized by brain demyelination, adrenal insufficiency, and accumulation of very long chain fatty acids. Deficiency of ALD protein in the peroxisomal membrane leads to the metabolic dysfunction, however, the mechanisms for degeneration and diversity of phenotype remain to be elucidated. Hematopoietic stem cell transplantation (HSCT) is the only practical strategy to treat, however, outcomes of HSCT for ALD is not necessary good. Here we investigated the outcome of patients with ALD who received HSCT in Japan.

Methods: Forty-seven patients with ALD were investigated. Mean age at HSCT was 8.6 y.o. Twenty-eight patients showed parieto-occipital demyelination and 9 patients showed frontal demyelination. Twenty patients received marrow from related-donor, 11 from unrelated-donor, and 15 received umbilical cord stem cells. Preparative regimens were BU/CY-based (18), Mel/ATG/TAI (12), Flu/Mel-based (14), and others (2). Nineteen patients showed Loes MRI score less than 10, and 11 showed the score over 10. Data were collected from 10 major centers for HSCT in Japan under the permission of IRB.

Results: Of 47 patients, 8 were dead, 6 were rejected, 13 were deteriorated, 13 were stabilized, and 7 remain asymptomatic (HSCT at the stage of asymptomatic). Total survival rate was 79.9%. There were no significant differences of results between the type of donor, the type of degeneration, and the score of IQ, however, patients with lower MRI score and those who received Flu/Mel-based preparative regimen showed better results. Patients who received HSCT after 2005 have better results.

Conclusions: With the advancement of general management of patients and HSCT procedure, we archived a better outcome of HSCT for ALD.

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Phase I/II Clinical Trial of Enzyme Replacement Therapy with GC1111 in Hunter Syndrome

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Objective: Mucopolysaccharidosis (MPS) represents a group of lysosomal storage disorders caused by the deficiency of enzymes which are responsible for the degradation of glycosaminoglycan (GAG). In affected patients, GAGs accumulate in the lysosomes of many organs and tissues, contributing to the pathology associated with MPS. Recently, GC1111, a new drug for MPS II patients has been developed in Korea. We finished preclinical and clinical studies. Here, we try to show the efficacy and safety of recombinant human iduronate-2-sulfatase beta (GC1111) in the treatment of MPS II.

Methods: Thirty-one MPS II patients between 6 and 35 years of age were enrolled in a randomized, single-blinded, active comparator-controlled phase I/II trial for 24 weeks. Patients were randomized to active comparator infusions, 0.5 mg/kg of GC1111 infusions or 1 mg/kg of GC1111 infusions. The primary efficacy variable was the level of urinary glycosaminoglycan (GAG) excretion. The secondary variable was the distance walked in 6 minutes (6-minute walk test, 6MWT) and the pharmacokinetic studies (PK study) of GC1111 and active-comparator drug were performed in six patients (two patients from each group).

Results: Patients in all three groups exhibited reduction in urine GAG and the reduced GAG level was maintained after 24 weeks. Moreover, Patients in all three groups experienced significant increase in 6MWT comparing with baseline. GC1111 infusions were generally safe and well tolerated. There was no serious adverse drug reaction. The most frequent adverse event was urticaria and skin rash which were easily controlled with administration of antihistamines.

Conclusions: In conclusion, we show that enzyme replacement therapy is the mainstay of therapy for the treatment of MPS in Korea. However, we speculate that novel drugs may be needed to overcome the limit of currently used therapeutic modalities. The clinical trial of patients with MPS II younger than or equal to 5 years old of age (GC1111) is scheduled.

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April 3rd 2012

SIV-1
Symposium IV “Amino acid & Energy metabolism”

3-SIV-1 Phenylketonuria
M. Demirkol (Istanbul, Turkey)

Symposium IV “Amino acid & Energy metabolism”

The Treatment and Outcome of Organic Acidurias

Yanling Yang, MD, PhD
Organic acidurias comprise many various disorders. They are considered the most frequent metabolic disorders among severely ill children. Improvements in the understanding of the biochemical and molecular basis of the organic acidurias have led to significant improvements in our ability to treat many of these disorders.

The improvement, coupled with an ability to make more rapid diagnosis and advances in general medical care, particularly intensive care, are resulting in better long-term prognosis for many patients. In this study, the current treatment and outcome of organic acidurias was reviewed.

Classical organic acidurias include methylmalonic aciduria, propionic aciduria, isovaleric aciduria, glutaric aciduria type 1 and maple syrup urine disease. Reducing the load on affected pathway substrate deprivation by diet can promote anabolism and growth of the patients. Medical foods, such as amino acid-based formulas, provide a key source of nitrogen, energy, vitamins and minerals. To remove toxic metabolites, a number of medications are used, such as L-carnitine in most patients with organic acidurias, sodium benzoate and sodium phenylbutyrate in disorders associated with hyperammonemia, glycine in isovaleric aciduria. In some disorders which are caused by mutations that affect the metabolism or binding of a coenzyme or cofactor, treatment with the coenzyme may lead to a complete return of a clinical phenotype to normal. For example, in the patients with biotinidase deficiency and holocarboxylase synthetase deficiency, the effect of biotin supplementation is dramatic. Most disorders with coenzyme-responsive variants show a more limited improvement. A majority of patients with vitamin-B12-responsive methylmalonic aciduria continue to produce abnormal, albeit smaller, quantities of methylmalonic acid. The patients with vitamin-B12 non-responsive methylmalonic aciduria should be treated by medical diet, vitamin B12 and L-carnitine. But, the patients of methylmalonic aciduria combined with homocysteinemia should be treated by cobalamin, L-carnitine, calcium folinate, betaine with normal diet. As new trends, liver or hepatocytes transplantation has been used as the successful therapy for a number of patients with vitamin-B12 non-responsive methylmalonic aciduria and propionic aciduria. Despite recent advances, therapies that directly effect pathology remain unavailable for many disorders. Improving the quality of life for patients and their families often relies on symptomatic treatment. Although, the outcome of many organic acidurias remains poor at present, there will be significant advances in the future to offer more effective treatment.
Objective: Methylmalonic aciduria (MMA) is the most common symptomatic organic aciduria found in Chinese patients. High incidence of combined MMA and homocystinuria (HC) among Northern Chinese MMA patients has been reported. The defects in the MUT and MMACHC genes may cause mut-type isolated MMA and cblC-type combined MMA and HC, respectively. In order to understand the molecular defects found in Chinese patients with MMA, the spectrum of mutations in MUT and MMACHC genes were studied.

Methods: Genomic DNA Samples were collected from unrelated Chinese MMA patients, 42 of them with isolated MMA and 79 of them had combined MMA and HC. All the exons and exon-intron boundary sequences of the MUT and MMACHC genes were analyzed by PCR-based sequencing for the isolated MMA and the combined MMA and HC patients, respectively. Short tandem repeat (STR) markers, D6S269 for MUT gene and D1S2677 for MMACHC gene, were used for linkage analysis.

Results: All the 42 isolated MMA patients were found to have at least one MUT mutation. On the other hand at least one MMACHC mutation has been detected in all the 79 combined MMA and HC patients. Sequence analysis identified 94% and 98% of MUT and MMACHC disease alleles, respectively. A total of 41 MUT mutations, including 20 novel ones, were identified. At least 66.7% of Chinese isolated MMA patients carried one of the five common MUT mutations, namely, c.1280G>A, c.729_730insTT, c.1106G>A, c.1630_1631GG>TA, and c.2080C>T, among which the c.729_730insTT and c.1280G>A are the most common mutations found in Northern and Southern Chinese, respectively. The results of STR analysis suggest that the spread of c.729_730insTT among the Northern Chinese and of c.1280G>A and c.1630_1631GG>TA among the Southern Chinese may have undergone founder effects. A total of 24 MMACHC mutations, including 9 novel ones, were identified. The c.609G>A, c.658_660delAAG, c.482G>A, c.394C>T and c.80A>G mutations were the most common mutations and accounted for 80% of the MMACHC disease alleles. Haplotype analysis suggests that the spread of the c.80A>G, c.609G>A and c.658_660delAAG mutations in Chinese patients were caused by a founder effect.

Conclusions: The results indicate that defects occurring in the MUT and MMACHC genes are the major cause of this disease in Chinese patients with isolated MMA and combined MMA and HC, respectively. Direct mutation analysis can therefore be used as a rapid confirmatory differential diagnosis among...
these Chinese MMA patients and could be applied for carrier detection and prenatal diagnosis among Chinese family at risk.

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April 3rd 2012

Symposium IV “Amino acid & Energy metabolism”

Inborn Errors of Metabolism and the Heart

Professor Anibh M. Das, MD PhD

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Energy demand of the heart is high and may vary enormously according to physical activity. The main fuels of the heart are fatty acids and to a lesser extent glucose. The heart relies on aerobic energy metabolism therefore the mitochondrial respiratory chain performing oxidative phosphorylation is essential. Energy supply has to be commensurate with energy demand. Energy flux is not only passively determined by substrate (ADP) saturation, but active regulation is operative as well including several regulatory elements. The mitochondrial ATP synthase (complex V of respiratory chain) has been shown to be actively regulated in response to cellular energy demand. Increased contractility and/or heart rate rapidly lead to up-regulation of the mitochondrial ATP synthase which is mediated by calcium. Also enzymes of the citric acid cycle are up-regulated in response to elevated calcium levels. Inborn errors of energy metabolism can compromise heart function. Defects in fatty acid oxidation (including carnitine deficiency and carnitine cycle defects) and of respiratory chain are well known. Furthermore, defects in glycogen catabolism lead to reduced energy generation from carbohydrates, like in glycogen storage diseases type III a (Cori/Forbes) and type II (Pompe). The latter being based on a lysosomal enzyme deficiency, this leads us to the second class of inborn errors of metabolism affecting the heart, the so-called lysosomal storage diseases. By mechanical deposition of storage
material, heart function and anatomy are compromised. Examples for this group of diseases are M. Anderson-Fabry, M. Gaucher, MPS I, II and VI, Danon disease. Some of them lead to secondary dysfunction of respiratory chain enzymes, e.g. M. Anderson-Fabry.

Sirtuins (especially SIRT 3) have been shown to play a regulatory role in oxidative energy metabolism. In SIRT 3 knockout mice free fatty acid oxidation is down-regulated leading to cardiac dysfunction with heart hypertrophy.

Other systemic inborn errors of metabolism affecting the heart include organic acidurias, some of them cause secondary mitochondrial dysfunction. Congenital defects of glycosylation may affect the heart. Apart from pathophysiological aspects, clinical symptoms, diagnostic procedures and therapeutic options shall be discussed.

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**April 3rd 2012**

**SIV-5**

**Symposium IV “Amino acid & Energy metabolism”**

**Current topics in diagnosis and treatment of mitochondrial fatty acid oxidation disorders**

Seiji YAMAGUCHI, Shimane, Japan

Mitochondrial fatty acid oxidation disorder (FAOD) is a group of inborn metabolic diseases, and has been attracted attention with development of neonatal mass screening using tandem mass spectrometry (MS/MS). Pathophysiology and molecular basis have also been clarified recently. FAOD is associated with a deficiency of an enzyme, which is involved in the conversion from fatty acids to energy. Acute encephalopathy or even sudden death can occur during the episodes of long fasting or infectious illness. It is considered that fatty acid oxidation (FAO) system is consisted of 5 groups: (1) carnitine cycle to activate long-chain fatty acids for undergoing beta-oxidation (2) long-chain fatty acid oxidation by the enzymes connected to mitochondrial inner membrane; (3) medium-chain fatty acid oxidation, whose enzymes are located in the mitochondrial matrix; (4) electron transferring system to respiratory chain, and (5) ketone body synthesis.

Clinical features of FAOD may be roughly divided to the 3 groups: (1) severe form: often fatal in early infancy due to profound energy deficit; (2) intermediate form: episodic attacks like lethargy, encephalopathy; (3) late onset form: often onset after school ages or adulthood with hypotonia, myalgia,
lethargy, myopathy-like symptoms, or liver dysfunction. In acute phase of the every type, hypoglycemia, liver dysfunction, increase of CK, or LDH, hyperammonemia, or myoglobulinuria are seen. Detection of these diseases is based on the acylcarnitine profiles analysed by MS/MS at the first step. As the second steps to confirm, GC/MS analysis, enzyme determination (assay or immunoblotting), or gene analysis may be required. Recently, in vitro probe (IVP) assay using cultured cells and MS/MS is available for the diagnosis of FAOD. In this method, acylcarnitines in the culture medium, which is carnitine-rich, glucose-free, and fatty acid-free, are measured after 96-h culture with palmitate loading, and the defective sites in the FAO can be identified. Treatments for FAODs are: (1) avoiding a “long fasting” to prevent the increased requirement of fuel from FAO: (2) early infusion of glucose for the metabolic stress resulting from infection, diarrhea or overexercis; (3) carnitine therapy in many cases showing low level of free carnitine; and (4) dietary therapy, including high carbohydrate/low lipid diet which may often be less strict. Recently, we are trying a hypolipidemic drug, Bezafibrate which is a PPAR agonist, for children, and observed the dramatic effect. We have further investigated the effectiveness using IVP assay of cultured cells from various types or severities of FAOD. There still issues to be resolved, relation between phenotype, genotype, or disease type, and long term adverse effects. At the present, however, it is believed that Bezafibrate can be a new treatment option for FAOD.
Glycogen storage disorder (GSD) comprises a group of inherited defects characterised by abnormal glycogen metabolism in various tissues. This group of GSDs are divided into 9 major types: type 0 to X. **GSD type I.** glucose 6-phosphatase system defect is subdivided into genetically distinct four forms: type Ia, Ib, Ic and Id, deficiency of glucose 6-phosphatase, glucose 6-phosphate translocase, pyrophosphate translocase and glucose translocase respectively. **GSD type II** commonly known as Pompe disease is caused by deficiency of lysosomal glucosidase (GAA). Mutations in the GAA gene, mapped to chromosome 17q25.2–25.3, induce disease manifestations at every life span from the foetal period to the adult age. **GSD type III.** amylol.6-glucosidase (AGL) deficiency, the most common GSD among Francoarabic populations is subdivided roughly into six forms according to clinical manifestation. The AGL gene is located on chromosome 1p21 with 35 exons spanning more than 85kb and mRNA consists of a 4596-bp coding region and 1 2371-bp 3'-nontranslated region. **GSD type IV** known as Andersen disease or amyllopectinosis, is a rare autosomal recessive disorder caused by deficiency of glycogen branching enzyme (GBE). The gene for this enzyme is located on chromosome 3q14. This GSD presents as numerous forms according to the clinical severity and organ involvement: liver, muscular, neuromuscular and generalized form. **GSD type V,** commonly known as McArdle disease is caused by muscle phosphorylase (PYGM) deficiency. This type is divided into two forms, severe infantile and mild
adult form. **GSD type VI** is caused by liver phosphorylase (PYGL) deficiency. **GSD type VII**. phosphofructokinase (PFKM) deficiency is commonly named as Tarui disease has two different subtypes: severe infantile form with respiratory failure and mild adult form with exercise intolerance combine with haemolysis, hyperuricemia and myoglobinuria. **GSD type VIa, VIII, IX or X**, the most diverse disease form of GSDs, supposed to be caused by tissue specific phosphorylase kinase (PHKA 1 and 2, PHKB, PHKG 1 and 2) deficiency. However, the biochemical as well as molecular aberration has been identified up to now only for the liver forms. The possible involvement of other enzymes or genes such as altered binding sites of phosphorylase b or cAMP dependent protein kinases has yet to be elucidated. **GSD type 0**, glycogen synthase deficiency, is extremely rare and divided into two subtypes, muscular (GYS1) and liver (GYS2) form. We report here molecular aspects of about 600 GSD patients especially type I, II and III focusing on the diagnostic methodology without tissue biopsy.
involvement of basal ganglia, impairment of brain energy metabolism, an imbalance of excitatory and inhibitory neurotransmission, altered transport across the blood–brain barrier and between glial cells and neurons (cerebral “trapping” of neurotoxic dicarboxylic acids), impairment of myelination or disturbed neuronal efflux of metabolic water.

Results: Signs and symptoms are non-specific and similar to those seen in infants with acquired conditions such as: sepsis, intrauterine infection, intracerebral haemorrhage or perinatal trauma. Newborns are normal at birth, but after few hours, days or weeks (asymptomatic interval), unexplained acute deterioration occurred. They become apathetic, start to poorly suck, vomit and develop tremor, convulsions, hypotonia, apnoeas, lethargy and coma. Occasionally, an unusual odor may suggest the diagnosis. If it is not established at this time, what results in the immediate introduction of specific treatment, patients deteriorated progressively and die or if not, present with irreversible brain damage. Diagnosis requires a variety of laboratory measurements such as: glucose, bicarbonate, ammonia (in the first line) and urine organic acid profile, amino acids and acylcarnitines in blood (in the second line), which allow to differentiate major causes of IEMs. There are two main groups of diseases, which present with intoxication type: organic acidurias (e.g. methylmalonic, propionic, isovaleric and glutaric aciduria type I) and urea cycle disorders, but also maple syrup urine disease. All these disorders can cause similar clinical manifestation, which can be life-threatening. The outcome of intoxication type IEMs is highly dependent on the speed with which the diagnosis is suspected and proper treatment started. Emergency therapy includes: stop protein (not longer than 48 hours, to avoid breakdown of endogenous proteins) in parallel with high energy intake, substrate removal acceleration, toxin removal procedures (extracorporeal detoxification) and additional therapies (if needed). Currently with tandem mass spectrometry use in newborn screening programs, many IEMs are identified in the presymptomatic stage.

Conclusions: IEM of intoxication type should be considered in differential diagnosis of a severely ill neonate, and special studies should be undertaken. Even in infants, in whom death seems inevitable, specific diagnosis is of great importance – for genetic counseling of the family.

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April 4th 2012

Symposium V “Basics of IMD”

The interaction of urea synthesis and acid base metabolism

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Inborn metabolic defects of urea synthesis, presenting with hyperammonemia are frequently accompanied by disturbances of acid base metabolism, either in the form of alcalosis or acidosis. Urea is synthesised from bicarbonate and ammonia. Therefore urea formation can be considered as a mechanism of bicarbonate excretion and is herewith involved in the regulation of acid base metabolism. The reactions of urea formation as well as of glutamine metabolism are clearly distributed within the
hepatic lobule. Urea synthesis and the glutaminase reaction occur mainly in the periportal area whereas glutamine formation is located around the central hepatic vein. Urea formation has a high capacity of ammonia detoxification but has only a low specificity. Glutamine formation in contrast has only a low detoxification capacity but is highly specific. Herewith is formed an anatomic sequence of a mechanism with high and low ammonia detoxification affinity. The periportal glutaminase is activated by \( \text{NH}_4^+ \) acting as amplificatory in dependence of the intramitochondrial pH. The periportal glutamine degradation increases the flux through the urea cycle. pH regulation is focused more on the bicarbonate elimination than on \( \text{H}^+ \) elimination. In diseases of urea formation ammonia and bicarbonate accumulate, supporting a tendency towards metabolic alcalosis. Alterations of urea formation which are induced by changes of the acid base metabolism are predominantly due to there gulation of hepatic glutaminase, which has a more alkaline pH optimum. Hyperammonemia in organic acidemias like propionic or methylmalonic academia in contrast, is very often accompanied by metabolic acidosis. In these cases, hyperammonemia is caused by an inadequate stimulation of carbamylphosphatesynthase, the first step of urea synthesis. Carbamylphosphatesynthase activity is dependent on the stimulation by \( \text{N}^- \)-acetylglutamate. An inadequate availability of acetate or glutamate for \( \text{N}^- \)-acetylglutamate formation, leads to an inhibition of the carbamylphosphatesynthase reaction. In for example propionic acidemia \( \text{N}^- \)-propionylglutamate is formed instead, acting as a competitive inhibitor of the first step of urea formation resulting in hyperammonemia. An inadequate glutamate availability on the other hand is the cause of carbamylphosphatesynthase inhibition in glutamate dehydrogenase overexpression, resulting in hyperinsulinemic hypoglycaemia and hyperammonemia.
neurotransmitters, and brain development and function. Sufficient folate intake during the periconceptual period of pregnancy is critical in prevention of neural tube defects. Dietary folate is taken up in the gut by the proton-coupled intestinal transporter (PCFT). From the liver, the main active metabolite 5-methyl-tetrahydrofolate (MTHF) is delivered to other organs by the vascular system. In addition to the PCFT, transmembrane MTHF transport can be mediated by the reduced folate carrier (RFC1), and by two GPI-anchored receptors, folate receptor alpha (FRα) and beta (FRβ). Nutritional folate deficiency and the hereditary folate malabsorption caused by PCFT-mutations cause systemic folate deficiency that manifests itself with megaloblastic anemia and variable neurological symptoms, such as mental retardation, movement disorder, and epileptic seizures. Several inborn errors of folate metabolism affect the regeneration or synthesis of the active MTHF, the most recently identified being dihydrofolate reductase (DHFR) deficiency. DHFR and most other defects of MTHF synthesis affect several organs including the brain.

CSF is the main folate source of brain cells and active MTHF transport into CSF occurs at the choroid plexus. However, the precise mechanism of MTHF transport across the blood-CSF-barrier is unclear. Deficiency of MTHF transport across the choroid plexus is caused by pathogenic mutations in the FOLR1 gene coding for FRα. Various stop mutations, splice mutations and missense mutations in the FOLR1 gene have been found in the DNA of patients with cerebral folate transport deficiency (CFTD). Patients might present with an epileptic encephalopathy including an atactic-dystonic movement disorder at the 3rd year of life or mights how a more protracted course with a progressive developmental delay, autistic behavior and ataxia in early childhood. No strict genotype-phenotype correlation can be inferred at present. But most patients reveal a very low MTHFCSF concentrations (<10nmol/L) as well as cerebellar and cerebral atrophy, hypomyelination and patchy WM lesions in the cranial MRI. Many patients with CFTD respond to supplementation with oral folic acid (up to 5 mg/kg daily) with improved motor function and reduced frequency of convulsions. Alternative routes of application should be considered in case of unsatisfactory clinical improvements.

In conclusion, CFTD should be considered for any patient with developmental retardation and atactic movement disorder. Since it can be treated by folic acid supplementation the diagnosis should be made as early as possible.
The eye is the fourth most common system affected by genetic disease. At least a third of hereditary systemic disorders have ocular abnormalities. Inherited metabolic disorders also involve the eye in various fashions and may cause significant visual disturbances and sometimes blindness. Furthermore, characteristic ocular findings can assist in the diagnosis of inherited metabolic disorders. Pathologic changes in the eye are characteristic of the underlying metabolic disease process. The detection of these ocular abnormalities depends on their prominence, the severity and the familiarity of the ophthalmologist with their nature and significance. In many instances, the patient is referred with a diagnosed systemic disease, and a search for the known ocular manifestations of the illness is undertaken. However, in other instances, a metabolic disorder is suspected but no diagnosis is offered, making the ophthalmologic findings, if present, even more valuable in the diagnostic process. Ophthalmologic findings such as corneal opacities, cataracts, cherry-red spot, and retinal degeneration may be the earliest signs of many inherited metabolic disorders. Prompt and accurate diagnosis of the systemic diseases aids in determining the prognosis and clinical expectations regarding career and life planning for the affected individual. It allows the early institution of treatment and the provision of genetic counseling on the risk of recurrence in siblings or in their children. The occurrence of ocular abnormalities in inherited metabolic disorders could be due to direct toxic mechanisms of abnormal metabolic products or accumulation of normal metabolites by errors of synthetic pathways or by deficient energy metabolism. A detailed ophthalmological assessment is essential. Recent advances in the diagnosis and treatment of inherited metabolic disorders have substantially improved the diagnosis, evaluation, and the prognosis of the various ocular conditions found in the inherited metabolic disorders.
Luncheon Symposium III
Oral Session Abstracts
Objective: Nonketotic hyperglycinemia (NKH) is a devastating neurometabolic disorder, leading to early death or severe disability. Classical treatment strategies for NKH included a reduction of glycine by sodium benzoate, and a blockade of the N-methyl-D-aspartate (NMDA) receptor by the NMDA receptor antagonist dextromethorphan.

Methods: Four patients (three with typical neonatal form and one with atypical late-onset form) are presented. Diagnosis was based on the finding of hyperglycinemia and hyperglycinuria in the absence of an organic acid disorder and on calculation the cerebrospinal fluid/plasma glycine concentration ratio greater than 0.08 (normal values below 0.04). All patients were treated with sodium benzoate 500mg/kg/day, dextromethorphan 5mg/kg/day and L-carnitine 50mg/kg/day. In one infant with neonatal form of NKH we started treatment with ketogenic diet at the age of 18 months after informed consent of parents.

Results: After classical treatment of NKH, glycine concentration in plasma decreased and condition of our 3 infants only temporarily improved. One died at the age of 34 days, other have suffered from severe brain damage at the age of 6 years. Only patient with atypical late-onset form have only moderate brain damage at the age of 7 years. In one male patient with neonatal form of NKH despite classical treatment extremely hypotonia and severe clinical state persisted, so we used a new treatment with ketogenic diet. Shortly after its institution, clinical state promptly improved and after one year of ketogenic diet, cerebrospinal fluid glycine markedly decreased from 124umol/L to 52.2umol/L (normal < 10.1), hypotonia and muscle strength improved, alertness increased and seizures ceased.

Conclusions: Our experience showed, that classical treatment of NKH favourably modified only early neonatal course, but does not prevent poor long-term prognosis of patients. In one patient with severe neonatal form of NKH a new treatment with ketogenic diet improved his clinical state and decreased cerebrospinal fluid glycine. Experience of other patients with NKH are required to confirm this beneficial effect of ketogenic diet.

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Mutations in Genes Encoding the Glycine Cleavage System Predispose to Neural Tube Defects in Mice and Humans

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Objective: Folate metabolism is associated with neural tube defects (NTDs). The glycine cleavage system (GCS) is a mitochondrial multienzyme complex, breaking down glycine to donate one-carbon units to tetrahydrofolate. The GCS is expressed during embryogenesis and, owing to the potential effect of the GCS on folate metabolism, we hypothesized that the GCS could affect susceptibility to NTDs.

Methods: The coding regions of two GCS genes, AMT and GLDC, were sequenced in UK and Japanese NTD patients and matched controls. GLDC cDNAs containing putative mutations were expressed in COS7 cells for enzymatic analysis. Mouse embryos with NTDs were recovered following knockout of Amt, which encodes an essential GCS component. Possible preventive effects of folic acid and related metabolites were tested by maternal supplementation of Amt mutant mice.

Results: We identified two unique non-synonymous changes in AMT that were absent from controls. We also identified a splice acceptor site mutation and six different non-synonymous variants in GLDC, which were found to significantly impair enzymatic activity and represent putative causative mutations. In order to functionally test the requirement for GCS activity in neural tube closure we generated mice which lack GCS activity, through mutation of AMT. Homozygous mutant mice developed NTDs at high frequency. NTDs were not preventable by supplemental folic acid, but there was a partial rescue by methionine.

Conclusions: Our results suggest that loss-of-function mutations in the GCS genes predispose to NTDs in mice and humans. These data highlight the importance of adequate function of mitochondrial folate metabolism in neural tube closure (Hum Mol Genet, 2012 in press)

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**O-03**

**Long-term Outcome and Intervention of Urea Cycle Disorders in Japan**

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Objective: Urea cycle disorders (UCDs) are one of the most frequently inherited metabolic diseases in Japan, with an estimated prevalence of 1 per 50,000 live births. Here, we investigated the clinical manifestations, treatment, and prognosis of 177 patients with UCDs who were evaluated and treated from January 1999 to March 2009.

Methods: In 2009, we sent a questionnaire to 928 institutions, including the departments of pediatrics, endocrinology and metabolism, neonatology, genetics, and transplant surgery, asking doctors if they had diagnosed or provided medical care to UCD patients. These included 77 cases of neonatal-onset UCDs and 91 cases of late-onset UCDs.

Results: The most common UCD was ornithine transcarbamylase deficiency (OTCD), which accounted for 116 out of 177 patients. This result is similar to a previous study performed between 1978 and 1995 in Japan: OTCD accounted for about two-thirds of the total number of UCD cases. We studied the relationship between prognosis and the peak blood ammonia level at the onset in 151 UCD patients. Compared with a previous survey conducted in Japan, we found that a greater number of patients survived without any mental retardation despite their peak blood ammonia levels being greater than 360 μmol/l. The 5-year survival rate of patients with OTCD improved to 86% for those with the neonatal-onset type and to 92% for those with the late-onset type.

Conclusions: This result is similar to a previous study performed between 1978 and 1995 in Japan: OTCD accounted for about two-thirds of the total number of UCD cases. We studied the relationship between prognosis and the peak blood ammonia level at the onset in 151 UCD patients. Compared with a previous survey conducted in Japan, we found that a greater number of patients survived without any mental retardation despite their peak blood ammonia levels being greater than 360 μmol/l. The 5-year survival rate of patients with OTCD improved to 86% for those with the neonatal-onset type and to 92% for those with the late-onset type.

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Application of Haplotype Analysis Employing SNPs in the Human OTC Locus to the Study of Origin of Mutant Allele

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Objective: Haplotype analysis of human ornithine transcarbamylase (OTC) gene was cumbersome due to lack of appropriate DNA markers. Availability of single nucleotide polymorphism (SNP) in the OTC gene, however, has enabled to construct haplotype easier. The aim of the present study is to test whether or not haplotyping employing SNPs is useful firstly to identify origins of alleles carrying a specific mutation, and secondly to identify mutation-bearing allele in pedigree where no specific mutation is found by conventional sequencing.

Methods: A total of 9 tagged SNPs selected on the Haploview with r² of 0.80 and MAF of 0.05 were used. These SNPs are all located within the OTC gene. The SNPs were determined by the TaqMan probe-based real-time PCR.

DNA specimens were obtained from patients carrying specific mutations, c.119G>A, c.163T>G and c.829C>T, found in discrete families worldwide and one Japanese family with OTC deficiency with no known mutation.

Results: A total of 10 haplotypes were constructed in patients and their families. In 14 families carrying the c.119G>A mutation, 8 families had haplotype 1, each 2 had haplotypes 2, 3 and 4. Two families with the c.163T>G both had haplotype 1. In 5 families with the 829C>T mutation, 3 families had haplotype 3 and the remaining two had haplotypes 2 and 6, respectively.

In the Japanese family with no known mutation, the allele with haplotype 9 was found to be the mutation-carrying allele, allowing genetic counseling.

Conclusions: Haplotype analysis employing SNPs is useful in identifying origin of an OTC allele. It can be applicable to identification of a mutation-carrying allele in family with no known mutation with a minimum risk of erroneous judgment because of minimum recombination frequency among the markers used.

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SLC25A13 Gene Analysis in Citrin-deficient Patients: Experience on an Eighty-nine-case Cohort in a Chinese Pediatric Center

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Objective: Citrin deficiency is an autosomal recessive disorder with SLC25A13 as the causative gene which is located at 7q12.3 and encodes citrin, a mitochondrial aspartate/glutamate carrier (AGC) protein mainly expressed in the liver. This paper aims to summarize the mutation spectrum of SLC25A13 gene in patients with citrin deficiency in an Asian Pediatric center.

Methods: High-frequency mutations of SLC25A13 gene in Chinese population, including 851del4, 1638–1660dup, IVS6+5G>A and IVS16ins3kb, were screened by routine approaches such as PCR/LA–PCR and PCR–Restriction Fragment Length Polymorphism (RFLP), in patients with clinical and laboratory presentations suggestive of intrahepatic cholestasis. In some cases with just one SLC25A13 mutation uncovered, direct genome DNA sequencing was performed to sequence all the 18 exons and their flanking sequences to identify novel mutation/variations in SLC25A13 gene.

Results: By end of the year 2011, eighty-nine citrin-deficient patients have been diagnosed by SLC25A13 analysis in our Department of Pediatrics. Eighty-seven of the patients are Chinese, and the remaining two Malaysian. Twenty-one mutations in total were uncovered in this Asian citrin-deficient cohort. Among these mutations, 851del4, 1638–1660dup, IVS6+5G>A and IVS16ins3kb take account for 84.0% of all the mutated alleles, while the remaining 17 mutations/variations, including R467X, IVS11+1G>A, R319X, A541D, G333D, R360X, V411M, G283X, Q259X, Q159X, M1T, D350N, R355G, G139R, R455L, Ex7–1G>A and Y24X as well, just occupied 12.6%. Moreover, mutations in 6 alleles (3.4%) of SLC25A13 gene still remain to be identified in this citrin-deficient cohort.

Conclusions: SLC25A13 mutations/variations presented with remarkable heterogeneity, while 851del4, 1638–1660dup, IVS6+5G>A and IVS16ins3kb constituted high-frequency SLC25A13 mutations in this Asian citrin-deficient cohort. So far as know, D350N, R355G, G139R, R455L, Ex7–1G>A, and Y24X identified in this paper are six novel mutations that have not been described yet in any other references.

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Simple and Rapid Genetic Testing for Citrin Deficiency by Screening 11 Prevalent Mutations in SLC25A13

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Objective: Citrin deficiency is an autosomal recessive disorder caused by mutations in the SLC25A13 gene and has two disease outcomes: adult-onset type II citrullinemia and neonatal intrahepatic cholestasis caused by citrin deficiency. The clinical appearance of these diseases is variable, ranging from almost no symptoms to coma, brain edema, and severe liver failure. Genetic testing for SLC25A13 mutations is essential for the diagnosis of citrin deficiency because chemical diagnoses are prohibitively difficult. Eleven SLC25A13 mutations account for 95% of the mutant alleles in Japanese patients with citrin deficiency. Therefore, a simple test for these mutations is desirable. The goal of this study was to establish a rapid and simple test for the detection of the 11 most common SLC25A13 mutations.

Methods: We employed a 1-hour, closed-tube assay for the 11 SLC25A13 mutations using real-time PCR. Each mutation site was amplified by PCR followed by a melting-curve analysis with adjacent hybridization probes (HybProbe, Roche). The 11 prevalent mutations were detected in seven PCR reactions. Six reactions were used to detect a single mutation each, and one reaction was used to detect five mutations that are clustered in a 21-bp region in exon 17. To test the reliability, we used this method to genotype blind DNA samples from 50 patients with citrin deficiency. For the estimation of the heterozygous carrier frequency, 420 genomic DNA samples from healthy volunteers were screened using the HybProbe analysis for the 11 prevalent mutations. Results: Our genotyping results were in complete agreement with those obtained using previously established methods. Furthermore, the mutations could be detected without difficulty using dried blood samples collected on filter paper. We found 10 mutations in 420 Japanese healthy controls.

Conclusions: We have established a rapid and simple detection system using the HybProbe assay for the 11 prevalent mutations in SLC25A13. This system could be used to screen newborns for citrin deficiency and may facilitate the genetic diagnosis of citrin deficiency, especially in East Asian populations.

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Hign Risk Screening on Inborn Error Metabolism Disorders in National Hospital of Pediatrics in North Vietnam

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The first time we have a survey for high risk screening on inborn error metabolic disorders (IEMs) at National Hospital of Pediatrics, Hanoi (NHP), the highest level of Pediatric system in Vietnam. No data inform IEMs in Vietnam previously.

Objective: To study the spectrum of IEM, prevalence and survival rate of patients with IEMs at NHP.

Patients and Methods: 877 high-risk patients were treated at NHP from 2005 to 2011. Blood and urine samples are analyzed by Tandem MS & GC/MS at Shimane University, Japan, and gene Beta- ketothiolase deficiency (BKT) are analyzed in Gifu University, Japan.

Results: 101/ 877 (11%) high risk cases, out of 315,000 inpatients, identified with 21 kinds of IEMs. Age range from 2 days to 8years, group < 12 months accounted 75%. Among 101 cases (+), 61% were organic acidemia with 10 different kinds, and common is 16% BKT, others are methylmalonic academia MMA, 5-oxoprolinuria, propionic academia PA, glutaric academia type 2, 3-methylglutaconic aciduria, congenital lactic acidemia, multiple carboxylase deficiency, glutaric academia type 1, 3-methylcrotonylCoA carboxylase deficiency : 19% aminoacidopathy with 2 different kinds: MSUD & PKU; 11% fatty acid oxidation disorders include 6 different types: deficiency of MCAD, SCAD VLCAD, CPT2, or CPT1; & 6% ure cycle defect with 3 different kinds: OTC deficiency, Citrulinemia type 1 or Arginosuccinic aciduria. In 12/17 families of BKT gene analysis, 4 types of mutations were found: R208X, the most common in Vietnam 70% with 10/12 cases of R208X: 7 cases of homozygous, 3 cases of heterozygous; IVS10-1g>c; A410V & one novel mutation 163_167del5ins2 from maternal allele. Management saved lives without complication in 22%, with complication in 18%, and the remains (60%) died.

Conclusions: Prevalence of high risk screening IEMs is high 11%. Organic acid disorders are the most common 61%. Mutation R208X (70%) in BKT deficiency is common in Vietnam & one novel mutation 163_167del5ins2. Management is poor with death rate still high at 60%. Early detection of these disorders is essential for prevention from impairments.
Phenotype and Genotype of Vietnamese Patients with Maple Syrup Urine Disease

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Objective: Maple syrup urine disease (MSUD) is caused by decreased activity of the branched-chain alpha-ketoacid dehydrogenase complex (BCKAD), the second enzymatic step in the degradative pathway of the branched-chain amino acids (BCAAs). Mutations in any of the three different genes BCKDHA, BCKDHB, and DBT encoding for the E1alpha, E1beta, and E2 catalytic components of the BCKAD complex can cause MSUD. Severity of the disease ranges from the classical to the mildest variant types. Here, we describe the phenotype and genotype in a cohort of 15 Vietnamese patients with MSUD.

Methods: This is a case series study. Clinical manifestations, biochemical changes and brain MRI lesion were evaluated at Vietnam National Hospital of Pediatrics (NHP) in Hanoi, Vietnam. Tandem mass spectrometry (MS/MS)-based amino acid profiling of dried blood spots and analysis of urine organic acids by gas chromatography-mass spectrometry were performed at Shimane University – Japan. Mutation analysis of BCKDHA, BCKDHB, and DBT were performed for 7 cases at Samsung Medical Center, Korea.

Results: 15 cases from 15 unrelated families included 9 boys and 6 girls. Age of onset was from 3 days to 6 months of age. 12/15 cases had symptoms before 15 days of age. Age of diagnosis was 7 days to 2 years of age. 8/15 cases had family history. Clinical manifestations were maple syrup odor in cerumen (15/15); poor feeding (12/12), lethargy (12/12), coma (8/12) in neonatal period; mental retardation in 3 cases (2 onset at 4 months and 1 onset at 6 months of age). Biochemical changes were metabolic acidosis (8/14), ketonuria (2/8), hypoglycemia (3/15), increased plasma concentrations of leucine (15/15) and large quantity branched-chain ketoacids of leucine and isoleucine in the urine (15/15). Brain MRI lesion was seen in 9/9 cases. 5 patients have only one identified mutant allele in BCKDHA (c.868G>A: p.Gly290Arg, and c.1211A>G: p.Asn404Ser), BCKDHB (c.1159C>T: p.Arg387X and c.1159C>T: p.Arg387X), or DBT (c.263_265delAAG: p.Glu88del) while only 2 cases are homozygous for either BCKDHA (novel mutation c.1280_1282delTGG: p.Leu427_Ala428delinsPro) or BCKDHB (c.564T>A: p.Cys188X). Mutations in BCKDHA (c.868G>A: p.Gly290Arg and c.1211A>G: p.Asn404Ser) were inherited from father. Both parent of the patient with mutation in BCKDHB (c.1159C>T: p.Arg387X) did not have the identified mutation in their child. One of the two DBT variants in one case was inherited from his father and testing for another variant is ongoing. Parents of two patients with homozygous mutations for either BCKDHA or BCKDHB are carrier.

Conclusions: The results of this study indicate heterogeneous clinical and molecular phenotypes in Vietnamese patients with MSUD.

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The clinical and molecular genetic characteristics of Korean patients with Fabry disease

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Objective: Fabry disease is caused by an alpha-galactosidase A (GLA) deficiency. This study was performed to investigate the clinical and molecular genetic characteristics of Korean patients with Fabry disease.

Methods: Clinical presentations, clinical courses with enzyme replacement therapy, and GLA mutation spectrum were reviewed.

Results: Thirty-five unrelated Korean families, comprised of 44 males and 11 females, had been enrolled in the Korean Fabry Registry. The presenting signs were acroparesthesia (23%), proteinuria (19%), hypertrophic cardiomyopathy (HCMP; 8%), angikeratoma (4%), heat stroke (4%) and renal failure (4%). The remaining 38% of the patients were identified by familial screening. The average onset age was 14.3 ± 8.3 yrs, and the age at diagnosis was 28.8 ± 13.3 yrs. HCMP, hearing difficulty, proteinuria (>300mg/day), and end stage renal failure were noted in 46%, 62%, 23%, 8% of the patients, respectively. Enzyme replacement therapy was commenced at 31.4 ± 13.6 yrs of age. During 47.4 ± 25.1 months of therapy, no patient experienced the aggravation of HCMP or proteinuria, deterioration of renal function, or stroke-like episode. Thirty distinct mutations in the GLA gene including 8 novel mutations (p.W47X, p.D61EfsX32, p.Y86H, p.C90X, IVS4-11T>A, p.G274R, p.D322E and p.W349) have been identified. The GLA activity of each mutant was decreased either in patient's leukocytes or in transiently over-expressed COS-7 cells. Besides, five subjects from four unrelated families carried the p.E66Q variant, known to be a functional polymorphism rather than a pathogenic mutation.

Conclusions: More efforts are needed to identify more cases with Fabry disease, which shows a wide spectrum of clinical manifestations. The variable molecular genetic heterogeneities of Fabry disease were also noted in this cohort, which might reflect ethnic diversity and affect their correlations to the phenotypes.

Contact Information: Han-Wook Yoo (bhlee@amc.seoul.kr)
Plasma Globotriaosylsphingosine (LysoGb3) is a Reliable Biomarker for Cardiac Variant Fabry Disease Causing by Chinese hotspot mutation (IVS4 + 919G→A)

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Objective: Fabry disease is an X-linked lysosomal storage disorder due to deficiency of alpha-Galactosidase A, causing accumulation of globotriaosylceramide(Gb3) and globotriaosylsphingosine (lysoGb3). The diagnostic value and clinical relevance of metabolite concentration was investigated. In Taiwan, newborn screening result for Fabry disease has revealed a high incidence of the cardiac variant GLA mutation, IVS4+919G>A (~1 in 1,500-1,600 males). However, our preliminary data showed that their plasma and urinary globotriaosylceramide (Gb3) were not elevated in most of these IVS4+919G>A cardiomyopathy patients. In contrast, noticeable elevations of plasma and urinary Gb3 were observed as early as in their neonatal period from patients with classical-type mutations. In the follow-up investigation presented here, patients with classic and cardiomyopathy patients of Fabry disease were analyzed with plasma lysoGb3 content. The aim was to determine the relationship between plasma lysoGb3 and clinical manifestations of Fabry disease.

Methods: In this study, we examine the plasma lyso-Gb3 and GB3 levels by HPLC-MS/MS method for total 154 patients, including 21 classical-type mutation, 92 carrying IVS4+919G>A mutation, and 41 with novel mutation.

Results: All the patients with classical-type could be identified by a significantly elevation in plasma lyso-Gb3. In IVS4+919G>A mutation, plasma lyso-Gb3 levels also significantly increases in both presymptomatic and symptomatic patients. We also found that the plasma lyso-Gb3 elevated gradually, as the IVS4+919G>A patients got older; and there is a correlation between lyso-Gb3 level and left ventricular mass in both males and females. Besides, among these IVS4+919G>A adults, males with plasma lyso-Gb3 levels greater than 6.5nM and females greater than 3.0 nM, have already developed prominent hypertrophic cardiomyopathy. But plasma lyso-Gb3 levels were variant from different types of novel mutation patients.

Conclusions: The plasma level of lyso-Gb3 increases significantly in classical-type and cardiac-type patients, and it was well related to the clinical manifestations of IVS4+919G>A patients. Thus, lyso-Gb3 is expected to be a useful biomarker for assessing for Fabry disease.

Contact Information: Hsuan-Chieh Liao (laiojoyce@gmail.com)
Mechanism of Endoplasmic Reticulum Stress-Independent Autophagic Activation
In Pompe Disease Fibroblasts

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Objective: Pompe disease is an autosomal recessive lysosomal storage disorder caused by a deficiency of acid α-glucosidase. Activation of autophagy is a prominent feature in skeletal muscles and fibroblasts from patients with Pompe disease, and massive accumulation of autophagosomes has been shown to interfere with efficacy of the ERT. We previously found that mutant GAA protein-induced endoplasmic reticulum (ER) stress is involved in induction mechanism of autophagy in fibroblasts from Pompe disease patients. However, since autophagic buildup is observed in GAA-KO mice, which totally lack GAA protein, ER stress is not sole factor in induction of autophagy in Pompe disease. In this study, we focused on akt signaling pathway, which regulates both autophagy and glycogen metabolism, and analyzed this pathway in patient fibroblasts to clarify their involvement in regulation of autophagy.

Methods: Skin fibroblasts from Pompe disease patients and healthy individual were cultivated in media containing high or low glucose, and were treated with insulin. These cells were lysed with 2% SDS, and analyzed by Western blotting using the antibodies against phosphorylated akt, phosphorylated S6 kinase and LC3.

Results: Both levels of phosphorylated akt and phosphorylated S6 kinase in patient fibroblasts were lower than those in normal fibroblasts, whereas levels of LC3-II were higher in patient cells than normal cells. Low glucose condition decreased the both levels of phosphorylated akt and phosphorylated S6 kinase in normal fibroblasts, and increased the levels of LC3-II in them. However, no significant changes were observed in patient fibroblasts which were cultivated in low glucose medium. In addition, treatment with insulin not only increased the levels of phosphorylated akt, but also decreased the levels of LC3-II in patient fibroblasts.

Conclusions: These results suggest that down-regulation of akt signaling pathway is involved in the induction mechanism of autophagy in the Pompe disease patient fibroblasts.

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Differentiation into Skeletal Muscle Cells from Mouse Pompe-iPS cells and Possible Application for Cell Therapy

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Objective: Pompe disease accumulates glycogen into somatic cells that induced cardiac hypertrophy, muscle weakness and respiratory failure because of acid α-glucosidase (GAA) deficiency. We aim to generate skeletal muscle cells from iPS cells derived from model mouse of Pompe disease (Pompe-iPS cells) in order to elucidate of pathophysiological mechanism of Pompe disease.

Methods: First, we generated iPS cells from model mouse of Pompe disease (GAA K.O. 8week-old), using reprogramming three factors (Klf4, Oct3/4 and Sox2). These iPS cells stained periodic acid-Schiff (PAS; indicating the accumulation of glycogen) and acid phosphatase (AP; lysosomal activation marker) and assayed level of GAA activity. Thereafter, we carried out to differentiate into skeletal muscle like cells from our iPS cells using Matrigel-coated plates. Differentiated cells were stained with myosin heavy chain and analyzed of transmission electron microscopy.

Results: We confirmed iPS colonies from 27 days after transfection and these iPS cells were expressed 13 ES marker genes by analysis of RT-PCR. These iPS cells also showed decreased levels of GAA activity and strong positive staining with PAS stain and AP stain. Next, differentiated cells showed skeletal muscle cell-like spindle-shaped fiber cells and spontaneous contraction. In addition, these cells were strongly positive for myosin heavy chain stain (marker for specific skeletal muscle protein). Furthermore, differentiated skeletal muscle like cells showed typical construction, including Z-bands, I-bands, A-bands and we confirmed accumulation of glycogen granules-like high electron density structure generated from Pompe-iPS cells.

Conclusions: We successfully generated iPS cells from a mouse model of Pompe disease and these iPS cells reflected pathology of Pompe disease. Furthermore, the Pompe-iPS cells differentiated into skeletal muscle like cells that were positive for myosin heavy chain stain. The differentiated cells showed accumulation of glycogen granules-like inclusions surrounded by single unit membrane. Our study may be useful in studies investigating the pathogenesis of Pompe disease, and also might eventually be enable to produce rejection-free development of autologous cell transplantation therapy.

Contact Information: Shiho Kawagoe (kawagoe@jikei.ac.jp)
Prenatal Counseling and Diagnosis of Gaucher Disease in Egypt

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Objective: Prenatal counseling and diagnosis of Gaucher disease in pregnant females with previous affected sibling(s).

Methods: • Primary intervention: from June 2000 to November 2011, prenatal counseling was done in 62 pregnancies among 48 females. Five times in 3 females and 4 times in another 2. Positive consanguinity found in 40 (83\%) couples. • Secondary intervention: chorionic villus sampling (CVS) was done between 11–12 weeks gestational age in 44 pregnancies among 34 females. Three times in 3 females and 2 times in another 4. B-glucocerebrosidase activity was measured in CVS.

Results: • Primary outcome: 44 (71\%) of 62 counseled pregnancies proceeded to CVS. 18 (29\%) were not subjected to CVS. Ten did not show up at scheduled time, 4 already came late for P.D, 3 had missed abortion and one refused. • Secondary outcome: 31 (72\%) of 43 had normal B-glucocerebrosidase activity and 12 (28\%) had no or low activity i.e affected fetus. In one case we could not retrieve enough chorionic villi.

Conclusions: In spite of the presence of enzyme replacement therapy for GD, it is not effective for all types in addition to high cost and variable response. So, our responsibility in prenatal counseling is to offer early diagnosis by CVS, as one of the options, to the pregnant/couple.

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Pseudodeficiency Alleles of Iduronate 2-sulfatase Gene and the Structural Modeling of the Enzyme Protein

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Objective: Enzyme replacement therapy (ERT) for mucopolysaccharidosis Type II became available in Japan. As it is speculated that ERT will show more efficacy before the symptoms appear, we carried out newborn screening of mucopolysaccharidosis type II against the babies born in Osaka City University Hospital. In this study, we found two novel pseudodeficiency alleles (P284L and P260H) of iduronate 2-sulfatase gene (IDS). We made structural models of IDS with P284L or P260H to confirm these amino acid substitutions are non-pathogenic.

Methods: Newborn screening, and enzyme and molecular analysis: Dried blood spots (DBS) of newborn babies were collected and analyzed the enzyme activity of IDS by ELISA using IDS polyclonal antibody to capture the IDS protein and 4-MU-sulfate to measure the IDS activity. For the male babies with low activity of IDS in DBS, the IDS activity in peripheral lymphocytes was analyzed using 4-MU-α-L-iduronide-2-sulfate, and molecular analysis of IDS gene was performed. Structural modeling: A structural model of human IDS was constructed using the crystallographic structures of putative sulfatase from Rhizobium leguminosarum (PDB ID: 2VQR) and arylsulfatase from E. coli (PDB ID: 3ED4) as the templates by means of the molecular modeling software of MODELLER (http://www.salilab.org/modeller/). Then, the structural models of IDS with P284L or P260H were constructed on this homology model of the human IDS protein by the molecular modeling software of TINKER (http://dasher.wustl.edu/tinker/), and the energy minimization was performed on these constructed molecules.

Results: Newborn screening was done in 892 babies (M:F=456:436) from 2009 to 2011. We found two male babies from unrelated families with deficient iduronate 2-sulfatase activity. They showed normal pattern of urinary glycosaminoglycan secretion and have the same genetic abnormality (P284L) in IDS gene. Each of the babies had healthy male family members with P284L. Furthermore, we carried out a screening of IDS activity in 150 healthy male volunteers, and found another pseudodeficiency allele (P260H) of IDS. The results of structural modeling of IDS with P284L or P260H suggested that a small structural change occurred on the molecular surface by either of the amino acid substitutions, but that the active site of IDS was not affected.

Conclusions: Screening of lysosomal storage diseases (LSD) is updated issue because the ERT is now available for some of the LSD. The screening of LSD is usually performed by the enzyme analysis in DBS. However, a deficient enzyme activity does not necessarily mean the affected metabolism in vivo, because the low enzyme activity may be sufficient for the metabolism in vivo or the altered enzyme may have a considerable activity against the natural substrates. To make the diagnosis without symptom is not easy in LSD because the abnormal metabolites are not easily detected in blood or urine.
Intraventricular Enzyme Replacement Therapy (ERT) for MPS II Murine Model

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The mucopolysaccharidosis type II (MPS II), as you know Hunter syndrome, is a lysosomal storage disorder caused by a deficiency of iduronate-2-sulfatase (IDS), characterized by the accumulation of glycosaminoglycans (GAGs). The MPS II has been treated by hematopoetic stem cell therapy (HSCT) / enzyme replacement therapy (ERT), but this effectiveness in central nervous system (CNS) is limited because of poor uptake of enzyme to cross the blood brain barrier (BBB). To increase the efficacy of ERT in the brain, we tested intraventricular ERT procedure repeatedly of IDS in IDS knockout MPS II murine model.

Methods: We tested multiple intraventricular ERT procedure (20ug/mice/3 weeks) of IDS in IDS knockout MPS II murine model. The IDS enzyme activities were measured in the brain and other tissues. The total GAGs storage was measured in the brain. The effects of intraventricular ERT on murine behavior were examined by Y-maze. Some tissues treated with/without intraventricular ERT of IDS MPS II mouse were analyzed pathologically.

Results: The IDS activities were significantly increased in MPS II mice and the accumulation of total GAGs was decreased in the brain treated with IDS multiple administration of intraventricular ERT. In MPS II mice, the high level IDS activities were maintained in other tissues (liver, spleen and testis etc.) by intraventricular administration of enzyme. IDS treated mouse groups induced the recovered of short-term memory, and behavior and activity. Although large and small vacuoles were found at the margin of cerebellum Purkinje cell in disease control mouse, these vacuoles disappeared treated with IDS. Loss of vacuoles was also observed in the other tissues (liver, kidney and testis).

Conclusions: Using intraventricular ERT administration, IDS was distributed not only in brain but also in various tissues (liver, spleen and testis etc.). And the IDS improved brain functions which include short-term memory and behavior. These results demonstrate the possibility and efficacy of novel ERT procedure with intraventricular administration for MPS II treatment.

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Optimal Clinical Outcome in Early Initiation of Enzyme Replacement Therapy for a Pre-symptomatic Newborn Patient with Mucopolysaccharidosis VI

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Objective: Preclinical and clinical studies suggest early initiation of systemic therapies like hematopoietic stem cell transplantation (HSCT) or enzyme replacement therapy (ERT) are beneficial in patients with mucopolysaccharidoses. The aim of the study is to investigate therapeutic efficacy of early initiation of ERT by a sibling study of mucopolysaccharidosis VI (MPSVI).

Methods: Two siblings with MPSVI started ERT with weekly infusion of recombinant human arylsulfatase B (ASB) at 1mg/kg. Sibling 1 started ERT 5.6 years of age and sibling 2 was 6 week old. The disease status in these two siblings prior to and for 36 months of ERT was followed up and compared.

Results: The treatment was well tolerated by both siblings. During 36 months of ERT, symptoms typical of MPSVI including short stature, progressive dysmorphic facial features, hepatosplenomegaly, hearing impairment, corneal clouding, and dysostosis multiplex were largely absent in the younger siblings. Her cardiac functions and joint mobility were well preserved. On the other hand, her affected brother had typical MPS phenotypic features described above before starting ERT at the equivalent age, of 3 years. There was significant improvement in the shoulder range of motion and hearing loss after 36 months of ERT and cardiac function was largely preserved. His skeletal deformity and shot stature remained unchanged or slightly advanced. The results showed that early ERT initiated at newborn is safe and effective in preventing or slowing down disease progression of MPSVI including bone deformities.

Conclusions: Based on these observations, we conclude that early diagnosis and treatment of MPSVI before development of an irreversible disease is critical for optimal clinical outcome.

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Pulmonary Function Assessment in Patients with Mucopolysaccharidoses: Experience in Taiwan

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Objective: To evaluate the pulmonary function in patients with mucopolysaccharidoses (MPS) in Taiwan.

Methods: We performed spirometric studies in 35 patients (22 males and 13 females; 1 with MPS I, 12 with MPS II, 16 with MPS IV, and 6 with MPS VI; mean age, 14.6 ± 5.9 years; age range, 6.4 years to 33 years). Forced vital capacity (FVC), forced expired volume in 1 sec (FEV1), FEV1 to FVC ratio (FEV1/FVC), peak expiratory flow (PEF), and mean forced expiratory flow during the middle half of FVC (FEF25–75) were measured.

Results: The average FVC, FEV1, PEF, and FEF25–75 were 74.2%, 73.9%, 64.7%, and 37.1% of the predicted values, respectively. By spirometric classification, fifteen patients (43%) had normal pulmonary function, three (9%) with obstructive lung disease, and 17 (48%) with restrictive lung disease. Thirty-two patients (91%) had small airway disease (FEF25–75 < 65%). The pubertal and postpubertal subjects had significantly lower values for FVC, FEV1, PEF, and FEV1/FVC than did the younger subjects. The values of FVC, FEV1, PEF, and FEV1/FVC were all negatively correlated with increasing age (p < 0.01). Follow-up pulmonary function test was also performed for 8 patients who underwent enzyme replacement therapy for 1.5 to 7.4 years. Six and five patients had improvement in the FVC and FEV1 values after treatment, respectively.

Conclusions: These findings and the follow-up data will help to determine the abnormalities of pulmonary function more precisely and will help with the quality of care for patients with MPS.

Contact Information: Hsiang-Yu Lin (lxc46199@ms37.hinet.net)
Objective: For improvement of patients QOL by develop radical treatment of Lysosomal storage disease (Krabbe disease), we investigate the method of lentivirus mediated gene therapy. Recombinant lentiviral vector can be delivered in vivo and integrate efficiently into the genome of dividing and non-dividing cells, and provide long-term expression of the transgene.

Methods: The lentiviral vector SMPUR-MND-GALC-IE was constructed by cloning the human b-galactocerebrosidase (GALC, the deficient in Krabbe disease) cDNA into the Bam HI and Sal I site and IE indicates the presence of the ECMV IRES followed by the eGFP gene. After checking the efficient and dose dependent expression of GALC enzyme in vitro, we have tried gene transfer in vivo. Newborn mice genotyped as homozygote (-/-) were injected via the facial vein with 50 ml of SMPUR-MND-GALC-IE. For evaluate the synergy effect of gene therapy and substrate reduction therapy, we tried subcutaneous injection of L-Cycloserine (L-CS), 25-75mg/kg. Mice were sacrificed at 5 weeks of age and tissues were harvested immediately and analyzed for GALC enzyme activity and psychosine storage by HPLC assay.

Results: In vitro, we have succeeded in dose dependent transduction for cell lines including fibroblast and oligodendrocytes cell line originally from Krabbe patient. And in vivo, we succeeded in gene transfer for Krabbe model mice, resulted in efficient improving of their body weight at 14-15 days -old, and the pathology of their sciatic nerve. And administering lentiviral vector with L-CS, we had some improvement in nutrition and life span.

Conclusions: The lentiviral gene therapy for Krabbe disease was thought to be effective in Krabbe disease, including efficient gene transfer into the central nervous system and peripheral nervous system. And some effects in the combination therapy with L-CS suggested synergism of gene therapy and substrate reduction therapy.

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Laboratory Diagnosis of Glycogen Storage Disease in Egypt: Ten Years Experience

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Objective: This is a retrospective study, to find out the commonest type of GSDs among a group of high risk children with liver disease.

Methods: The present study included 204 patients with liver disease. Glycogen level and the activity of the different enzymes were determined for each patient. The enzyme activities were measured in peripheral blood according to Prof. Shin methods.

Results: From the 204 patients, 125 (61.3%) patients had been diagnosed with different types of glycogen storage disease as follow:

- GSD I: results from a deficiency of the enzyme glucose-6-phosphatase 9 (4.4%) patients.
- GSD III: results from a deficiency of the debranching enzyme (amylol-1,6-glucosidase, 69 (33.8%) patients.
- GSD IV: results from a deficiency of branching enzyme, 9 (4.4%) patients.
- GSD VIa: results from a deficiency of hepatic enzyme phosphorylase kinase, 38 (18%) patients.

Conclusions: GSD represents 61% of cases with liver disorders. The commonest type among the studied patients is type III representing 38.8% of the diagnosed cases.

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Clinical and Molecular Aspects of Japanese Children with Medium Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency

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Objective: Medium chain acyl-CoA dehydrogenase (MCAD) deficiency is a relatively common inherited metabolic disorder of mitochondrial fatty acid oxidation among Caucasians. Recently, a number of cases with MCAD deficiency have been reported in Asian countries since tandem mass screening become available. Herein, we report the clinical and molecular basis of MCAD deficiency in 18 Japanese patients, identified in Shimane University.

Methods: Eighteen Japanese patients from 16 families including previously reported cases were investigated. The diagnosis was based on mass spectrometric and mutation analyses.

Results: Ten of 18 patients with MCAD deficiency were symptomatic and the other 8 were asymptomatic. Seven of the 8 asymptomatic patients were detected in neonatal screening and the other was diagnosed by sibling screening. Hypoglycemia was seen 7 of 10 symptomatic patients. Five of the symptomatic patients were triggered by infection such as common cold or gastroenteritis. Two patients of the symptomatic patients had sudden infant death syndrome. Acylcarnitine analysis was available in 16 cases, and all these cases showed significant elevation of octanoylcarnitine (C8) in blood filter paper. Organic acid analysis was determined in 13 patients including 7 asymptomatic cases, and increased excretion of hexanoylglycine and suberylglycine in urine were detected in all except 1 case. Thirteen mutations in 32 alleles from 16 families were identified, and eight of them (c.275C>T (P67L), c.422A>T (Q116L), c.1085 G>A (G337E), c.212 G>A (G46D), c.820 A>C (M249V), IVS3+2T>C, IVS3+5G>A and IVS4+1G>A) were novel. 449–452delCTGA (34.4%, 11/32 alleles), deletion of exons 11 and 12 (4/32 alleles), c.157C>T (2/32 alleles) and c.843A>T (2/32 alleles) were detected.

Conclusions: The results suggest that Japanese patients with MCAD deficiency show allelic heterogeneity, and genetic background is a different from other countries. The 449–452delCTGA was detected in 34.4% of alleles of Japanese patients. The symptoms of patients with homozygous 449–452delCTGA mutation were extremely heterozygous, ranging from without symptoms to life-threatening metabolic decompensation, indicating that the genotype does not necessarily predict MCAD phenotype. We consistently found that there was no association between genotype and phenotype in Japanese patients with MCAD deficiency.

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Clinical and Molecular Analysis of Chinese Patients with Primary Carnitine Deficiency

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Objective: The aim of this study was to test mutations of the SLC22A5 gene in 15 Chinese subjects with primary carnitine deficiency (PCD) and analyze a potential correlation between genotype and phenotype.

Methods: The subjects in this study included fifteen unrelated patients suspected of having PCD recruited from eight different areas of China, and five asymptomatic patient identified by newborn screening. We analyzed their clinical and biochemical phenotypes. The entire coding region of the SLC22A5 gene was amplified with polymerase chain reaction (PCR) and sequenced for all patients.

Results: A total of 15 different mutations were found in 14 PCD patients. Six mutations are novel, in which only c.433_434insA is homozygous and others are heterozygous namely c.338G>A (p.C113Y), c.797C>T (p.P266L), c.497+1G>T, c.745_748delTTTG, c.1372delG. The mutation c.760C>T (p. R254X) detected in one homozygous patient and in four heterozygous patients, is the most frequent mutation with a frequency of 21.4% (6/28). No correlation between genotype and phenotype was confirmed in the present study.

Conclusions: This article reviews the clinical presentations, biochemistry, and molecular analysis in 15 Chinese PCD patients, which should be helpful for the future therapeutic trials.

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**Diagnosis and molecular basis of mitochondrial respiratory chain disorders in Japan: the experiment of systematic analysis for causative genes**

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Objective : Mitochondrial respiratory chain disorders (MRCD) are a group of the most common (1/5,000) congenital metabolic disorders of energy production. Our aim is to make a prompt and correct diagnosis of MRCD.

Methods : MRCD was diagnosed using both in vitro isolated enzyme assays and Blue native polyacrylamide gel electrophoresis (BN-PAGE). Activities of the individual respiratory chain complexes were measured in liver, heart and muscle homogenates and mitochondrial fractions isolated from fibroblasts. Tissue homogenates or mitochondria isolated from skin fibroblasts were solubilised in n-dodecyl-maltoside and subjected to 4–13% BN-PAGE and western blotting using monoclonal antibodies specific for Complex I to IV subunits. Mitochondrial DNA and nuclear DNA copy numbers within tissues were determined by quantitative polymerase chain reaction to find MtDNA depletion syndrome (MDS). Subsequently, mtDNA analysis and systematic search for nuclear causative genes are investigated using high-speed sequencers.

Results : Two hundred patients were diagnosed to have MRCD out of 601 candidate patients. Most frequent was complex I deficiency, of whom many patients had tissue-specific type deficiency. Thirty three out of 113 analysed patients had mitochondrial DNA pathogenic mutations, which meant the majority of childhood-onset MRCD was nuclear origin. Patients with mtDNA mutations had milder symptoms than those suspected to have nuclear mutation. MDS was a prevalent cause of multiple MRCD. Thirteen patients in 11 families were diagnosed to have hepatic MDS, and 10 patients were diagnosed to have myopathic MDS. Out of 13 hepatic MDS, compound heterozygous MPV17 and POLG mutations were detected in one family, each, and homozygous DGUOK deletion (c.143-308_169del335) in the other. This DGUOK deletion might be a common mutation in Japanese. Twenty two cases were investigated the systematic search of nuclear gene by high-speed sequencers. Out of 22 cases, we could find six known gene, and twelve Mitocarta-gene, and we are now confirming them.

Conclusions : We must have a suspicion that almost every disease may be a MRCD. Then, the clinical, biological, and enzymological estimations are all important to lead to greater confidence in the diagnosis achieved. Systematic search to MRCD gene including the high-speed sequence method is the fundamental approach to the diagnose the MRCD.
Objective: Smith–Lemli–Opitz syndrome (SLOS) has strikingly different incidences among various ethnic groups. It is known to be most prevalent in populations of northern and central Europe and low in Asian and African. Clinical spectrum and genotype of SLOS is highly variable. The purpose of this study is to determine the clinical, biochemical and molecular characteristics of SLOS patients from Slovakia as a typical middle European country. All fifteen analysed patients were followed up in University Children’s Hospital, Bratislava.

Methods: The clinical suspicion of SLOS was biochemically confirmed in all patients according to a typical sterol pattern in serum determined by gas chromatography–mass spectrometry. Lipids and apolipoproteins, with hormonal profile in case of ambiguous genitalia, were examined in serum samples collected from fasting children. A rapid PCR/RFLP technique and sequencing was used to detect mutations in the 7-dehydrocholesterol reductase (DHCR7) gene.

Results: The most common clinical features of our patients were microcephaly (14/15), 2–3 toe syndactyly (14/15) with high frequency of polydactyly, cleft palate (13/15), micrognathia (13/15), cardiac defects (10/15) and ophthalmological abnormalities (8/15) including cataract. Three patients had sacral dimple. Plasma concentrations of the cholesterol precursor 7-dehydrocholesterol and 8-dehydrocholesterol were markedly elevated in every patient. One patient with severe form of SLOS had an extremely low plasma cholesterol (0.017 mmol/l), the lowest value ever recorded in a human being. There was an inverse relationship between the clinical severity and the absolute level of cholesterol in plasma. We found reduced, and in 3 patients even extremely low Apo A-I levels, which rised after cholesterol supplementation. Four 46,XY individuals with severe manifestations of SLOS had "sex reversal" showing female external genitalia. In four cases the hypocorticism was detected, in two of them the aldosterone/renin ratio declared a relative aldosterone production insufficiency.

A total of six different mutations was found in 14 families, including five missense (V326L, G410S, R352Q, S397L, L109P) and one nonsense (W151X) mutation. The most frequent SLOS mutation W151X was found in 15 mutant alleles (53%). The other prevalent mutation V326L accounts for 21% of mutant alleles.

Conclusions: The spectrum of DHCR7 mutations in the Slovak patients is distinctly different from the ones in British and German/Austrian, but is comparable with Polish SLOS patients. In the phenotype there is high frequency of microcephaly, cleft palate, cataract and cardiac defects. We include sacral dimple as a new feature in the phenotypic
spectrum of SLOS. In addition to their primary defect in cholesterol biosynthesis, children with SLOS have also complex secondary changes in lipoprotein metabolism probably in order to compensate disturbances in cholesterol homeostasis. The relative adrenal insufficiency could be result of impaired transmission of signal caused by abnormal sterol cell membrane composition.

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O-24

Study on pathogenic mechanisms underlying Wilson disease using an animal model, LEC rats.

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Objective: Using Long-Evans Cinnamon (LEC) rats, an animal model of Wilson disease, we sought to identify proteins involved in the pathogenic process of Wilson disease.

Methods: The proteomic profiles of hepatic tissues and the whole genomic expression profiles of brain tissues of LEC rats were compared to the control rats in an age-dependent manner.

Results: The hepatic proteins differentially expressed in LEC rats are related to mitochondrial injuries or in oxidative stresses. Notably, the differential expressions of malate dehydrogenase 1 and annexin A5 contrasted in an age-dependent manner. In addition, during later stages, the differential expressions of the antioxidant enzymes were remarkably pronounced. Especially, the decreased expression of S-adenosylhomocysteine hydrolase, also involved in monoamine neurotransmitter metabolism, indicates that this protein might be related to the development of neurological manifestations in Wilson disease. The investigation of whole gene expression profiles of brain tissues in LEC rats in age-dependent manner revealed the 178 genes differentially expressed in LEC rats: 68 genes are involved in neuronal development and activities, 23 genes in inflammatory reactions, 33 genes in oxidative stress and apoptotic conditions, and 4 genes in visual pathways.

Conclusions: These results reflect not only the early molecular pathogenic changes but also the important leading process to the development of hepatic and neurological manifestation accompanying Cu-accumulation, which will ultimately help to understand the pathogenic mechanisms of Wilson disease.

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Poster Session
Abstracts
**P-01**

**Application of Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in Screening of Newborn in the NICU and High Risk Children with Inherited Metabolic Disease in and around Beijing**

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**Objective:** To screen of inherited metabolic disease (IMD) of newborns in the NICU and high risk children of the Han nationality in and around Beijing by LC-MS/MS in order to understand the positive rate and distribution of types of IMD.

**Methods:** LC-MS/MS was used to examine 3848 newborn blood samples from NICU of our hospital and 1684 children blood samples with high risk of IMD from hospitals in and around Beijing. The positive results were further confirmed by recheck, gas chromatography-mass, other laboratory tests and clinical symptoms.

**Results:** 5 inborn (1.3‰, 5/3848) were confirmed with IMD in NICU, with 4 cases of methylmalonic academia (MMA) and 1 case of isovaleric acidemia, which all belong to organic acidemias. 43 children (25.5‰, 43/1684) were confirmed with 12 diseases, including 1 (2.3%, 1/43) case with fatty acid disorders, 11 (25.6%, 11/43) with amino acid diseases, and 31 (72.1%, 31/43) with organic acidemias. Out of 31 patients with organic acidemias, there are 26 (83.9%, 26/31) cases of methylmalonic academia or propionic acidemia.

**Conclusions:** From two sources of cases, the patients with organic acidemias were significantly more than fatty acid disorders and amino acid diseases. And methylmalonic academia is in the highest frequency among the diseases of organic acidemias. We speculate methylmalonic academia would be the most common type of IMD among northern Han Chinese population.

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Objective: There have been a number of studies on many inherited metabolic disorders. However, systemic evaluations of newborn screening programs’ efficacy are rarely carried out and limited in scope.

Methods: In an eleven-year-long single laboratory observational study, 224 cases with confirmed diagnosis of a metabolic disorder between 2000 and 2011 were investigated and followed up. Medical history, diagnostic and confirmatory tests, treatment, developmental and clinical status were evaluated.

Results: Early detection and treatment of amino acid metabolic disorders produced comparatively better outcome. Approximately 75% of urea cycle disorders presented complications despite the newborn screening program. As for organic acid metabolic disorders, 90% of MMA and PPA patients experienced complications while early diagnosis of MSUD helped almost 90% of the affected patients to grow normally. Early diagnosis of fatty acid metabolic disorders like EMA and medium-chain acyl-CoA dehydrogenase deficiency was associated with good outcome. However, VLCAD patients had the low survival rate.

Conclusions: Discussion
Clinical outcomes seem inevitable in a large number of patients with metabolic disorders. Lack of comprehensive governmental supervision for tandem newborn screening may be one of big issue despite more than 80 percent of Korean newborns have been screened. This may be attributed to patients not visiting until there are very acute symptoms of the disorders, limited understanding and clinical experience of metabolic disorders by physicians, inappropriate countermeasures to control the clinical manifestations, and delays in treatment where harmful metabolites were not removed in a timely manner.

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P-03

Quality Assurance Program for Neonatal Screening of Glucose-6-Phosphate Dehydrogenase Deficiency

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Objective: The nationwide neonatal screening of Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency in Taiwan was started on July 1, 1987. The effective collection rate has reached >99% of all newborns since 1996 and the overall incidence rate of G6PD deficiency is about 2%. A network of referral hospitals distributed all around Taiwan was organized. In order to assess the reliability and assure the quality of the confirmatory and screening tests, an external quality assurance (EQA) program for G6PD assay was developed.

Methods: For screening test, the QC materials were prepared from whole blood by spotting on to Guthrie cards. For confirmatory quantitative test, lyophilized quality control (QC) materials were prepared from human red blood cells. Periodically (1–2 month), 3–5 QC samples and 10 QC blood spots were sent to referral and screening laboratories, respectively. The external QA results were evaluated and compared to the reference value (and medium/mean for quantitative test). The test results were submitted through internet and the summary reports were published on the webpage within two weeks for each survey.

Results: Twenty-nine screening laboratories (3 in Taiwan, 10 in Mainland China, 4 in Philippines, 2 in German, 2 in India, 2 in Mexico and 1 each in Australia, Greece, Lebanon, Thailand, Turkey, and Vietnam) and 20 referral laboratories in Taiwan are participating in the QA program at the present time. From 1999.3 to 2011.12, 80 surveys for screening test were performed and 1051 reports were received. One hundred and forty (13.3%, 140/1051) abnormal QA reports were found. One hundred and thirty false negative and 274 false positive results were reported from the 10,510 blood spots tested. From 1988.1 to 2011.12, 172 QA surveys were sent to referral laboratories and 3,120 reports were received in reply to these QA surveys. Three hundred and one (9.6%, 301/3,120) abnormal QA results were found. Interlaboratory C.V. for the quantitative test has reached below 10% in recent years. Between 2007.1 and 2011.12, 3 QC materials with different G6PD activities (5.1, 8.1, and 12.7 U/gHb) have been used 8 times in different surveys during this 4 years period of time. The long term intra-laboratory between run CV of the G6PD confirmatory test in those referral laboratories were found to be between 4.1% and 17.3%. Since July 2009, 15 surveys (2009.7–2011.12) have been carried out for the newly established network of confirmatory testing laboratories (n=10) in Philippines. Thirty-three (24.4%, 33/135) abnormal QA results were found from 135 reports.
Interlaboratory C.V. were between 9.9% and 22.7% (1.9 ~ 20.5 U/gHb), which is lower than those found in CAP surveys.

Conclusions : The external quality assurance program has been useful for monitoring the performance of the referral hospitals and screening laboratories, and might be a guidance for the participating laboratories to correct the analytical errors.

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P-04

Newborn Screening for Infantile Pompe Disease in National Center for Child Health and Development Hospital: A Pilot Study

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Objective : Newborn screening (NBS) for Pompe disease has been initiated in several countries and regions, and is reportedly successful. However, the comparatively high frequency of pseudodeficiency allele makes NBS for Pompe disease complicated in Japanese population. We demonstrated our modified screening procedure was able to distinguish the pseudodeficiency from Pompe disease patients. Based on this research, we have started a pilot study of NBS for infantile Pompe disease and reported the result of this study.

Methods : Healthy newborns born from Jan. to May in 2011 in National Center for Child Health and Development were screened. Informed consent was obtained from all newborn’s family members. Dried blood spot (DBS) samples were obtained from newborns in 3~5 days after deliver. GAA activities were analyzed under the following conditions: (1) total GAA measured at pH 3.8, (2) GAA measured at pH 3.8 in the presence of acarbose, and (3) neutral glucosidase activity (NAG) measured at pH 7.0 without acarbose. The % inhibition and NAG/GAA ratio were calculated. Samples with 30% of the normal GAA activity mean, % inhibition 60%, and NAG/GAA ratio 30 were considered as 1st screening positive. To identify pseudodeficiency, DBS samples of 1st screening positive individuals were analyzed sequence variants c.1726G>A (p.G576S) in GAA gene using PCR direct sequencing.

Results : 361(Male: 185, Female: 176) neonates were screened in this pilot study. 15 neonates were found with less than 30% of normal GAA activity level. 14 of 15 newborns were diagnosed as “Pseudodeficiency” with the result of gene analysis. The frequency of “Pseudodeficiency” was 3.9%. One newborn was underwent the measurement of GAA activity in lymphocytes and GAA gene analysis. This newborn was diagnosed as normal healthy individual in the end. Specificity of the pilot study was 95.8%.

Conclusions : NBS for Pompe disease in Japanese population could be successfully conducted by using a new cut-off value and including genotyping and lymphocyte GAA assay.

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Newborn Screening for Fabry Diseases in Japan

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Objective: Fabry disease is an X-linked disorder of alpha-galactocidase A which causes the accumulation of glycolipids in lysosomes. The incidence of the classical type of the disease is approximately 1 in 40,000 males. Recent studies have revealed the late-onset type of the disease to have a higher frequency than previously known.

Methods: To determine the disease incidence in Japan, we screened newborns to measure alpha-galactosidase A activity in dried blood spots from Japanese neonates. Enzyme-deficient infants were retested, and infants who were double-screening positive were diagnostically confirmed by enzymatic activity and mutation analyses.

Results: Thirty eight neonates had a deficiency in alpha-galactosidase A activities and specific mutations, including 5 neonates with classical mutations identified previously. Based on our newborn screening in Japan, the incidence of alpha-galactosidase A deficiency was 1 in 8,000 male. These results suggest that the late-onset phenotype of Fabry disease is underdiagnosed among both males and females in Japan.

Conclusions: The recognition of the existence of these patients suggests the need for both early diagnosis and therapeutic intervention. However, ethical issues need to be taken into consideration in terms of when and whom the screening should be performed.

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Objective: Lysinuric protein intolerance (LPI) is a rare inherited metabolic disorder of dibasic amino acid transport, caused by mutations in the SLC7A7 gene. Renal tubular, intestinal, and hepatocellular transport is deficient, resulting in decreased circulating dibasic amino acid levels and a lack of sufficient ornithine to support activity of ornithine transcarbamylase in the urea cycle. Growth retardation and signs of episodic hyperammonemia are the earliest manifestations of LPI.

Results: We encountered a 3.7-year-old Korean girl with LPI. She was born with birth weight of 3.38 kg at 38+6 weeks of gestation and she was the first child born to healthy and non-consanguineous parents. She visited our hospital with the chief complaint of short stature and increased somnolence for several months. There had been no event of acute metabolic crisis including altered consciousness on her past medical history. She had no abnormal neurological sign and no evidence of significant neurodevelopmental delay. Upon the findings of physical examination, her height was below 3rd percentile, and the liver was palpable as 5 cm width below the subcostal margin. Her lab findings showed microcytic hypochromic anemia, leukopenia, elevated liver enzyme, hypoproteinemia, elevated ferritin and LDH levels, and hyperammonemia. Low levels of lysine, arginine, and ornithine were noted by plasma amino acid analysis, and urine amino acid analysis showed elevated excretion of lysine, arginine, and ornithine. On the ultrasonographic findings, hepatosplenomegaly was observed, but there was no focal parenchymal lesion. The SLC7A7 gene analysis revealed that she was a homozygote with a known mutation c.625+1G>A in intron 3. This mutation was previously reported in Japanese patients. Her younger brother and parents were heterozygous carriers of this mutation. Low protein diet, sodium benzoate, citrulline and L-carnitine supplementation were started. During the follow-up period of 9 months, decreased ferritin levels, normalized ammonia levels, and normal growth velocity in height has been observed.

Conclusions: Here, we report a girl with LPI for the first time in Korea. LPI is a multisystemic disease, and the phenotypic variability of LPI could result in various misdiagnoses. In particular, LPI should be considered for differential diagnosis of hyperammonemia.
P-07

Amino Acid Disorders Detected by Quantitative Amino Acid HPLC Analysis in Thailand: An Eight-Year Experience

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Objective: Amino acid disorders are a major group of inborn errors of metabolism (IEM) with variable clinical presentations. This study was aimed to provide the data of amino acid disorders detected in high-risk Thai patients referred to our metabolic lab from all over the country.

Methods: From 2001 to 2009, we analyzed amino acids by high-performance liquid chromatography (HPLC) in 1,214 plasma and cerebrospinal fluid specimens. These specimens were obtained from patients with clinical suspicion of IEM or with positive newborn screening. The clinical data of the patients with confirmed diagnoses of amino acid disorders were also analyzed.

Results: Fifty-eight patients were diagnosed with amino acid disorders, including 20 cases (34.5%) with maple syrup urine disease, 13 (22.4%) with phenylketonuria and hyperphenylalaninemia, 13 (22.4%) with nonketotic hyperglycinemia, 9 (15.5%) with urea cycle defects, 2 (3.4%) with classical homocystinuria, and 1 (1.7%) with ornithine aminotransferase deficiency. There was considerable delay in diagnoses which led to the poor outcomes in most patients.

Conclusions: The prevalence of amino acid disorders in Thailand is distinct from other countries. This will guide the selection of the prevalent IEM for the future newborn screening program in this country.

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Glutathione Synthetase Deficiency (5 Oxoprolinuria) in Vietnamese Patients: Clinical Manifestations and Outcome

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Objective: to describe clinical features, laboratory finding and to evaluate outcome of Vietnamese patients with glutathione synthetase deficiency

Methods: This is case series of 12 patients during 6 years (2006 - 2011) at Vietnam National Hospital of Pediatrics (NHP). Tandem Mass and GC/MS were performed in Shimane University School of Medicine – Japan

Results: 12 patients from 11 unrelated families were diagnosed as 5 oxoprolinuria and the condition was the second common organic aciduria at NHP. 6 patients with severe type had neurological symptoms such as seizure, psychomotor retardation, spasticity. Remain six patients were moderate type. More half of patients presented with clinical symptoms in the neonatal period. The mean age of onset were 4.6 months of age (range 1 – 22 months). Male/female was 6/6. Clinical symptoms were lethargy (10/12), acidosis symptoms (10/12), refused feeding (10/12), vomiting (5/12), diarrhea (4/12), dystonia (4/12), convulsion (2/12), psychomotor retardation (2/12), respiratory/intestinal infections (6/12). The investigations showed hemolytic anemia (12/12), chronic metabolic acidosis (12/12), leukocytosis (6/12), hyperlactatemia (8/12), hyperammonemia (6/12), elevated transaminase (6/12), ketonuria (2/12). GC/MS showed all patients having excessive amounts of 5 oxoproline in urine. All patients were treated with correction of acidosis, polyvitamins supplement, symptoms treatment. The outcome was very bad: 100% patients died on the first admission due to recurrent infections, persistent severe metabolic acidosis (PH<7.0), septic shock.

Conclusions: 5 oxoprolinuria disease is one of the common organic aciduria and poor prognosis was seen in Vietnamese patients. It is important to make early diagnosis and to have adequate treatment to reduce mortality

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P-09

Clinical Features of Propionic Acidemia in Vietnamese patients: A report of 7 Cases

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Objective: To describe clinical features and laboratory finding of patients with PPA and to evaluate outcome of management.

Methods: This is a case series report. Clinical features, biochemical finding and management outcome of 7 Vietnamese cases from 7 unrelated families were study. GS/MS and Tandem mass were performed in Shimane University - Japan.

Results: 4/7 families had more than 2 affected children. 6/7 cases had acute metabolic decompensation with onset age < 1 year, among them 4/7 cases had clinical symptoms in neonatal period. Clinical manifestations of the acute episodes were lethargy/coma (6/7), convulsion (6/7), hypotonia (6/7), dehydration due to vomiting (4/7). In acute episodes, laboratory findings showed metabolic acidosis (6/7), hyperamonemia (6/7), thrompenia (6/7) and neutropenia (5/7) ketouria (4/5). There were poor prognosis in our patients with PPA: 5/7 cases died during acute episodes, median age of death were 9.9 months (min 16 days – max 28 months). Among them, 4 cases died in the neonatal period. 1 case has motor and mental retardation, 1 case has normal development at 3 months of age.

Conclusions: Propionic acidemia is an organic acidemia known to bring about a syndrome of ketoacidosis, lethargy/coma, convulsion, hypotonia and vomiting, to present in the newborn period. This disorder results in neurological impairment or death, if not properly controlled. It is important to perform screening for high risk infants, newborn screening and early diagnosis to reduce mortality and neurologic consequence of patients with PPA.

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Hepatic Imaging Features of Neonatal Intrahepatic Cholestasis Caused by Citrin Deficiency: Investigation of 18 Patients Diagnosed by SLC25A13 Gene Analysis

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Objective: Neonatal Intrahepatic Cholestasis caused by citrin deficiency (NICCD) is the major clinical phenotype at pediatric age for citrin deficiency, an inborn error of metabolism with SLC25A13 as the causative gene. The molecular, clinical and biochemical features of NICCD have been described in many papers. However, the medical imaging presentations of this disease still remain an issue that was poorly understood at the current stage. This paper aims to explore the hepatic radiological features of NICCD to facilitate the clinical recognition of this disease.

Methods: Eighteen NICCD patients including 9 females and 9 males, were enrolled as research subjects in this study. The clinical and biochemical information were collected and investigated by means of cross-sectional study, and SLC25A13 mutations were analyzed using PCR/LA–PCR and PCT-RFLP approaches for all subjects with their informed consents. In radiological investigation, twelve cases were studied by unenhanced CT, four by in-phase/out-of-phase MRI, and two by unenhanced CT and in-phase/out-of-phase MRI as well. The attenuation values of liver and spleen at CT and the signal intensity values of liver on in-phase and out-of-phase MR images were measured, and then liver-to-spleen attenuation ratio at CT and fat index on MRI were calculated to evaluate hepatic steatosis condition, respectively. Patients with liver-to-spleen attenuation ratio less than 1 at CT and/or fat index over 9% on MRI were considered to have hepatic steatosis.

Results: All patients presented with clinical signs and biochemical changes in consistence with intrahepatic cholestasis, and SLC25A13 mutation homozygotes or compound heterozygotes were revealed by SLC25A13 analysis. All subjects demonstrated various degrees of hepatomegaly at imaging study. Hepatic steatosis was detected in fifteen patients, cirrhosis in one, and normal hepatic density in the remaining two. In the patients with hepatic steatosis, liver-to-spleen attenuation ratio ranged from 0.47 to 0.98 at CT, while fat index between 10.03% and 18.86% on MRI.
Conclusions: NICCD diagnoses were confirmed in 18 patients based on their clinical, biochemical and molecular presentations. All NICCD subjects present with various imaging features on CT and MRI and hepatic steatosis is the commonest radiological presentation. Our findings in this paper suggest that CT or MRI might be considered as the reliable, rapid, objective and noninvasive approaches to evaluate the liver conditions in NICCD patients.

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P-11

Analysis of Clinical Features, Biochemical Analysis and Gene Mutations in One Chinese Pedigree with Infant-Onset Isolated Methylmalonic Acidemia

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Objective: This study aimed at understanding clinical features, biochemistry and gene mutations in one Chinese pedigree which had an infant-onset isolated methylmalonic acidemia (MMA) boy, and exploring the significance of gene diagnosis in prenatal diagnosis.

Methods: The clinical and biochemical data of one case were analyzed. The concentrations of acylcarnitines and homocysteine in blood and organic acids in urine were analyzed by mass spectrum technology. The MUT gene mutation was detected using polymerase chain reaction (PCR) and DNA direct sequencing for the case, his parents and the fetus amniocyte and her blood after birth.

Results: The age of onset was 6 months. He began to have poor reaction, vomiting, tachypnoea, noticeable metabolic acidosis. Family history showed that he had a sister who died of the same manifestations at 8 months. The diagnosis of this disease mainly depends on the measurement of propionylcarnitine (C3), C3/free carnitine (C0) and C3/acetyl carnitine (C2) in the dry blood spo released mass spectrometry (MS/MS), and the level of methymalonic acid in urine by gas chromatography–mass spectrometry (GC-MS). We identified the types of MMA by detecting the level of total homocysteine in the serum and confirmed the diagnosis of isolated MMA. One heterozygous missense mutation was found at the exon 6 (c.1106 G>A, p.R369H) in the patient and his father, which was located in the N-terminal substrate binding domains. Another heterozygous missense mutation was found at the exon 11 (c.1853 T>C, p.L618P) in the patient and his mother, which was located in the C-terminal cobalamin binding domains. But the analysis of these target mutations of the fetus amniocyte and her blood were normal.

Conclusions: Isolated MMA results from either MCM apoenzyme defects or defects in synthesis of its cofactor cobalamin. Two heterozygous missense mutations were found in MUT gene in this Chinese pedigree with infant-onset isolated methylmalonic academia. The DNA analysis of MUT gene could be utilized for the prenatal diagnosis.

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Isovaleric Acidemia in Korea

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Objective: Isovaleric academia is an autosomal recessive inborn error of the leucine metabolism that is caused by a deficiency of isovaleryl-CoA dehydrogenase. Recent application of tandem mass spectrometry to newborn screening in Korea has allowed a significant expansion of the recognition with IVD deficiency.

Methods: We characterized IVD mutations in seven Korean IVA patients from six unrelated families and their clinical characteristics.

Results: 6 patients are male and 1 patient is female. Their age distribution is from 15 month to 8 years. 3 patients were diagnosed by tandem mass spectrometry at neonate and now there is not developmental delay and any other symptoms. Another patients were diagnosed at his 22 days after birth, 3 years, 4 years and 8 years respectively, with recurrent vomiting, poor feeding, sweaty foot odor and refractory metabolic acidosis. Now they have developmental delay.

Bi-directional sequencing analysis identified two novel variations affecting consensus splice sites and three novel variations altering coding sequences (c.149G>T;Arg21Leu, c.832A>G;Ser249Gly, and c.1135T>G;Phe350Val). Five patients from four families were found to be compound heterozygotes while two unrelated patients were homozygous for the c.457-3_2CA>GG variation. Reverse-transcription polymerase chain reaction confirmed that both intron variations cause aberrant splicing. Analysis of cultured lymphocyte extracts of the seven patients showed no detectable enzyme activity and reduced levels of IVD protein in all samples compared with normal control.

Among 7 patients, 3 unrelated patients had c.457-3_2CA>GG and all of 3 lived in Jeju island, which is the biggest island in Korea, suggesting a possible founder effect in this population. Clinically, Ser249Gly/Phe350Val mutation is better prognosis, there was no developmental delay and sweaty foot odor. And they had more IVD enzyme activity than other patients.
Conclusions: In conclusion, Korean patients with IVA have been shown to have mutations in the IVD gene but the mutation spectrum is completely different from previously reported in other countries. Therefore, mutation analysis of the IVD gene may be helpful for rapid confirmatory diagnosis, prenatal diagnosis and carrier detection. Furthermore we will be suppose the prognosis of the patient using IVD gene mutation.

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P-13
Clinical and Mutation Analysis of the Ornithine Transcarbamylase (OTC) Gene in Six Korean OTC Deficiency Patients Revealed Two Known and Two Novel Mutations

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Objective: To analyze the clinical and genetic characteristics of six children with ornithine transcarbamylase deficiency (OTCD)

Methods: All exons of the ornithine transcarbamylase (OTC) gene were screened by polymerase chain reaction-DNA direct sequencing in the six OTCD patients.

Results: One patient firstly showed vomiting at 22 month of age. Mutation analysis revealed one novel mutation such as heterozygote deletion on exon 1 which was detected by MLPA. Her mother had no mutation without any clinical symptoms. She had elevated blood ammonia (233mg/dl) and urinary orotic acid levels (7.5 mmol/mol c). The second patient presented as altered consciousness at 14 months of age, and had a missense mutation of R129H. Biochemical findings revealed the elevated ammonia (200mg/dl) and orotic acid levels (59.3 mmol/mol c). The third patient presented as seizure at 23 months of age, harbored a missense mutation of Y183C. She had elevated blood ammonia (495mg/dl) and urinary orotic acid levels (200.1 mmol/mol c). The fourth patient showed vomiting and seizure at 24 months of age. Direct genetic testing was not done due to parental refusal. She had elevated blood ammonia (850mg/dl) and urinary orotic acid levels (60.1 mmol/mol c). In the remaining cases, which were two late-onset male siblings, aged 19 and 20 years, one of whom, younger brother showed altered consciousness at 18 years of age. He had elevated blood ammonia (258mg/dl) and urinary orotic acid levels (10.4 mmol/mol c). DNA studies revealed a splicing mutation, IVS8(-3)T>C, which has never been previously reported.
Conclusions: Molecular analysis is a practicable way for diagnosing OTCD. We report two known and two novel mutations of the OTC gene in six Korean patients including two late-onset and four symptomatic female patients.

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P-14
Pathogenetic Mechanisms of PTS Mutations Associated with Mild Clinical Phenotype

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Objective: Phenylketonuria (PKU) and hyperphenylalaninemia (HPA) may be caused by deficiency of phenylalanine-4-hydroxylase or its important cofactor, tetrahydrobiopterin (BH4). BH4 is also involved in nitric oxide synthases, tyrosine hydroxylation and tryptophan hydroxylase, the latter two are crucial enzymes for the biosynthesis of neurotransmitters dopamine and serotonin, respectively. BH4 deficient patients without early diagnosis and proper treatment show impairment in phenylalanine catabolism accompanying with a deficiency in neurotransmitters. Depletions in any of the enzymes involved in BH4 biosynthesis or regeneration leads to BH4 deficiency. Among these, 6-pyruvoyl-tetrahydropterin synthase (PTPS, gene symbol: PTS) deficiency (MIM 261640) is the most common form of BH4 deficiency. To date, 36 mutations, including 27 missense, 2 nonsense, 1 small deletion, 1 frameshift, 4 splicing and 1 initiation mutations, were identified in PTS gene in Chinese populations. The c.166G>A and c.842-291A>G mutations were found to associate with mild clinical phenotype of PTPS deficiency. Here we investigated the pathogenesis mechanism of the two mutations.

Methods: Wild type PTS cDNA was first cloned into vector pcDNA3. The missense mutant alleles were induced into the plasmid by site direct mutagenesis. The plasmid carrying wild type and/or mutant PTPS were transfected into COS-1 cells followed by analyzing PTPS enzyme activity in lysate. On the other hand, the cDNA of cells carrying the intronic mutation were isolated, amplified and subcloned into the vector followed by sequencing analysis.

Results: The COS-1 cells overexpressing c.166G>A mutant protein presented normal PTPS enzyme activity (106.53±22.3% of that in cells transfecting wild-type plasmid). However, the activity of PTPS was dramatically
deceased to approximately 10% when c.166G>A were cotransfected with other mutant alleles while no enzyme activity reduction was observed in cells cotransfected with plasmid carrying c.166G>A and wild type PTS cDNA. ESE finder predicted an alteration of the SRp40 splicing protein binding site when the c.84–291A>G mutation is present. A 79bp pseudoexon flanked by consensus splice site can only be identified in cells carrying the c.84–291A>G mutation but not in the control cells. Furthermore, in subcloning of PTS cDNA from the skin fibroblasts of a patient heterozygous for the c.259C>T and c.84–291A>G mutations, 55% of clones represented the c.259C>T mutation or exon 3 skipping. Intriguingly, only 9% of the clones contained the 79bp pseudoexon, whilst the other 37% of clones represented a normal PTS cDNA.

Conclusions: We speculate that the c.166G>A may be susceptible to other mutant PTPS protein but still retained low PTPS enzyme activity. On the other hand, the c.84–291A>G mutation may only create a weak binding site for SR splicing factors and allow a significant portion of normal transcript to be formed, resulting in a mild form hyperphenylalaninemia.

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P-15

Glutaric Acidemia Type II, Neonatal Onset Type in 2 Thai Patients

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Objective: Glutaric acidemia type II (GA2) is an inherited disorder of fatty acid, amino acid, and choline metabolism resulting in interfering the body’s ability to use proteins and fats for energy. GA2 is classified in 3 groups; neonatal onset with congenital anomalies, neonatal onset without anomalies, and later onset. In the neonatal onset form, patients usually present with hypoketotic hypoglycemia, metabolic acidosis and hyperammonemia. Urine organic acids analysis reveals massive excretion of lactic acid, glutaric acid and other organic acids. The aim of our study is to study clinical features and biochemical analysis in 2 Thai patients.

Methods: We reviewed clinical features of 2 Thai patients with GA2, neonatal onset type. The diagnosis was made by analysis of organic acids in the urine and immunoblot analysis in skin fibroblast.

Results: There are two patients: one female and one male. Patient 1 presented with hypoketotic hypoglycemia at 44 hours of age, which was treated as sepsis with clinical improvement. At 4 months, she developed fever, lethargy and hypoglycemia. Physical examination revealed dysmorphic facial features, hepatomegaly and hypotonia. Patient 2 presented with recurrent episodes of hypothermia and hypoketotic hypoglycemia at 2 hours and 48 hours after birth that resulted in cardiac arrest requiring cardiopulmonary resuscitation. He did not have congenital anomalies or dysmorphic features. The first patient had increased excretion of glutaric acid, 2-hydroxyglutarate and adipic acid in the urine typically found in GA2. However, the diagnosis of GA2 in the second patient was made by using immunoblot analysis in the skin fibroblast performed in Japan. The treatment was started including riboflavin, glyicine, carnitine and diet restricted in fat and protein. However, the first patient died at 6 months of age. The second patient is currently alive, but has developed hepatomegaly and jaundice since 4 months of age.
Conclusions: Previously, we reported 1 Thai patient with GA2, neonatal onset type which is the first patient in this study as well as 2 Thai patients with GA2, later onset type presenting with myopathy. Herein we report one more patient with GA2, neonatal onset type.

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P-16

Proteasome inhibitor improves the function of mutant lysosomal alpha-glucosidase in fibroblasts from Pompe disease patient

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Objective
Pompe disease is an autosomal recessive lysosomal storage disorder caused by a deficiency of acid α-glucosidase (GAA). We previously found that mutant GAA in patient fibroblasts carrying c.546G>T mutation is degraded by proteasome, and stabilized by treatment with proteasome inhibitor as well as imino sugar N-butyldeoxyojirimycin. However, detailed effects of proteasome inhibitor on patient GAA functions are not clarified. In this study, we investigated the effects of proteasome inhibitor on maturation, subcellular localization and residual activity of mutant GAA in the patient fibroblasts carrying c.546G>T mutation.

Methods
Skin fibroblasts from Pompe disease patients carrying c.546G>T mutation were cultured for 24h with or without proteasome inhibitors, bortezomib or MG132 (1 uM, 100 nM, 10 nM, respectively). These fibroblasts were lysed and analyzed by Western blotting and assay of GAA activity. Subcellular localization of mutant GAA in proteasome inhibitor-treated patient fibroblasts was analyzed by immunocytochemical techniques. Cytotoxic effects of proteasome inhibitors were determined by WST–8 assay.

Results
Each proteasome inhibitor promoted the stabilization of patient GAA and processing of them to mature forms at any concentration tested. In addition, lower concentrations of bortezomib and MG132 (100 nM and 10 nM, respectively) showed no cytotoxic effects in patient fibroblasts. Increased colocalization of GAA with the lysosomal marker LAMP2 were observed in patient fibroblasts treated with proteasome inhibitors. Furthermore, proteasome inhibitors also increased enzyme activity in the patient fibroblasts. Especially, bortezomib was more effective than MG132 in enhancing GAA activity in patient fibroblasts (about 4-fold and 2-fold increase of residual activity, respectively).

Conclusions
Bortezomib has already been approved by U.S. Food and Drug Administration, and used for the treatment of multiple myeloma patients. Therefore, our findings indicate that bortezomib may be a novel drug for the treatment of Pompe disease patient carrying chaperon–responsive mutation.

P-17

Analysis of gene mutations in Chinese patients with maple syrup urine disease

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Objective: The aim of this study was to screen the mutations of BCKDHA, BCKDHB, and DBT genes in 16 Chinese subjects with MSUD and analyze a potential correlation between genotype and phenotype.

Methods: BCKDHA, BCKDHB, and DBT genes were analyzed by polymerase chain reaction (PCR) and direct sequencing in sixteen MSUD patients.

Results: In 16 patients, six mutations of BCKDHA gene were detected in 4 cases, 10 mutations of BCKDHB gene in 8 cases, and 4 mutations of DBT gene in 3 cases. Among the 15 cases, 13 were non-responsive to thiamine, whose genotypes include BCKDHA(3 cases), BCKDHB(8 cases), and DBT(2 cases). Two cases were thiamine-responsive, whose genotypes include BCKDHA(1 case)1 1and DBT(1 case).

Conclusions: Twenty gene mutations were detected, including sixteen novel mutations. We found a part of gene mutations spectrum in Chinese patients with maple syrup urine disease.

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Objective: Citrin is a liver-type mitochondrial aspartate-glutamate carrier, which plays an important role in urea synthesis and translocating cytosolic NADH into mitochondria. Two phenotypes can occur by citrin deficiency, neonatal intrahepatic cholestasis by citrin deficiency (NICCD: OMIM #605814) and adult-onset type II citrullinemia (CTLN2: OMIM #603471), and some patients with NICCD develop CTLN2 in their later lives. This study was performed to investigate the clinical and genetic characteristics of Korean patients with citrin deficiency.

Methods: A total of 22 patients, 13 males and 9 females, were enrolled in this study. Clinical manifestations, laboratory findings, clinical courses and results of genetic study were reviewed.

Results: Sixteen patients manifested as NICCD, and Six patients as CTLN2. Prolonged neonatal jaundice was noted in 83.3% of the patients with NICCD. Two patients with NICCD were identified by neonatal screening. Hyperammonemic encephalopathy was accompanied in 5 patients with CTLN2. Atypical manifestations such as severe coagulopathy (1 pt), dyslipidemia (2 pts), and peripheral neuropathy (1 pt) were also noted. At initial evaluation, most patients showed abnormal serum liver enzyme levels (88.9%), hyperammonemia (76.5 %), and hypoproteinemia (76.5%). All the patients had citrullinemia (336.2 ± 316.1 umol/L; nl, 1–46 umol/L), normo/hyper-argininemia (116.6 ± 68.0 umol/L; nl, 10–140 umol/L), and increased threonine to serine ratios (3.8 ± 3.0; nl, <1.1 umol/L). Methioninemia and galactosemia were observed in 10 and 3 cases, respectively. Genetic analyses of
SLC25A13 revealed that five common mutations, IVS16ins3kb (13 alleles, 31.0%), 851del4 (10, 23.8%), IVS11+1G>A (8, 19.0%), IVS13+1G>A (2, 4.8%), and S225X (3, 7.1%), comprises 85.7% of all the mutations.
Prolonged jaundice in 6 pts with NICCD was spontaneously resolved by 3 to 7 months of age. Fatty liver or dyslipidemia were noted in 11 patients (84.6%). Recurrent episodes of hyperammonemic encephalopathy, liver cirrhosis and death were noted in 4, 2, and 1 pts with CTLN2, respectively. Liver transplantation was done in 1 pt with CTLN2. None with NICCD progressed to CTLN2 until 2.5 (0.4–7.6) years of age.

Conclusions: Typical presenting phenotypes and biochemical findings can be used as the hallmarks for the diagnosis of citrin deficiency. Genetic screening for 5 common mutations in SLC25A13 can be recommended for the Korean patients with citrin deficiency for the rapid diagnosis. Considering the poor clinical course of CTLN2, long-term follow-up evaluation for the patients with NICCD is crucial, and more efforts are needed to diagnose the patients with CTLN2 at early stage.

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P-19
Treatment Strategy of patients with Ornithine transcarbamylase deficiency (OTCD)

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Objective: Long-term survival rate of OTCD is remarkably improving by prompt diagnosis and better medical treatment including the liver transplantation as a radical treatment in Japan. The aim is to review clinical presentation and prognosis of OTCD patients in our hospital.

Methods: Six patients with OTCD followed in our hospital in the last five years. We examined our 6 cases about age at onset, severity of hyperammonemia, gene mutation, residual OTC enzyme activity, content of internal therapy, age at liver transplantation and prognosis to consider the treatment strategy for OTCD patients retrospectively.

Results: Among diagnosed 6 patients, neonatal onset was 3 boys and late onset was 2 boys and 1 girl. Two patients had marked hyperammonemia (2370μg/dl, 1936μg/dl) and received hemocatharsis, and 1 patient had mild hyperammonemia(361μg/dl). He could be received monitoring of blood ammonia level with using regular formula soon after birth. Therefore, he was diagnosed OTCD promptly and started medical treatment. The OTC activity was 1.3%, 5.4% and 4.7%, and gene mutation was R26Q, deletion including OTC gene and R26Q, respectively.

Other 3 patients with late onset type, age at onset was 7 months, 9 months and 13 months. Peak ammonia level was 546μg/dl, 732μg/dl and 688μg/dl, the OTC activity was 6.0%, 2.6% and 2.9%, and gene mutation was N161S, R129H
and F48V, respectively. All patients had protein-restricted diet, arginine or citrulline and benzoate. Four out of six patients were medicated the phenylbutyrate, too. Instead of medical treatment, 6 patients underwent the liver transplantation consequently, and have good course without severe complication. All of 3 with neonatal onset had liver transplantation at one year of age. Two patients with marked hyperammonemia have remained moderate neurological impairment and the other has been normal growth. Patients with late onset type had liver transplantation at the age of 3, 15, and 5 years, respectively. Two patients had ADHD or speech delay.

Conclusions: Our data suggest that each treatment such as prompt diagnosis involving prenatal diagnosis, early treatment before marked hyperammonemia, intensive care in acute phase including hemocatharsis, medical treatments in chronic phase and liver transplantation are important strategy to take better outcome.

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P-20

Beneficial Effect of Bezafibrate on Boy with the Late-onset Glutaric Aciduria Type 2

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Objective: Glutaric aciduria type 2 (GA2) is an autosomal recessive disorders caused by a defect of electron transfer flavoprotein (ETF) or ETF dehydrogenase (ETFDH). In GA2, multiple acyl-CoA dehydrogenases in fatty acid oxidation pathway, and of glutaryl-CoA dehydrogenase involved in branched-chain amino acid catabolism are impaired. Clinical presentation has been described in two types, neonatal-onset form (severe form) and late-onset form (milder form). Late-onset form presents stress-induced hepatic encephalopathy, hypoglycemia, or muscle weakness. Recently, efficacy of bezafibrate, a common hypolipidemic drug, on the patient with long-chain fatty acid oxidation disorders has been reported. However, the effect of bezafibrate on patient with GA2 is not reported. We have previously reported the effect of bezafibrate on fatty acid oxidation in cultured fibroblasts using in vitro probe acylcarnitine assay and MS/MS. In this report, we present a boy with late-onset GA2 treated with bezafibrate whose clinical features were dramatically improved.

Methods: Case report
A 2-year-9-month old boy, who was diagnosed as having GA2 by the MS/MS newborn screening, developed episodic hypoglycemia and muscle weakness, although he underwent the therapy with L-carnitine, riboflavin, and low-fat and high-carbohydrate diet. At 2 years and 3 months of age, he developed respiratory failure following RSV infection. His developmental quotient (DQ) in Enjoji system was 59 point, and muscle weakness became more obvious. Following initiation of bezafibrate at 100mg per day, with gradual increase to 300mg, his motor development was improved in a short period. Walking distance gradually extended in a week. His DQ was improved to 79 point in 6 months. Hypoglycemic episode was no longer observed. Acylcarnitine profiles were gradually improved with escalating doses of bezafibrate.

Results : Discussion
Bezafibrate improved the clinical features in the child with late-onset GA2. Recent study reported that bezafibrate increases the residual fatty acid oxidation capacity by activating peroxisome proliferator-activated receptors δ (PPARδ). Our study indicates that bezafibrate improves not only long-chain fatty oxidation defects but also whole fatty acid oxidation defect. It is expected that bezafibrate can be a new therapeutic option on GA2. However, its effect on neonatal-onset GA2 form and the safety for long term administration remains to be determined.

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**P-21**

**Chemical Diagnosis of Methylmalonate Semialdehyde Dehydrogenase (MMSDH) Deficiency: A First Case Report in East Asia**

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Objective: Methylmalonate semialdehyde dehydrogenase (MMSDH) deficiency is an inherited metabolic disease caused by a defect of MMSDH which catalyzes methylmalonate semialdehyde (MMS) to propionyl-CoA in the metabolic pathway of valine. Only a few cases with MMSDH-deficiency were reported in the world so far, and there is very little information on the clinical sketch and pathophysiology. Clinical findings in reported cases vary from asymptomatic, mild clinical courses including recurrent vomiting, or unconsciousness to life threatening episodes. Some cases showed severe developmental delay or external malformations. We found a Japanese boy with MMSDH-deficiency, who was detected in detailed work up examination including organic acid analysis because of hyperlipidemia indicated at regular school medical checkup.

Methods: The patient was an 11-year-old boy. He had no abnormality in his growth or development, and played a basketball at school. His family history was normal. Hypercholesterolemia (T-Chol 265 mg/dl) was detected at in
school medical checkup, which persisted a year after (T-Chol 280 mg/dl). Although he was essentially asymptomatic, he was further examined.

Results: Urinary organic acid analysis by GC/MS showed significant increase of 3-hydroxypropionate (3HPA) and 3-hydroxyisobutyrate (3HIBA). There was no elevation of ketone bodies or methylcitrate. Amino acid analysis demonstrated elevation of 3-aminoisobutyrate (3AIBA) and beta-alanine (b-ALA) in both serum and urine. Sequencing of the ALDH6A1 cDNA that encodes MMSDH demonstrated heterozygous skipping exon10, although the other mutation was not identified. Enzyme activities of 3-hydroxyisobutyl-CoA hydratase and 3-hydroxyisobutyric acid dehydrogenase, and respiratory chain complex-I in skin fibroblasts were normal.

Conclusions: Similar to metabolic pathways of MMSDH, ethylmalonate semialdehyde dehydrogenase (EMSDH) that catalyzes ethylmalonate semialdehyde to butyryl-CoA, and malonate semialdehyde dehydrogenase (MSDH) that catalyzes malonate semialdehyde to acetyl-CoA, are known. 3HIBA and 3AIBA are located up-stream of MMSDH reaction, 3HPA and b-ALA are up-stream of MSDH and 2-OH-methylbutirate exist up-stream of EMSDH. These metabolic pathway and excessive accumulation of 3HPA, b-ALA, 3HIBA and 3AIBA observed in our patient with MMSDH deficiency suggest that MMSDH and MSDH are identical. Furthermore, the relation between this disease and hypercholesterolemia seen in this case is still unknown.

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P-22

Citrulline Treatment for OTCD, CPSD, and LPI in Japan

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Objective: OTCD (ornithine transcarbamoylase deficiency), CPSD (carbamoyl phosphate synthetase I deficiency) and LPI (lysinuric protein intolerance) are inherited metabolic diseases with hyperammonemia and serious disorders which can cause severe impairment and death even if patients received proper treatment. Because liver transplantation for urea cycle disorders (UCDs) is not frequently performed, medicine treatment which include
arginine, sodium benzoate or and citrulline administration etc are generally performed and are effective for UCDs. Because sodium benzoate and citrulline is not government-approved drugs in Japan, most sodium benzoate and citrulline used for UCDs patients are reagents for research. Therefore, these drugs were uncertain for safety. Because we used citrulline supplement approved as healthy food and could confirm the safety and effect on the usage of citrulline in this time, we report this content.

Methods: This study was designed for patients with OTCD, CPSD or LPI who received citrulline supplement. This citrulline was provided patients with for free by the Japanese society of inherited metabolic diseases. We investigated the effect of citrulline administration for growth, blood ammonia concentration and liver disorder etc and researched the safety of citrulline administration.

Results: Eighteen patients with OTCD, 7 patients with CPSD and 21 patients with LPI received citrulline supplement in Japan. For these patients, citrulline supplement is very effective for hyperammonemia. In many patients with OTCD or LPI, digestive symptom was improved by citrulline supplement. Many patients with LPI suffered from growth impairment, hepatomegaly, impaired consciousness, convulsion, nausea/vomit, liver disorder or and anemia before citrulline administration, but these symptom were improved after citrulline administration. And the adverse effect for citrulline supplement was not reported.

Conclusions: Although arginine and sodium benzoate was generally used for UCDs patients, citrulline was not frequently used. Citrulline should be more frequently used for OTCD, CPSD and LPI. We think that citrulline can be a very effective treatment strategy for OTCD, CPSD and LPI.

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**P-23**

**Clinical and molecular characteristics of four patients with Glutaric aciduria type I**

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Objective: Glutaric aciduria type I (GA I) is caused by deficiency of glutaryl-CoA dehydrogenase (GCDH) which is involved in the catabolic pathways of L-lysine, L-hydroxylysine and L-tryptophan. This study was performed to report the clinical and molecular characteristics in 4 Korean patients with GA I, which has been rarely reported in the Asian population.

Methods: The study population consisted of 4 Korean patients with a confirmed diagnosis of GA I. Clinical manifestations at diagnosis, laboratory findings, clinical courses and results of genetic study by GCDH sequence analysis were reviewed.

Results: All patients, 2 males and 2 females, were unrelated Koreans. The median age at diagnosis was 13 months, ranged from 1 to 33 months. Macrocephaly and developmental delay were observed in three patients, whereas seizure was observed only in one patient. Urine organic acid analysis revealed elevated urinary excretion of 3-
hydroxyglutaric acid (3-OHGA) (23.4 (5.6 – 54) mmol/mol creatinine; nl, 0 mmol/mol creatinine) and glutaric acid excretion (3376 (55–3799) mmol/mol creatinine; nl, 0 – 15.7 mmol/mol creatinine) in all cases. Symmetrical cerebrospinal fluid space widening within the sylvian fissures and anterior to the temporal lobes and basal ganglia changes were noted on brain magnetic resonance imaging. Patients were treated with the Glutarex-1 (Abbott cooperation), L-carnitine (100 mg/kg/day) and riboflavin (100 mg/day). One patient showed severe neurological impairment with movement disorder. Two had mild intellectual impairment. The remaining patient, identified in the presymptomatic stage by neonatal screening test, showed normal development until age 8 months. Genetic study for the GCDH gene was available in three patients. p.Ser139Leu were found in four alleles, whereas p.Glu160X and p.Ser305Leu were detected in one allele each. The most common mutation in our study, p.Ser139Leu, was known as rare both in Western and other Asian populations.

Conclusions: GA I is a treatable but often missed inherited disorder with a previously underestimated prevalence in the Korean population. Early detection and treatment are associated with better patient outcome, reinforcing the importance of neonatal screening. The mutation spectrum of our patients indicates the possibility of ethnic differences in GCDH mutations.

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P-24

A Large Genomic Deletion Detected By SNP Array in One Methylmalonic Aciduria Family

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Objective: Methylmalonic aciduria (MMA) is an autosomal recessive metabolic disorder caused by impaired methylmalonyl-CoA mutase activity, leading to accumulation of methylmalonate in body fluids. MMA may be caused by mutations either in the MUT, MMAA, MMAB, or MMADHC genes. Mut-type MMA, with an incidence of about 1/100,000, is one of the most common organic aciduria disorders in Taiwan. Most of the mutations detected in the MUT gene in Chinese population are missense or small insertion/deletion. One of our mut-type MMA patients was
found to be homozygous for the c.1280G>A mutation in the MUT gene and was suspected to carry an exonic deletion since the c.1280G>A mutation was only present in the MUT gene of the father, and was not present in the mother.

Methods: The patient was detected by newborn screening with elevated blood propionylcarnitine and confirmed to be MMA by urinary organic acid analysis and methylmalonyl-CoA mutase activity determination. In order to characterize molecular defects in the MUT gene of this patient, mutation analysis, linkage analysis, and short tandem repeat (STR) analysis were performed. To identify the cause of loss of maternal allele, a genome-wide single nucleotide polymorphism (SNP) array with an average spacing between SNPs of 2.4kb was performed.

Results: One homozygous c.1280G>A mutation was identified in the MUT gene of the proband. However, the results of linkage analysis revealed that this mutation and c.636G>A SNP in the MUT gene were only identified in the MUT gene of the father indicating a deletion of genomic segment in this patient. A genome-wide SNP array with an average spacing between SNPs of 2.4 kb was then applied to characterize the deletion. The SNP analysis revealed a homozygosity between rs12176541 and rs2635727 for the patient and her mother. Additional SNPs analysis between rs12176541 and rs2635727 indicated that both patient and her mother harbored a deletion across genomic sequence of 2.2 Mb between SNP rs2052800 and rs6930924.

Conclusions: This study describes for the first time a large genomic deletion in the MUT gene. The use of SNP arrays provides an accurate and rapid tool for defining large genomic abnormality.

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**P-25**

**Determination of Propionyl-CoA Carboxylase Activity in Human Lymphocytes by High Performance Liquid Chromatography**

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Objective: Propionyl-CoA carboxylase (EC 6.4.1.3; PCC) is a biotin-dependent enzyme which converts propionyl-CoA to D-methylmalonyl CoA mitochondrial matrix. PCC is composed of two subunits, alpha and beta subunit. Defects in either subunits result in deficient PCC activity and lead to propionic acidemia (PA, MIM 232000, 232050). Determination of PCC activity from cell culture or mutation screening on the genes responsible for the alpha and beta subunits of PCC are the two methods of choice to confirm the disease status. Conventional method for PCC activity assay requires radioisotope-labeled reagents and is time-consuming. Previously, methylmalonyl-CoA mutase was shown to be determined with the aid of high performance liquid chromatography (HPLC) for
confirmatory diagnosis of methylmalonic acidemia. In this study we applied this non-radioisotope method for PCC measurement to test the application of HPLC in PCC enzyme assay for diagnosis of PA.

Methods: Phytohaemagglutinin (PHA) stimulation of lymphocytes were lysed by sonication 5mM (pH 7.0) phosphate buffer and used for PCC assay. The reaction mixture containing Tris buffer, NaHCO3, ATP, and propionyl-CoA was incubated at 37°C for 60 minutes, and the reaction was stopped by HClO4. After centrifugation, the supernatant was analyzed by reverse-phase HPLC to determine the methylmalonyl-CoA production of the enzyme reaction.

Results: The mobile phase of HPLC was composed of 100mM phosphate buffer (pH 4.0) with 7.5% (v/v) acetonitrile at 1.0 ml/min flow rate using Finepak SIL C18-5 column to separate methylmalonyl-CoA and propionyl-CoA. Normal PHA-stimulated lymphocytes had activities from 0.5 to 0.9 nmol/mg protein/min whilst PHA-stimulated lymphocytes from PA patient had undetectable PCC activity.

Conclusions: We have established a non-radioisotope assay for determining the activity of PCC in PHA-stimulated lymphocytes for diagnosis of PA. This method is clinically applied in the differential diagnosis for an affected baby detected by MS/MS with elevated propionylcarnitine.

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P-26

Pitfall for the Diagnosis of Fructose-1,6-bisphosphatase Deficiency: Urinary GC/MS Analysis after Organic Solvent Extraction May Miss the High Excretion of Glycerol-3-Phosphate in Lactic Acidosis Atta

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Objective: Fructose-1,6-bisphosphatase (FBPase) deficiency is a rare autosomal-recessive disorder. FBPase is a key enzyme in gluconeogenesis pathway and its defect causes hypoglycemia, ketonuria and lactic acidosis after a period of fasting. In this condition, all gluconeogenic precursors, including dietary fructose cannot be converted to glucose, and consequently, intermediates such as glycerol and glycerol-3-phosphate are excreted into the urine.
We present a case with FBPase deficiency in which routine urinary GC/MS analyses could not detect the elevation of excreted glycerol of glycerol-3-phosphate in lactic acidosis attacks.

Methods: Case: 1-day-old girl. Pre- and perinatal history: Not significant.

Present illness: Normally delivered girl with gestation of 38 weeks-6 days, birth weight: 2890g. Because she developed tachypnea and retraction after 24 hours after birth, she was transported to an NICU in a prior hospital. At the admission, severe lactic acidosis (pH: 7.159, HCO3: 3.5mmol/L, BE: -21.9mmol/L, lactate: 211.6mg/dl) was observed. CHDF and administration of vitamins and L-carnitine were performed and lactate reduced to the normal level. Amino acid analysis, urinary organic acid analysis by GC/MS and acylcarnitine analysis by tandem MS showed no abnormal results. She was referred to our hospital for farther study for inherited metabolic diseases, but she had no episodes such as lactate elevation or hypoglycemia with normal development. When she was 12 months of her age, she showed lethargy and tachypnea in the morning, and blood analysis revealed her lactic acidosis and hypoglycemia. Glucose infusion improved her symptoms, but similar severe lactic acidosis and hypoglycemic attacks were observed every 3 months (in the 2nd attack, pH: 7.118, BE: -20.9mmol/L, lactate: 112.7mg/dL, glucose: 1mg/dl). Routine urinary GC/MS analyses were performed in every attack, but no specific results were observed other than lactate elevation. We suspected mitochondrial disorders but muscle and liver biopsy showed no abnormality. Because severe hypoglycemia was observed in every attack without liver enlargement, alanine and glycerol loading tests were performed to detect the gluconeogenesis defect after informed consent.

Results: In the glycerol loading test, her blood glucose did not rise and lactate levels were increased with pH reduction. From this result, we measured her enzyme activity of FBPase and detect low activity of the enzyme. Re-analysis in the stocked urine sample by GC/MS using urease pretreatment without organic solvent extraction detects the high amount of glycerol-3-phosphate.

Conclusions: Urinary GC/MS analysis is important test for the diagnosis of FBPase deficiency, because glycerol-3-phosphatase is a key metabolite in this disease. This analysis usually performed after organic solvent extraction to detect organic acids, but in the high lactate condition, glycerol-3-phosphate may be hardly extracted into a sample fraction. For the diagnosis of FBPase deficiency, we should consider the patient’s lactate levels for urinary GC/MS analysis, and urease treated GC/MS method is useful even in high lactate condition.

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Glycogen storage disease type III in Malaysian children: clinical and laboratory findings

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Objective: Glycogen storage disease type III (MIM 2324000) is an autosomal recessive disorder that affects glycogen metabolism. It is caused by deficiency of glycogen debranching enzyme as a result of mutations of the AGL gene. Deficiency of the debranching enzyme resulted in impaired release of glucose from glycogen. The glycogen accumulated has a structure with short outer chains. The involvement of liver results in hepatomegaly and hypoglycaemia while muscle involvement results in progressive myopathy and exercise intolerance. GSD type III is characterized by variable liver, cardiac muscle, and skeletal muscle involvement. To date, there are limited knowledge of GSD III in South-east Asia. We aim to study the clinical features and laboratory findings of GSD III in Malaysian patients.
Methods: All patients with the diagnosis of glycogen storage disease type III under follow-up at the Genetics and Metabolic Clinic, University of Malaya Medical Centre, Kuala Lumpur were reviewed. A retrospective case review of 3 patients who performed. Clinical and laboratory data were collected and analysed.

Results: There were 3 patients—two Malay boys while the third patient was a Chinese girl. None of the parents reported any consanguineous marriage. The two Malay patients were diagnosed at the age of 6 years, while the third patient was diagnosed at 15 months, when they presented with progressive abdominal distension. The mean duration between clinical presentation and diagnosis was 3 and half years. None presented with symptoms of hypoglycaemia. All the patients had huge hepatomegaly with the liver margin 10–12 cm below the right subcostal margin. Two patients had growth delay while one patient had mild learning difficulties. None of the patients had effort intolerance, muscle weakness or symptoms of cardiac failure. The two Malay patients had low pre-prandial plasma glucose levels between 2.4 – 2.8 mmol/L. All the patients had elevated liver transaminases, fasting triglycerides and serum creatine kinase levels. The Malay patients had raised fasting serum lactate and uric acid levels. One of the patients had neutropenia. None had evidence of liver tumours or cardiomyopathy on imaging studies. All the 3 patients had liver biopsy performed which showed preservation of liver architecture and hepatocytes diffusely distended with glycogen. None had cirrhosis. The initial diagnosis was GSD type I or type III. The diagnosis of GSD III was confirmed with low blood levels of debrancher enzyme assay. All patients received genetic counselling, advice on symptomatic treatment of hypoglycaemia and uncooked cornstarch supplements.

Conclusions: To the best of our knowledge, this is the first report of GSD type III in the Malay population. All the patients had delayed diagnosis. It is vital to increase awareness of this condition amongst clinicians so that early diagnosis, treatment and monitoring of this condition can be achieved

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P-28
To Ascertain the Utility Of Serum Biotinidase Activity As A Potential Biomarker In Hepatic Glycogen Storage Diseases

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Objective: The aim of this study was to investigate the potential use of serum biotinidase activity in different types of hepatic GSDs

Methods: Serum Biotinidase activity was measured in serum by spectrophotometric techniques. Molecular diagnosis of GSDW’s was obtained whereever feasible

Results: Serum biotinidase activity was measured in a total of 27 GSD patients and was compared with that of healthy controls (7.7 +/- 1.0: range 6.0–10.8 mU/ml: n = 26). We found an increased biotinidase activity in patients
with GSD Ia (17.7 +/- 3.9; range: 11.4-24.8; n = 12), GSD I non-a (21.6 +/- 5.6; range 14.6-26.0; n = 2), GSD III (12.5 +/- 3.6; range 7.8-19.1; n = 9), GSD VI (15.4 +/- 2.0; range 14.1-17.7; n = 4).

Conclusions: The sensitivity of this test was 100% for patients with GSD Ia, GSD I non-a and GSD VI. Serum biotinidase appears to be as a reliable diagnostic biomarker for hepatic glycogen storage disorders. In resource poor countries where enzyme activities on both fresh and frozen liver tissue are not commonplace it may be useful in direct molecular diagnosis based on basic biochemical criteria.

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activity. The siblings are 6 years and 1 month of age. The younger brother showed high galactose level in newborn screening and enzyme assay revealed a loss of GALK activity without any clinical symptoms. Two point mutations, c.1025G>A, c.623_626delCCAG, were identified in DNA analysis of the GALK gene. His elder brother also showed the same mutations without any clinical symptoms and his serum galactose level was 0.6 mg/dL.

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P-30

Functional Analysis of Mutant MCAD Protein Found in Japan

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Objective: To date, over 20 Japanese patients with Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) have been found. Recently, c.449–452delCTGA (449del4) mutation had been reported as a common mutation among which found up to 60% Japanese patients. However, no other common mutations were found, suggesting that most of
the other mutations are unique and uncharacterized mutations. To characterize mutants found in Japanese patients, we performed analysis of their functions using gene expression system.

Methods: Eleven patients with MCADD were studied. Five patients were diagnosed after episodes of metabolic decompensation and 6 patients were detected to have MCADD from the results of newborn screening (NBS). We generated eleven mutations found among them by PCR-based site-directed mutagenesis. Plasmid DNAs carrying the WT or each mutants allele of ACADM were introduced into HEK293 cells. We evaluated n-octanoyl-CoA dehydrogenase activity of crude cell lysate including mutant MCADD protein by measuring 2-octenoyl-CoA formation at 37°C.

Results: Six mutants including 449del4 showed less than 10% residual activity. On the other hand, five showed over 50% activity. We examined residual activities of the latter 5 mutants at higher reaction temperature, and found that none of them showed significant decline. These 5 mutants had been found among screened infants and a patient suspected MCADD with slight symptom.

Conclusions: Our results suggest that some of those newborns with positive results in the screening of MCADD could have considerably high levels of residual enzyme activity. Minute genetic and enzymatic evaluation is essential for appropriate follow-up of cases suspected with MCADD by MS/MS–NBS in Japan.

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What do you think of Enzyme Replacement Therapy and Newborn Screening for Mucopolysaccharidoses? Opinions from Patients and Families of patients in Japan and Korea

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P-31

What do you think of Enzyme Replacement Therapy and Newborn Screening for Mucopolysaccharidoses? Opinions from Patients and Families of patients in Japan and Korea

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Objective: Discussion about introduction of some types of Mucopolysaccharidoses (MPS) in newborn screening (NBS), derived from the availability of effective therapy that may improve long-term outcome especially if started prior to irreversible organ damage. Although the clinical trials of enzyme replacement therapy (ERT) for those MPS have demonstrated amelioration of some clinical manifestations, ERT also has raised numerous practical, ethical, and even medical issues especially when there has been no clear benefit to the patients. The aim of this study is to find out and to clarify the issues in ERT and NBS for MPS by assessing the opinions of individuals with MPS and of their parents in Japan and Korea.

Methods: A questionnaire, including hypothetical clinical scenarios about ERT and NBS for MPS, was sent to members of MPS support groups in Japan. In Korea, same questionnaire (translated into Korean) were handed out to adult patients with MPS and parents of individuals with MPS. A part of the questionnaire was generated based on the results of previous studies (Hayes IM, et al., Clin Genet 2007; David JC, et al., J Pediatr. 2008).

Results: The questionnaire was completed by 208 members of MPS support group in Japan, 46 adult patients and parents of individuals with MPS in Korea. The previous study of USA and Australia showed that only 47% were in favor of ERT where severe physical and intellectual problems are well established. On the other hand, 81% of Japanese and 91% of Korean participants were in favor of ERT even in such severe conditions. Also, majority of participants in all countries gave an affirmative to use of NBS in all hypothetical scenarios, severe or mild, even if they would not promise outcomes affected by early diagnosis and treatment.

Conclusions: In Japan and Korea, majority of adult patients and parents of individuals with MPS considered that they want to receive ERT if they will have any effect of ERT, even if they will not promise an effect for the intellectual problems. They also concerned about various things that often associated with ERT (including ethical, psychosocial and physical problems, the economic burden and family function burden etc.). Furthermore, majority of parents of individuals with MPS gave an affirmative for NBS, and they expect an early treatment with an early diagnosis. However, in some cases that early diagnosis and treatment would not affect outcome, approximately 20% participants had negative opinions about an early diagnosis before appearance of any symptoms. We have to carefully examine about NBS for MPS (including what type of MPS would be introduced into NBS). It is necessary to establish the system to provide a chance for pre- and post-counseling (genetic counseling) and the system to obtain informed consent.

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P-32

Secondary Carnitine Deficiency in Patients with Fatty Acid Oxidation Disorders during Acute Crisis

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Objective: To evaluate the derangement of carnitine metabolism in patients with long-chain fatty acid oxidation disorders.
Methods : Using tandem mass spectrometry, we analyzed free carnitine and acylcarnitines in sera or dried blood spots (DBS) of patients with CPT-2 deficiency and VLCAD deficiency. Three patients with CPT-2 deficiency and 3 with VLCAD deficiency experienced acute metabolic decompensation, such as hypoglycemia, hyperammonemia, or sudden death in infancy. One patient with VLCAD deficiency had acute metabolic decompensation during newborn period. Eight patients with CPT-2 deficiency and 11 with VLCAD deficiency experienced rhabdomyolysis.

Results : All patient, who experienced acute metabolic decompensation in infancy, had severe deficiency of free carnitine (1.9–5.5 nmol/ml), while patients with myopathy–form of CPT-2 deficiency or VLCAD deficiency did not have carnitine deficiency (18.6–69.6, 27.3–59.8 nmol/ml, respectively). The VLCAD–deficient patient with acute life–threatening event had very mild elevation of C14:1–acylcarnitine (0.26 nmol/ml) together with severe carnitine deficiency (5.3 nmol/ml), and the diagnosis of VLCAD deficiency was confirmed by enzyme assay and DNA analysis. The CPT-2–deficient patient with sudden death had carnitine deficiency (5.5 nmol/ml) during apparently healthy period several months before death at the age of 13 months. The VLCAD–deficient patient with metabolic decompensation during newborn period did not show carnitine deficiency (27.9 nmol/ml). Retrospective analysis of the stored newborn DBS showed that all patients with acute metabolic decompensation in infancy were detected in newborn screening by MS/MS using modified screening markers for CPT-2 deficiency or VLCAD deficiency.

Conclusions : The present data suggested the importance of carnitine supplementation for the patients with severe type of long–chain fatty acid oxidation disorders in order to prevent acute crisis in infancy. With respect to the chemical diagnosis on acute crisis, the designated marker acylcarnitines, that is, C14:1–acylcarnitine in sera for VLACD deficiency or C16–acylcarnitine in DBS for CPT-2 deficiency, may not be elevated significantly due to carnitine deficiency, and wrong diagnosis as carnitine transporter defect may result. Newborn screening by tandem mass spectrometry is thought to play an important role to improve the prognosis of the patients with fatty acid oxidation disorders by the therapies including carnitine supplementation.

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P–33

Why LCHAD Deficiency is Frequently Detected in Poland?

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Objective : Long chain 3–hydroxyacyl–CoA dehydrogenase deficiency (LCHADD) is rare defect of mitochondrial fatty acid beta–oxidation , which is a major energy–producing pathway during fasting or metabolic stress. LCHADD
is inherited by autosomal recessive trait and associated in most cases with common mutation c.1528G>C. The condition considered initially as very rare, is now reported as rather frequent disorder of fatty acid beta-oxidation all over the world. The aim of the study was to answer why LCHADD is the disease so frequently identified in Poland.

Methods: The study was performed by retrospective analysis of all 59 patients from 55 families with diagnosed LCHADD. They were detected through: regular differential diagnosis, symptomatic screening by both GC/MS and MS/MS methods, pilot newborn screening (NBS) by MS/MS (performed since 2001), family at risk screening and post mortem investigation. Additionally genetic diagnostics using newborns’ filter paper has been performed in selected Kashubian region.

Results: In the whole group of 59 patients diagnosed during 17-year period, twenty eight were detected through selective screening (seventeen by GC/MS and eleven by MS/MS), nine patients during differential diagnostics, eleven as a result of pilot NBS by MS/MS (among them five neonates were detected by the routine phenylketonuria screening, as their dry blood spots were reexamined by MS/MS method), four due to family at risk investigations, four were post mortem diagnoses and three detected abroad. Most of these patients became from the Kashubian region. DNA analysis revealed that in this region carrier frequency of c.1528G>C mutation was high comparing to the frequency in the whole country and in the rest of Poland: 1:73 vs 1:189 vs 1:217. These results allowed for evaluation of LCHADD frequency as: 1:16 900, 1:118 336 and 1:153 664, respectively. Mean disease frequency determined on the basis of the number of detected patients in Poland in 2000-2008 was 1:90 512, and was similar to the mean frequency based on the pilot NBS (658 492 dry blood spots investigated) – 1:109 749.

Conclusions: In Poland LCHADD is the most frequently detected defect among mitochondrial fatty acid oxidation disorders, what seems to be explained by high frequency of the disease in one region and efficient active selective screening for inborn errors of metabolism in the whole country.

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P-34

Clinical Analysis of Mitochondrial Diseases in One University Hospital

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Objective: The aim of this study was to investigate clinical features of neurological mitochondrial diseases.

Methods: We reviewed medical records of the patients with mitochondrial diseases diagnosed in Department of Child Neurology, Tottori University Hospital between 1971 and 2010. The diagnoses were made according to the criteria proposed by a research group of the Japanese Ministry of Health, Labour and Welfare.
Results: We had diagnosed 19 patients (10 males and 9 females) from 16 families in the period. Their phenotypic categories were Leigh syndrome (9 patients), myoclonic epilepsy with ragged-red fibers (MERRF) (2 patients), infantile myopathy and lactic acidosis: fatal form (2 patients) and non-fatal form (1 patient), mitochondrial encephalomyopathy with lactic acidosis (MELAS), Leigh/MELAS overlap syndrome, Leigh/NARP (neuropathy ataxia and retinis pigmentosa) overlap syndrome, Leber hereditary optic neuropathy and nonspecific encephalopathy (1 patient, respectively). The onset age of the disease ranged from 0 day to 14 years old, and the first symptoms were failure to thrive (31.6%), psychomotor delay (31.6%), acute regression (10.5%), nystagmus (10.5%), seizure, hearing loss and visual impairment. Fourteen out of 19 patients had hypotonia and nine patients had seizures in the course and some patients had visceral involvements: heart, liver, kidney, gastrointestinal tract and pancreas. Twelve patients showed abnormalities in their brain image. Two out of 12 patients had had normal image at the initial study, and cortical atrophy or abnormal intensity lesion of the basal ganglia were detected later. The serum and cerebrospinal fluid (CSF) lactate levels were increased in 8 and 15 patients, respectively. Eight patients showed high lactate level only in the CSF. Mitochondrial DNA analyses were done in 10 patients and the mutations were detected in 6 patients. No nuclear DNA analyses were done.

Conclusions: Leigh syndrome was main phenotype of mitochondrial diseases. Failure to thrive, psychomotor delay, hypotonia and seizures were common symptoms. It is important that to examine both serum and CSF lactate level, and brain image should be studied repeatedly in the patients who were suspected to have mitochondrial diseases.

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P-35

The First Case of Creatine Transporter Deficiency with SLC6A8 Gene Mutation in Korea

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Objective: Creatine transporter deficiency (OMIM #300352) is a rare X-linked inborn error of creatine metabolism caused by a dysfunction of creatine transporter 1 (CT1 or CRTR). It is membrane transporter encoded by the SLC6A8 gene (OMIM *300036) mapped to Xq28, which works for creatine to enter cells and the brain. Creatine is an energy shuttle that is required for the utilization of ATP derived energy at sites of high energy utilization such as
brain, heart, muscle. A defect in the CT1 results in brain creatine deficiency, so it induces mental retardation, hypotonia, autism, behavioral problems, and seizures. We describe the first Korean patients with creatine transporter deficiency initially identified by brain proton magnetic resonance spectroscopy (MRS). The patient presented seizure at 11 years of age with severe mental retardation. MRS shows a markedly decreased creatine peak. However, creatine in plasma was normal and creatine treatment failed to improve the neurological symptoms. Three nucleotides deletion, c.1222–1224delTTC in exon 8, were identified in DNA analysis of the SLC6A8 gene. This is the first case of creatine transporter deficiency reported and confirmed by gene analysis in Korean patient. The clinical symptoms are usually non-specific and MRS followed by gene analysis can be useful tools for the clinical diagnosis of the disease.

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P-36

Newborn Screening for Infantile Pompe Disease: Report of a pilot study

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Objective: Newborn screening (NBS) for Pompe disease has been initiated in several countries and regions, and is reportedly successful. However, the comparatively high frequency of pseudodeficiency allele makes NBS for Pompe disease complicated in Japanese population. We demonstrated our modified screening procedure was able to
distinguish the pseudodeficiency from Pompe disease patients. Based on this research, we have started a pilot study of NBS for infantile Pompe disease and reported the result of this study.

Methods: Healthy newborns born from Jan. to May in 2011 in National Center for Child Health and Development were screened. Informed consent was obtained from all newborn’s family members. Dried blood spot (DBS) samples were obtained from newborns in 3-5 days after delivery. GAA activities were analyzed under the following conditions: (1) total GAA measured at pH 3.8, (2) GAA measured at pH 3.8 in the presence of acarbose, and (3) neutral glucosidase activity (NAG) measured at pH 7.0 without acarbose. The % inhibition and NAG/GAA ratio were calculated. Samples with 30% of the normal GAA activity mean, % inhibition 60%, and NAG/GAA ratio 30 were considered as 1st screening positive. To identify pseudodeficiency, DBS samples of 1st screening positive individuals were analyzed sequence variants c.1726G>A (p.G576S) in GAA gene using PCR direct sequencing.

Results: 361 (Male: 185, Female: 176) neonates were screened in this pilot study. 15 neonates were found with less than 30% of normal GAA activity level. 14 of 15 newborns were diagnosed as “Pseudodeficiency” with the result of gene analysis. The frequency of “Pseudodeficiency” was 3.9%. One newborn was underwent the measurement of GAA activity in lymphocytes and GAA gene analysis. This newborn was diagnosed as normal healthy individual in the end. Specificity of the pilot study was 95.8%.

Conclusions: NBS for Pompe disease in Japanese population could be successfully conducted by using a new cut-off value and including genotyping and lymphocyte GAA assay.

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P-37

Significant Accumulation of Cholesterol Ester in Fetal Tissues in Wolman Disease

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Objective: Background
Wolman disease is an autosomal recessive disorder of breaking down cholesteryl esters and triglycerides, resulting in accumulation of fat in the several organs. Major clinical manifestations are hepatomegaly and liver dysfunction. Enzyme replacement therapy is now under development. Its effectiveness is controversial because it is unclear whether significant accumulation and organ dysfunction initiate in fetal period or not. We had opportunity to analyze fetal liver and kidney of Wolman disease.

Methods: Case report

A 36-year-old woman had two babies diagnosed as Wolman disease based on clinical manifestations. Gene test showed that the proband is a homozygote of 890 insert G of acid lipase gene. Prenatal genetic diagnosis was performed and the fetus was diagnosed as affected and aborted at 16 gestational weeks. An autopsy was done.

Results: Liver and kidney tissue were obtained from the fetus. Cholesterol, Cholesteryl esters and triglycerides were separated by thin-layer chromatography. Apparent cholesterol ester accumulation was detected in liver and kidney tissue. In the liver, accumulation of triglycerides and cholesterol was also observed, indicating the accumulation had already started in fetal period.

Conclusions: Accumulation of fat in the several organs had already started in fetal period. But, as for Wolman disease, it is limited to body tissue that lesions appear, and the neurologic lesion does not progress. So enzyme replacement therapy for Wolman disease may be effective when it starts after birth.

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Objective: Wolman’s disease is a rare autosomal recessive lysosomal storage disease caused by deficiency of lysosomal acid lipase leading to accumulation of cholesterol ester. We explored clinical characteristics and diagnosis methods of Wolman disease based on a case of Wolman disease.

Methods: We retrospectively reviewed the clinical, biochemical, radiological and histopathological findings of an infant with Wolman’s disease confirmed by deficient acid lipase activity in the leukocytes, which was measured using 4-methylumbelliferyl oleic.

Results: The sixteen-day boy was failing to thrive with progressive vomiting, ditention and hepatosplenomegaly. Xanthomatosis were observed on enlarged liver, spleen and lymph nodes during abdominal surgery. Abdominal x-ray revealed adrenal calcifications which were confirmed on abdominal CT scan. Liver and lymph node biopsy showed foamy histiocytes. The acid lipase activity in leucocytes was 3.5 nmol/(mg.h) [control 35.5–105.8 nmol/ (mg.h) ]. Serum chitotriosidase activity was 315.8 nmol/ (ml•h) [control < 53 nmol/ (ml•h) ].

Conclusions: In suspected case of Wolman’s disease, a plain abdominal x-ray should be obtained to check for the typical pattern of adrenal calcification, especially in any young infant with failure to thrive and progressive hepatosplenomegaly. The estimation of acid lipase in leucocytes is critical to confirm the disease.

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P–39

Effect of Enzyme Replacement Therapy for Mucopolysaccharidosis type II Initiated in Early Infancy

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Objective: Mucopolysaccharidosis type II (MPS-II) is a lysosomal storage disorder which progressively involves multiple organs and tissues. It has been shown that enzyme replacement therapy (ERT) by recombinant idursulfase can improve several visceral symptoms. Some lesions seem to be irreversible by ERT started after they are developed, but little is known about preventive effects for pre-symptomatic patients yet.

Methods: We diagnosed an asymptomatic infant with MPS-II according to his family history. The index case was his elder brother who was diagnosed after typical symptoms including mental retardation had developed and was introduced into ERT at the age of 3 years. We could start ERT for the younger patient when he was 4 months old, and compared its effects with those observed in the elder brother. Recombinant idursulfase was administered weekly, at the dosage of 0.3 – 0.5 mg/kg for the elder patient and 0.5 mg/kg for the younger. The duration of ERT was 22 months and 20 months, respectively.

Results: After 20 months of ERT, the younger patients stayed free from most of the symptoms that had already appeared in his brother at the same age, only showing exudative otitis media. Radiological evaluation detected slight changes indicative of dysostosis multiplex and dilation of perivascular spaces. His developmental quotations were kept around lower limit of normal range. Symptoms in elder patient also improved or kept stable in general during ERT, but his mental development seemed to be deteriorating gradually.

Conclusions: These findings suggest that pre-symptomatic initiation of ERT can prevent or attenuate progression of visceral lesions of MPS-II. Further observations are required to clarify long-term outcome of preventive application of ERT, though we have to consider hematopoietic stem cell transplantation if progressive mental deterioration should become evident.

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P-40
Enzyme Replacement Therapy in Two Japanese Siblings with Fabry Disease and its Effectiveness on Angiokeratomas

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Objective: Fabry disease is an X-linked metabolic disorder caused by a deficiency in the lysosomal enzyme, alpha-galactosidase A (α-Gal A). The resultant inability to catabolize glycosphingolipids causes progressive multisystemic accumulations of globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) throughout life. The initial signs and symptoms of Fabry disease typically begin in childhood and adolescence with neuropathic pain crises, angiokeratomas, hypohidrosis, and gastrointestinal problems. However, because these signs and symptoms are not specific to Fabry disease, these patients are frequently misdiagnosed, and the correct diagnosis may be delayed.

Methods: We present the cases of two Japanese siblings with Fabry disease who were initially suspected by older brother’s angiokeratomas at age 13 years, although his younger brother did not have. It seemed to them and their parents that they had the hypohidrosis constitution and they did not complain of neuropathic pain. Their α-Gal A activities in leukocytes showed very low level (0.2 nmol/mg.protein/hr compared with a usual threshold of 49.8–116.4).

Results: They were started enzyme replacement therapy (ERT) with every other weekly infusions of agalsidase alpha (ReplagalTM, Dainippon Sumitomo Pharma, Osaka, Japan) dosed at 0.2 mg/kg in August 2007. After 3 months of therapy, their sweating abnormalities were improved, and after 1 years of therapy, they recognized the sense of pain which they had not recognized probably due to, paralysis of nerve function. After 4 years of therapy, older brother’s angiokeratomas partially disapperared. They experienced no drug-related adverse events or infusion-related reactions.

Conclusions: The clinical use of agalsidase alpha was demonstrated to be safe and effective in angiokeratomas.

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P-41

**Retrospective analysis of the clinical manifestations and survival of Korean patients with mucopolysaccharidosis type II: Emphasis on the cardiovascular complication and mortality cases.**

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Objective: Hunter syndrome (mucopolysaccharidosis II, MPS II) is a rare, X-linked disorder of glycosaminoglycan (GAG) catabolism caused by a deficiency in the activity of the lysosomal enzyme, iduronate-2-sulfatase (I2S).

Methods: In this study, the medical records of 75 Korean patients with Hunter syndrome (74 males, 1 female) were retrospectively reviewed to investigate the frequency of organ involvement and survival at a single center.

Results: The three most common symptoms of organ involvement were hepatosplenomegaly (99%), facial dysmorphism (97%) and frequent otitis media (91%). Cardiovascular involvement was also common including valvular abnormalities (89%), left ventricular hypertrophy (68%), and hypertension (30%).

The 19 patients who died had a median age of 16.8 years at the time of death. Four of them died within 1 year of the start of enzyme replacement therapy; autopsy showed myocardial infarction with severe coronary artery stenosis in 1 patient. Two other patients died due to pneumonia and sleep apnea. In one case, the cause of death was not investigated.

Conclusions: The high incidence of hypertension and the presence of valvular heart disease indicates that close cardiac monitoring is mandatory in all patients with Hunter syndrome, especially relatively older patients even if they are being treated with enzyme replacement therapy.

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**P-42**

**Novel Mutations in The GALC Gene in A Chinese Patient with Adult-onset Krabbe Disease**

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Objective: Krabbe disease is an rare autosomal recessive leukodystrophy. It is caused by a deficiency of galactocerebrosidase (GALC) activity.

To describe a 30-year-old woman with Krabbe disease, correlating clinical and biochemical abnormalities to two novel mutations on the GALC gene.

Methods: Clinical investigation was enriched by neurophysiological and neuroimaging data. The activity of GALC was assayed in white blood cells using methylumbelliferone (MU)-labelled fluorescent substrate. Genomic DNA was isolated from peripheral blood, and the GALC gene was sequenced.

Results: The patient had a 2-year history of gait problem and was experiencing weakness in the right leg. Intellectual capacity was normal. Krabbe disease was confirmed by markedly reduced leukocyte galactocerebrosidase (GALC) activity. Magnetic resonance imaging showed areas of demyelination in the white matter of the brain. T2-weighted MRI of the brain showed brain atrophy and diffuse high signal intensity of the cerebral white matter and the brain stem, while nerve conduction was completely normal. Two novel mutations were found in the GALC gene, a small deletion, 1908_1911+1delTAAGG and a missense mutation, 2041G>A(V681M).

Conclusions: Enzyme assay resulted in low, although not null, GALC activity, which can explain the protracted clinical course in this patient.

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P-43
Mutation Analysis of GALNS Gene for Prenatal Diagnosis of Morquio A (MPS IVA) Disease

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Objective: Mucopolysaccharidosis IVA (MPS IVA) is caused by deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS), leading to systemic skeletal dysplasia because of excessive storage of keratan sulfate (KS) in chondrocytes. In an effort to determine a precise prenatal diagnosis, we aim to identify mutations of GALNS gene for prenatal diagnosis of MPS IVA in a pregnant female with previously affected children.

Methods: Chorionic villi (CV) were obtained by transcervical aspiration from 3rd and 4th pregnancy at 11 weeks of gestation age. Mutation analysis of GALNS gene from genome of CV tissue and CV culture cells were performed using genome amplification and direct sequence. Enzyme assay from CV culture cells was performed using spectrofluorometry.

Results: The 3rd affected fetus was a compound heterozygous for mutations p.G116S (c.346G>A) in exon 4 and p.R386C (c.1156C>T) in exon 11 of GALNS gene. Mutant allele p.G116S was from the mother and mutant allele p.R386C was from the father. The enzyme activity was undetected in CV culture cells and the pregnancy was terminated. The 4th fetus was found to be carrier for the mutation p.G116S with detected residue activity of CV culture cells (2.99 UI/mg protein). The pregnancy was continued for 4th fetus.

Conclusions: Rapid and precise mutation analysis of GALNS gene is suitable for prenatal diagnosis in MPS IVA.

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Clinical, Radiological and Molecular Analyses in a Chinese Patient with Fucosidosis

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Objective: Fucosidosis is an autosomal recessive lysosomal storage disease in which a deficiency of α-L-fucosidase results in multisystem accumulation of fucose-containing glycolipids and glycoproteins. It is characterized clinically by progressive mental and motor deterioration, growth retardation, coarse facies, and often recurrent infections. The gene encoding α-L-fucosidase has been mapped to the short arm of chromosome 1 at position 1p34.1-36.1 and has been called FUCA1. Here we described a 2 year-old Chinese boy with fucosidosis confirmed by measuring α-L-fucosidase activity and gene mutation analysis.

Methods: The activity of α-L-fucosidase was assayed by fluorescence method in peripheral blood leukocytes. His spine and brain were detected by X-ray and MRI/MRS, respectively. Analysis of FUCA1 gene mutation was performed on cDNA by RT-PCR from whole blood and directly sequencing.

Results: The 2 years-old boy, the first child of non-consanguineous parents born at 40 weeks of gestation after an uneventful pregnancy, had normal psychomotor development until the age of 8 months. Thereafter, psychomotor development progressed slowly with kyphosis, mild coarse facies. Radiological studies showed anterior beaking of vertebrae and kyphosis. MRI revealed mild hyperintense signals on T2 weighted and fluid-attenuated inversion recovery (FLAIR) images, hypointense signal on T1-weighted images in both internal capsules. An abnormal peak at 3.8 ppm was found in both internal capsules and globus pallidus. The α-L-Fucosidase activity in leukocytes was deficiency (0 nmol/h. mg protein; control 56.5-113). Other lysosomal enzyme activities were normal. Urine glycosaminoglycan and organic acids were negative. Genetic analysis of FUCA1 showed that a known homozygous polymorphisms, Q281R (860C>G), was detected and a homozygous single-base substitution (396T>A) was found in exon 2, changing the tyrosine to a stop codon (Y126X).

Conclusions: Fucosidosis is a very rare lysosomal storage disease. The Y126X mutation of FUCA1 predicts a lack of 314 amino acids at the carboxyl terminus of the protein. The same mutation was reported in a Hong Kong patient and a Taiwanese patient, both of them were from southern China. MRS detected a spectral peak at 3.8-3.9 ppm which was reported in a Fucosidosis patient by A. Y. Oner in 2007.

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P-45

Combined Effects of Enzyme Replacement and Adipose-Tissue derived Mesenchymal Stem Cells on Mouse Model of the Mucopolysaccaridosis II
Objective: Mucopolysaccharidosis type II (MPSII or Hunter syndrome) is a progressive, multisystemic disease caused by a deficiency of iduronate-2-sulfatase (IDS). Neurological involvement is indicative of more severe disease, but is not attenuated by current enzyme replacement therapy due to the blood-brain barrier. Mesenchymal stem cells (MSCs) possess self-renewal capacity and they are multipotent progenitor cells capable of differentiating into mesenchymal lineages. Moreover, MSCs are also known to differentiate into neurons and glial cells in vitro, and in vivo following transplantation into the brain of animal models of neurological disorders. Therefore, we postulated that the neurological defects in MPSII may be corrected by MSC transplantation or the combination therapy with IDS (Elaprase®) and MSCs. The purpose of this study was to evaluate the therapeutic efficacy of combination therapy of stem cell transplantation and enzyme replacement in MPSII.

Methods: In this study, IDS knock-out mice (MPSII mice) was used and the treatments were initiated at different ages, including 8 weeks (Experiment 1) and 16 weeks (Experiment 2). Each experiment has five groups (n=4~7 per each group): IDS wild type mouse group (WT), IDS knock-out mouse group (KO), KO-IDS group, KO-MSC group, and KO-MSC/IDS group. WT and KO groups were given saline, while IDS (0.5 mg/kg), MSCs (2×10^6) and both of IDS and MSCs were administered in KO-IDS group, KO-MSC group and KO-MSC/IDS group, respectively. Test materials were administered intravenously weekly (saline and IDS) or once every 2 weeks (MSC) up to 3 months. At 6 weeks after initial i.v. treatment, subsequent intracerebroventricular injection with same material (5 μl saline (WT and KO groups), 5 μl IDS (KO-IDS group), or 2×10^5 MSCs (KO-MSC and KO-MSC/IDS groups)) was performed using a stereotaxic apparatus.

Results: The KO-IDS group showed decreasing tendency of GAG concentration in brain, suggesting the possibility of direct intracerebroventricular IDS replacement for the neurological defects in MPSII, although the difference did not reach statistical significance. Early treatment (Experimental I) did not show any synergistic effects of combined therapy on reducing GAG accumulation. Late treatment (Experiment II) also showed the same result pattern of GAG accumulation in liver and urine samples on day 90 with experimental I. However, MSC/IDS combined therapy significantly reduced GAG concentration in urine on day 60 compared to the KO group whereas the IDS treatment alone couldn’t. This finding suggests that the combined stem cell-IDS therapy is more effective treatment than IDS only.

Conclusions: In conclusion, this study might be useful for development of combined stem cell therapies, especially for lysosomal storage disease.

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Significant Accumulation of Cholesterol Ester in Fetal Tissues in Wolman Disease
Objective: Background

Wolman disease is an autosomal recessive disorder of breaking down cholesteryl esters and triglycerides, resulting in accumulation of fat in the several organs. Major clinical manifestations are hepatomegaly and liver dysfunction. Enzyme replacement therapy is now under development. Its effectiveness is controversial because it is unclear whether significant accumulation and organ dysfunction initiate in fetal period or not. We had opportunity to analyze fetal liver and kidney of Wolman disease.

Methods: Case report

A 36 year-old woman had two babies diagnosed as Wolman disease based on clinical manifestations. Gene test showed that the proband is a homozygote of 890 insert G of acid lipase gene. Prenatal genetic diagnosis was performed and the fetus was diagnosed as affected and aborted at 16 gestational weeks. An autopsy was done.

Results: Liver and kidney tissue were obtained from the fetus. Cholesterol, Cholesteryl esters and triglycerides were separated by thin-layer chromatography. Apparent cholesteryl ester accumulation was detected in liver and kidney tissue. In the liver, accumulation of triglycerides and cholesterol was also observed, indicating the accumulation had already started in fetal period.

Conclusions: Accumulation of fat in the several organs had already started in fetal period. But, as for Wolman disease, it is limited to body tissue that lesions appear, and the neurologic lesion does not progress. So enzyme replacement therapy for Wolman disease may be effective when it starts after birth.

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Study on newborn screening for glycorgen storage disease type II, mucopolysaccharidosis type I and type II by using dried blood spots
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Objective: There have been significant advances in the development of enzyme replacement therapy (ERT) for lysosomal disorders such as glycogen storage disease type II (GSD II or Pompe disease), mucopolysaccharidosis type I (MPS I or Hurler & Scheie disease), and type II (MPS II or Hunter disease). These diseases are rare and some patients are not diagnosed until irreversible tissue damage has occurred. Pompe disease usually die within the first year of life from cardiomyopathy, muscular hypotonia, and/or pulmonary disease, therefore, it has been necessary to establish an appropriate newborn screening program to give early ERT for patients with this disease. We found that the measurement of activity of acid α-glucosidase (GAA), α-iduronidase (ID) and iduronate-2-sulfatase (IDS) in dried blood spots (DBS) from newborns is useful in the early detection of patients with GSD II, MPS I and MPS II, respectively.

Methods: Material and Methods: We measured enzyme activity in the DBS of the Following: 240 healthy volunteers as controls and 5 previously diagnosed cases with GSD II, 7 cases with MPS I and 3 cases with MPS II, and 916 newborn babies of Osaka city university hospital. Activity of GAA for GSD II and ID for MPS I in DBS were measured by the Chamoles’ method using fluorogenic substrates. For early detection of GSD II and MPS II, GAA and IDS in DBS respectively, were measured by using immuno-capture assay.

Results:
1) Results of GSD II screening
The mean level of GAA of 916 neonates was 2.678±1.54 μmol/hr/L. The activity in 22 of these cases were lower than cut off values, and 19 cases of the 22 were diagnosed as pseudo GAA deficiency by DNA analysis. GAA levels in patients with GSD II measured by Chamole method as well as immune-capture method were clearly distinguishable from normal subjects.

2) Results of MPS I and MPS II screening
Measurements of ID activity for MPS I and IDS activity for MPS II were clearly distinguished in 7 patients with MPS I and 3 patients MPS II from the control subjects. In 4 out of the 916 cases, ID activity was below the cut off values, however, these results were not reconfirmed in the second blood specimen. IDS activity in two severe cases and one milder case with previously diagnosed MPS II were almost zero, and the residual activity of IDS in DBS was not found in the milder case. Among eleven male cases showing activity below cut off value, 2 cases were diagnosed as pseudodeficiency of IDS (A284L) by DNA analysis.

Conclusions: In summary: We developed reliable newborn screening methods for GSD II, MPS I and II than can detect patients before irreversible tissue damage has occurred.

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Globotriaosyl sphingosine (Lyso-Gb3) as a biomarker for Fabry disease; Negative electrospary ionization tandem mass analyses

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Objective: To date, a reliable biomarker that reflects disease severity and progression to help guide the management of Fabry disease has not been discovered. It is necessary to develop rapid analytical method for screening and/or correct confirmation diagnosis of Fabry disease. Following the recent publication on the determination of plasma globotriaosyl sphingosine (lyso-Gb3) by Aerts et al, we developed De Novo analytical method for Lyso-Gb3 in urine samples by tandem mass spectrometry (MS/MS).

Methods: A rapid negative ion MS/MS analytical method was developed for Lyso-Gb3 detection without any long preparation step with good sensitivity and specificity on human urine. LC-MS/MS was set with specific MRM ions (m/z 281, m/z 263 m/z 84, m/z 96, and m/z 60) for Lyso-Gb3 and m/z 310, m/z 280, m/z 110, m/z 84, and m/z 82 for internal standard, N,N-Dimethyl-D-erythro-sphigosine.

Results: Calibration curves for quantification showed an excellent linear relationship for Lyso-Gb3 in pooled urine. R² was 0.9978 in the range of 0.01 – 200 ng investigated.

Conclusions: The new method was simple as well as time and labor saving without SPE or liquid-liquid extraction. The described method could be useful for rapid monitoring of clinical application after enzyme therapy with high specificity, excellent sensitivity and rapidity of analysis.

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Clinical Screening of X-linked Adrenoleukodystrophy in Indonesia
Objective: The childhood cerebral form of X-linked adrenoleukodystrophy (X-ALD) are seen most commonly in males between ages four to eight years. It initially resembles attention deficit disorder or hyperactivity: progressive impairment of cognition, behavior, vision, hearing, and motor function follow the initial symptoms and often lead to total disability within two years. MRI is always abnormal in males with neurologic symptoms and often provides the first diagnostic lead. VLCFA levels are elevated in 99.9% of males with X-ALD of all ages regardless of the presence or absence of clinical symptoms. Unfortunately in developing countries such as Indonesia, the facilities of VLCFA examination were unavailable locally and too expensive for sending abroad, for that reason we proposed clinical screening that lead to suggested diagnosis.

Methods: We have evaluated 5 patients who were presented with psychomotor regression since school age (5–10 years old) who were previously reported healthy. All patients were subjected to detailed history, physical examination and brain MRI imaging. Basal cortisol and serum levels of VLCFA were measured if brain MRI suggested X-ALD. VLCFA measurement were sent to Kennedy Krieger Institute, Baltimore if financially possible.

Results: Patients all boys aged 5–7 years old, presented with progressive impairment of cognition, behavior, vision, hearing, and motor function. Brain MRI studies performed in all of our cases have shown characteristic bilateral extensive demyelination in posterior brain white matter, especially in the parieto-occipital regions. Our results are fairly comparable to those reported by other author. (Melhem et al., 1997). Basal cortisol were performed in 2 patients, one showed deficiency of cortisol and treated by corticosteroid. Only two patients could afford the VLCFA measurement and both were definitively confirmed by significant high concentration of VLCFA.

Conclusions: Brain MRI studies could be used as clinical screening tool for X-ALD among progressive impairment of cognition, behavior, vision, hearing, and motor function school aged boys, before VLCFA confirmation diagnostic test.

Contact Information: Damayanti Rusli Sjarif (ukk.npm.idai@gmail.com)
Objective: Wilson’s disease (WD) is an autosomal recessive disorder of copper metabolism. In Japan, there are few reports of long-term follow-up studies of Wilson’s disease. This study evaluated the current state of patients with Wilson’s disease (WD) at our hospital. In the present study, we have retrospectively reviewed all children with WD treated in the Pediatric Department of Kumamoto University Hospital.

Methods: The patients were treated pediatric WD patients who were followed by our Department. The 18 patients were analyzed.

Results: The age ranged from 3–21 years old (median 13 years). There were 7 girls and 11 boys. Side effect of D-penicillamine were occurred in 4 patients. There were 4 acute hepatic failure patients. Only 2/18 patients were grafted in our hospital otherwise liver transplantation can be lifesaving for WD with acute hepatic failure. 2 patients with acute hepatic failure were saved without LDLT regardless of severe predicting scores.

Conclusions: Recent progress of treatments allows better prognosis for severe WD patients. In the future, other clinical markers that should be established.

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**Spectrum of inherited metabolic diseases in the Southern Chinese population: a 10 year review**
Objective: The first integrated metabolic clinic in Hong Kong was setup in 1997 in this hospital. After over 10 years of operation, more than 100 patients and families had been diagnosed and treated in this metabolic clinic. And a profile of prevalent IMDs in the Southern Chinese population has emerged from our case registry. Here, we review and estimate the incidence of these more prevalent IMDs.

Methods: Case review and analysis of case registry of a Metabolic Clinic in Hong Kong serving a catchment area of a population of 1.2 million. Only those 109 Han Chinese patients were included in the review.

Results: The category of diseases are comparable to other countries, e.g. small molecular disease accounts for about half of all IMDs. Prevalent IMDs with estimated incidences of higher than 1 in 60,000 include glutaric aciduria type 1, multiple carboxylase deficiency, primary carnitine deficiency, carnitine-acylcarnitine translocase defect, glutaric aciduria type 2, citrin deficiency and PTS deficiency.

For large molecular diseases, most of the types of storage diseases have been diagnosed but no specific pattern is evident. However, glycogen storage diseases type 1 appears to be more common.

Conclusions: The spectrum of IMDs in the Southern Chinese is different from that of Northern Chinese. It is generally accepted that genetic difference exists between the Northern and Southern Chinese. It has been hypothesized that Northern Chinese are descendants of settlements from taken North migration route, while the Southern Chinese are the offsprings of another migration event using routes through Southeast Asia. Therefore, our data is not only useful for future service development for Southern China, it may also be of reference to other countries in the SE Asia region.

Contact Information: Nelson Tang (nelsontang@cuhk.edu.hk)
Objective: Background. Familial hypercholesterolemia (FH) is an autosomal dominant disorder which is characterized by increased levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C). The clinical phenotype of FH has been shown to be associated with increased coronary heart disease (CHD) and premature death. Fortunately, FH can be detected at any age, from clinical and/or genetic testing plus family history. There are three groups who have developed diagnostic tools for FH: MedPed Program, Simon Broome Register, and Dutch Lipid Clinic Network. These tools use the combination of family history of CHD, elevated LDL cholesterol and DNA analysis.

Objective. To compare FH screening and identification in children based on criteria of MedPed Program, Simon Broome Register, and Dutch Lipid Clinic Network.

Methods: Method. This is a cross-sectional study. Children, age 2–18 years, from families in which their parents had a premature CHD and hypercholesterolemia are eligible for this study. Parents and children are conducted physical examination and lipid (total cholesterol, triglycerides, LDL-C, HDL-C and Apolipoprotein B) profile determination. They also conducted some tests to rule out the possibility of hypercholesterolemia due to other conditions, such as diabetes mellitus, kidney, thyroid, or hepatic disorders or on lipid-lowering medications or any drugs that affect lipid metabolism. Subjects are classified into three different criteria based on MedPed Program, Simon Broome Register, and Dutch Lipid Clinic Network criteria.

Results: Results. There are 28 subjects and 20 index cases from 20 families. The mean age of the children is 11.6 ± 4.75 years, while the mean age of index cases is 47.8 ± 5.50 years. The mean age of index case had their first heart attack is 45.3 ± 5.65 years. According to MedPed criteria 15% of subjects may suffer from FH, while only 10% of subjects are suspected to have FH with Simon Broome Register criteria. Whereas Dutch Lipid Clinic Network criteria found 50% subjects are very likely (probable) had FH and 30% children are possible qualified as FH. Genetic testing is not included in this study.

Conclusions: Conclusion. Dutch Lipid Clinic criteria can detect more FH children compared with two other criteria based on history, clinical and cholesterol profile only.

Contact Information: Titis Prawitasari (tprawitasari@yahoo.com)
Objective: Background: Mucopolysaccharidosis (MPS) I, is a debilitating and often fatal disease. Early diagnosis and treatment may help prevent irreversible damage. Enzyme Replacement Therapies (ERT) is known as one of the therapeutic choices. This case is aimed to demonstrate the obstacle providing ERT for MPS I in Indonesia.

Methods: Case: A twelve year old girl is referred to the hospital because of obstructive sleep apnea (OSA) since few months ago. Actually, she already had chronic recurrent ENT infections since she was three and followed by snoring symptoms since two years ago. The ENT doctor in the local hospital thought that large of tonsils and adenoid are the root of problems. Unfortunately, tonsil-adenoidectomy is never being done because of very tricky intubation procedure. Doctor recommends doing some diagnostic examination in tertiary hospital. However, because of economical constrain, diagnostic procedure was done after sometime. On physical examination we found coarse thick hair, flat nasal bridge, broad mouth, large tongue, cloudy cornea, pansystolic murmur, hepatosplenomegaly, umbilical hernia, and shortened phalanges. Bone survey showed dysostosis multiplex and valvular deformity on echocardiography. It also revealed that patient was born normal, from unrelated parents. The early development was unremarkable and parents did not observe any changes in child’s facial feature. According to all data, we think about MPS I and plan to do enzyme analysis abroad. Result showed a deficiency of α-L-Iduronidase. We manage the OSA with Continuous Positive Airway Pressure (CPAP) and try to get ERT. Parents were counsel about the risk and benefit of ERT and try to get some donations to afford the drug. We asked manufacturer to provide ERT, but they need import permit from The National Agency for Food and Drug Control (NA-DFC) and should be asked by the physician incharge. It took several weeks to be approved. After spartan discussion, parents decided to quit due to financial problem. Patient was died one month later.

Results: Discussion. It is very challenging to manage MPS patient in Indonesia. Ignorance, economical constrains, limited facility and comprehensive health services are the most common problems in Indonesia. Thus, many patients are delayed to manage and admitted to the hospital with severe condition that will reduce survival and quality of life.

To prevent such condition, socialization and education among health workers and community about MPS should be done. Coarse facial, skeletal dysplasia, organomegaly, severe skin changes and elevated glycosaminoglycan (GAG) in urine are common manifestations that lead to MPS. Another problem arise is how to get ERT’s drugs, which are very expensive and the submission for import permit, which takes some time.

Conclusions: Conclusion. Although ERT have already help MPS I patient around the world, there is some obstacles to afford this drug in Indonesia.

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Objective: Background

Absence of sufficient laboratory examination to screen and establish the diagnosis of Inborn Error of Metabolism cases in our country has become a challenge for us, pediatricians, to be able to make clinical and laboratory assessment. Isovaleric Acidemia (IVA) is one of the branched-chain aminoacidopathies due to isovaleryl-coenzyme A dehydrogenase deficiency. Early diagnosis and appropriate management are significant in improving survival rate.

Objective: To report a case of Isovaleric Acidemia due to isovaleryl-coenzyme A dehidrogenase deficiency.

Results: Case. A 36-month-old child, came to the emergency unit with profuse vomiting and decreased consciousness. Her mother reported that she had been in good health until 2 days before admission. History of past illness, she had been hospitalized when she was 3 weeks due to septic condition and she had motoric developmental delay. Later, she was admitted to the Pediatric Intensive Care Unit of Sardjito Hospital and presented severe dehydration and intractable metabolic acidosis. Physical examination revealed hyperthermia, tachycardia, kussmaul respiration, and prominent “sweaty feet odor”, filling the examination room. Laboratory evaluation exhibited a very high anion gap level, increased lactate serum, ureum and creatinin, and liver function test were within normal limits. Despite a normal glucose value from serial examination, the tests for urine ketones were positive. Blood culture showed positive pseudomonas aeruginosa. Our working diagnosis at that time were organic acidemia suspected Isovaleric Acidemia, sepsis, and acute renal failure. Then, the sample blood paper was sent to Genetic Metabolic Disease Laboratory Academic Medical Center Amsterdam, which took 2 weeks before the result was known. While waiting for the examination result, we managed this patient by giving Intravenous fluid therapy of 10% dextrose in normal saline, antibiotic, sodium bicarbonate, carnitine supplementation, and restricted protein diet especially Leucyn for 6 days. Acylcarnitine analysis suggested the C5-carnitine was extremely high, while level of free carnitine decreased. Eventually, the patient was discharged against medical advice, and died in two days.

Conclusions: IVA due to isovaleryl-coenzyme A dehydrogenase deficiency could be managed optimally. Early detection and appropriate management became a necessity for better prognosis.

Contact Information: Neti Nurani (neti_pediatrician@yahoo.co.id)
A Suspected Wilson Disease Girl Undergone For Liver Transplant

Maria Mexitalia1, Ninung RD Kusumawati2, I. Hartantyo3, Hirlan4, Moedrik Tamam5, Yulianto6, Christie Mannopo7, AG. Soemantri8

1Pediatrics, Dr. Kariadi Hospital, Diponegoro University, Semarang, Indonesia , 2Department of Pediatrics Faculty of Medicine Diponegoro University / Dr. Kariadi Hospital, 3Department of Pediatrics Faculty of Medicine Diponegoro University / Dr. Kariadi Hospital, 4Department of Internal Medicine Faculty of Medicine Diponegoro University / Dr. Kariadi Hospital, 5Department of Pediatrics Faculty of Medicine Diponegoro University / Dr. Kariadi Hospital, 6Department of Pediatrics Surgery Faculty of Medicine Diponegoro University / Dr. Kariadi Hospital, 7Department of Pediatrics Faculty of Medicine Sam Ratulangi University Manado, 8Department of Pediatrics Faculty of Medicine Diponegoro University / Dr. Kariadi Hospital

Objective: Wilson disease (WD) is a genetic disorder in which copper accumulates in tissues; this manifests as neurological or psychiatric symptoms and liver disease. The objective of the case report is to diagnose and prepare child with Wilson disease for liver transplant.

Methods: A seven years girl referred to Dr. Kariadi Hospital Semarang on April 2010 to undergo the liver transplant. She was born from un-consanguinity couple, full term baby, and normal delivery. She suffered from jaundice since 2 years old and the abdomen became distended, difficult to walk and failed to gain weight. Physical findings revealed Kayser-Fleischer ring diagnosis by oculist, enlargement of the liver and spleen, movement disorders including tremor, mild dysarthria, rigid dystonia and drooling. The lunulae ceruleale appeared on her skin. The result of laboratorium were mild anemia, Bilirubin 7.62 mg/dl, AST 252, ALT 137, GGT 111, Alkaline Phosphatase 729, albumin 2.4 g/dl, Plasma Prothrombin Time 1.3x control, Partial Tombroplastin Time 1.5x control, Fibrinogen 363.4 mg/dl, INR 1.58, ceruloplasmin serum 50 mg/dl, blood copper 1.31 ug/L, 24h-urine copper 41.17 ug/L. Liver biopsy appeared steatosis microvascular appearance, ballooning degeneration, parenchymal necrosis, sufficient billiary duct, billiary stasis, parenchymal fibrosis and peace meal necrosis, this appearances due to initial pseudolobulus, and mallory bodies in hepatocytes. This histology appearance is due to Wilson disease. The liver copper was not measured.

Results: According to the guideline for diagnosis and treatment of Wilson disease (American Association for the Study of Liver Diseases) the diagnosis of the girl was suspected of Wilson disease. Since the ceruloplasmin level of the girl is 50 mg/dl, we need the confirmed diagnosis by the liver copper level that did not done to the patient due to limited facility. The recommend treatment of WD is chelating agents (D-penicillamine, trientine, zinc and tetrathiomolybdate). Unfortunately due to unavailability of the donor and the condition of her liver end stage, she died before got the liver transplant.

Conclusions: Wilson disease is one of treatable metabolic disease with treatment by reduce copper absorption or removes the excess copper from the body. Occasionally a liver transplant is required. Due to the later referral and liver end stage condition, our patient died before got the liver transplant.

Contact Information : Maria Mexitalia (maria_mexitalia@yahoo.com)

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A Case of Morquio A Syndrome

Julius Anzar¹, INGGRIANI TOBARASI², INGGRIANI TOBARASI, ADITIAWATI³

¹Child Health, MOH. HOESIN HOSPITAL, PALEMBANG, FACULTY OF MEDICINE, UNIVERSITY OF SRIWIJAYA, Palembang, Indonesia ; ²FACULTY OF MEDICINE, UNIVERSITY OF SRIWIJAYA, ³FACULTY OF MEDICINE, UNIVERSITY OF SRIWIJAYA

Objective : To report morquio A syndrome as a rare case

Results : A 4 years old boy with short and disproportional stature, appeared healthy at birth (weight 3800gr, height 52cm) with normal growth and development velocity. First symptoms were spinal and sternum deformity. No history of consanguinity in family. Physical examination: body weight 12kg

Conclusions : Morquio A syndrome should be considered in children with normal growth and development at birth, but at 2nd year of life shows growth retarded and bone deformity. It’s important to measure growth velocity regularly for early detection.

Contact Information : Julius Anzar (juliusanzar@gmail.com)
Three Korean Patients with Maple Syrup Urine Disease: Four Novel Mutations in the BCKDHA Gene

Hyung-Doo Park\textsuperscript{1}, Dong Hwan Lee\textsuperscript{2}, Yong Hee Hong\textsuperscript{2}, Dong Hee Kang\textsuperscript{3}, You Kyoung Lee\textsuperscript{3}, Junghan Song\textsuperscript{4}, Soo-Youn Lee\textsuperscript{1}, Jong-Won Kim\textsuperscript{1}, Chang-Seok Ki\textsuperscript{1}, and Yong-Wha Lee\textsuperscript{3}

\textsuperscript{1}Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan, University School of Medicine, Seoul, Korea; \textsuperscript{2}Department of Pediatrics, Soonchunhyang, University College of Medicine, Seoul, Korea; \textsuperscript{3}Department of Laboratory Medicine and Genetics, Soonchunhyang University Bucheon Hospital and Soonchunhyang University College of Medicine, Bucheon, Korea; \textsuperscript{4}Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea

Objective: Maple syrup urine disease (MSUD) is a rare, autosomal recessive disorder of branched chain amino acid (BCAA) metabolism caused by dysfunction of the multienzyme branched-chain alpha-ketoacid dehydrogenase (BCKDH) complex. Although a few cases of MSUD have been reported in the Korean population, the genetic background of MSUD is not well understood.

Methods: We investigated three newborn males who were diagnosed with MSUD using a standard newborn screening test and amino acid analysis. We screened all coding regions of the BCKDHA, BCKDHB and DBT genes for abnormalities using direct sequencing. Changes in these genes are associated with MSUD. For one patient with complex deletion/duplication mutations, we also performed TOPO TA cloning sequencing.

Results: Amino acid analysis showed elevated levels of all branched chain amino acids (BCAAs) in all patients. Three patients were either homozygous or compound heterozygous for the BCKDHA mutations. Patient 1 was homozygous for c.1036C>T (p.R346C); patient 2 was heterozygous, with c.632C>T (p.T211M) and c.659C>T (p.A220V); and patient 3 had c.1204_1209dupAAACCC (p.L402_P403dup) and c.1280_1282delTGG (p.L427_A428delinsP). Among these mutations, c.1036C>T, c.632C>T, c.1204_1209dup and c.1280_1282del were novel. These patients had no mutations in either the BCKDHB or the DBT gene. Although this study included only three patients, the five different mutations in these patients may indicate mutational heterogeneity in Korean patients with MSUD.

Conclusions: The BCKDHA gene may present a primary target for clinical genetic analysis. To the best of our knowledge, this is the first report of genetically confirmed MSUD in Korea.
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Multiplex Ligation-Dependent Probe Amplification Assay for Diagnosis of Congenital Adrenal Hyperplasia

Ja-Hyun Jang¹, Dong-Kyu Jin², Jong-Hwa Kim³, Hyun-Kyung Tan⁴, Jong-Won Kim¹, Soo-Youn Lee¹, Chang-Seok Ki¹, and Hyung-Doo Park¹

¹Departments of Laboratory Medicine & Genetics, ²Pediatrics, ³Obstetrics & Gynecology, and ⁴Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Objective: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder characterized by androgen overproduction and impaired cortisol and aldosterone synthesis. More than 90% of CAH cases are caused by a defect in 21-hydroxylase, which is encoded by the CYP21A2 gene. The molecular diagnosis of CAH requires deletion/duplication analysis in addition to targeted mutation or sequence analysis since approximately 20% of mutant alleles are deleted for a 30-kb gene segment. In this study, we tried to detect deletions/duplications in the CYP21A2 gene by multiplex ligation-dependent probe amplification (MLPA) assay.

Methods: We analyzed 13 DNA samples that were submitted for CYP21A2 sequence analysis for the molecular diagnosis of CAH. Selective amplification of the CYP21A2 gene was performed by long PCR using allele-specific primers modified a previously described method and sequencing was subsequently performed. MLPA KIT consists of 5 probes specific for the CYP21A2 gene containing the 5' region and exons 3, 4, 6, and 8 (MRC Holland, Netherlands). The MLPA data were analyzed with Gene Marker v.1.9 software (Softgenetics, USA).

Results: MLPA confirmed the complete deletion of one CYP21A2 allele in 5 of 13 patients (Patients 1, 3, 4, 5, and 6). These 5 patients all carried a single mutant allele peak in sequence analysis: 1 patient with IVS2-13A/C>G, 3 with I172N, and 1 with R356W, respectively. In cases of I172N mutation in exon 4 (Patients 3, 5 and 6), the peak corresponding to CYP21A2 exon 4 was absent in the MLPA electropherogram since the MLPA exon 4 specific probe was unable to bind to its target.

Conclusions: All patients in our study who had a single peak of a mutant allele in sequence analysis carried large gene deletions. Direct sequencing alone is inadequate for the diagnosis of CAH and gene dose analysis should be performed simultaneously.
Suspected Kearns-Sayre Syndrome Accompanied with Severe Malnutrition and Failure To Thrive

Aidah Juliati Wahyudin¹, Damayanti Rusli Sjarif²

¹Pediatrics, Wahidin Sudirohusodo, Hasanuddin, Makassar, Indonesia , 2Pediatrics Department, Medical Faculty, University of Indonesia

Objective: Kearns-Sayre Syndrome (KSS) is a collection of symptoms characterized by a triad of progressive external ophthalmoplegia, retinal pigment degeneration and onset before age 20. This disease was first reported by Kearns and Sayre in 1958. KSS is a mitochondrial disease caused by a mutation in mitochondrial DNA. The purpose of this report is discussing the KSS case of a child with severe malnutrition and failure to thrive.

Methods: I, a girl, 1 yr 7 mo, referred by ENT department due to laryngomalacia and malnutrition. Growth chart plot revealed failure to thrive condition. No consanguinity. Vital signs within normal limit. Physical examination revealed wrinkled skin, bilateral ptosis, low set ear, xylophone ribs, dimple on back, wasting, baggy pants, no edema. Body weight 5.25 kg, body length 69 cm. Nutritional status: Severe malnutrition, marasmic type.

Results: Lab; Hb 10.4 g/dl, WBC 13,800/ul, platelet 340,000/ul, ureum 13 mg%, kreatinin 0.51 mg%, ammonia 31 umol/L, lactate 7.20 mmol/L. Blood gas analysis: pH 7.285, pCO2 29 mmHg, pO2 112.9 mmHg, HCO3 13.9 mEq/L, BE -10.7, Sat 97.9%. Electrolyte: Na 138 mmol/L, K 3.6 mmol/L, Cl 90 mmol/L. Renal tubular acidosis and lactic acidosis. Chromosome analysis: 46 XX (normal)

Conclusions: A diagnosis of suspected Kearns-Sayre syndrome accompanied by marasmic type malnutrition, failure to thrive, renal tubular acidosis and lactic acidosis was established. She was treated with Pediasure diet, Sodium bicarbonate, Carnico Q.

Contact Information: Aidah Juliati Wahyudin (aidah_juliaty@yahoo.com)
Social Program

Entertaining programs and events in beautiful harmony of Korea await participants and accompanying persons of the 2nd ACIMD & 12th AEWIEM Meeting.

Gala Night- Night to Cherish

Venue: Sapphire Ballroom (3rd fl)

Time & Date: 18:30 ~ 21:00, April 2(Mon), 2012

Attendees: Open to all participants and accompanying persons

Program: The Harmony Chorus

The Harmony Chorus is a group of young medical students (Soonchunhyang School of Medicine) who sing for the joy of themselves and others. Formed 34 years ago, the group has given countless performances. Divided into 4 parts, the chorus will end their performance by singing traditional folk songs of the Asia-Pacific region, in hopes to become one in harmony.

Banquet- Night to Enjoy

Venue: Sapphire Ballroom (3rd fl)

Time & Date: 18:30 ~ 21:00, April 3(Tue), 2012

Attendees: Open to all participants and accompanying persons

Program: The Queen (Fusion Gugak Group)

The fusion Gugak(traditional Korean music) band Queen plays traditional Korean instruments and sings Chang(a traditional Korean method of singing), but in a very new and unique way. The way they arrange and orchestrate the songs, western as well as eastern, turn music which would seem not to match into an amazing harmony. They are a renowned group in Korea and have performed at numerous international stages.
Tour Program

Full day and half day tour programs will offer participants a great opportunity to feel the beauty and wonder of Seoul. You will be able to experience the traditional and modern Seoul by attending the optional tour programs.

Optional Tour Program

FT-1 Seoul City Tour (Full-Day)

<table>
<thead>
<tr>
<th>Hours</th>
<th>09:00 - 17:30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Price</td>
<td>₩76,000/P (min. 1 person/tour) (lunch included)</td>
</tr>
</tbody>
</table>

Course:
- Hotel ➔ Jogyesa Buddhist Temple ➔ Cheongwadae Sarangchae (closed on every Monday) ➔ Presidential Blue House (Photo time) ➔ Changing of the Guard Ceremony
- Gyeongbok Palace (Deoksu Palace Tuesday only) ➔ Amethyst/ Ginseng Center ➔ Lunch ➔ Changdeok Palace (Namsan Hanok Village Monday only) ➔ Insadong Arts and Crafts Market ➔ Amethyst Center, Namdaemun Traditional Market ➔ Hotel

Remarks: with an English speaking guide assistance

Do you want to explore the Korean history and culture? This city has a lot to offer the first-time visitor. Changdeok palace is one of the oldest Royal Palaces built in 1405. For the Joseon Dynasty (1392-1910) it served as the main palace for over 300 years. Insadong is the most reliable place to purchase antiques in Seoul. You may browse around the antique shops, art galleries, and secondhand book stores.

HT-1 Morning Seoul Tour (Half-Day)

<table>
<thead>
<tr>
<th>Hours</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Price</td>
<td>₩33,000/P (min. 1 person/tour)</td>
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</table>

Course:
- Hotel ➔ Jogyesa Buddhist Temple ➔ Cheongwadae Sarangchae (closed on every Monday) ➔ Presidential Blue House (Photo time) ➔ Changing of the Guard Ceremony ➔ Gyeongbok Palace (Deoksu Palace Tuesday only) ➔ Amethyst/ Ginseng Center ➔ Arrive at Itaewon or City Hall area

Remarks: with an English speaking guide assistance

Extend an invitation to you! First visit in Korea? You can start with Korea’s representative structure. Sarangchae is a history center that give visitors a chance to follow in the footsteps of presidents of Korea and gain insight into the history of Seoul. Gyeongbok Palace, the oldest palace, built in 1394 is the main palace of the period.
Exhibition Directory

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Booth No.1 Genzyme Korea

Website: www.genzyme.co.kr  www.genzyme.com

E-mail: jeeyong.lee@genzyme.com

Tel: 82-2-2186-0216
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One of the world's leading biotechnology companies, Genzyme is dedicated to making a major positive impact on the lives of people with serious diseases. Since its founding in 1981, the company has introduced breakthrough treatments across several areas of medicine that have provided new hope for patients. Today, approximately 10,000 Genzyme employees serve patients in nearly 100 countries.

Genzyme's products are focused on rare genetic diseases, multiple sclerosis, cardiovascular disease, and endocrinology. The company's commitment to innovation continues today with a substantial development program focused on these fields, as well as other areas of unmet medical need. Genzyme is a Sanofi company.

About Sanofi. Sanofi, a global and diversified healthcare leader, discovers, develops and distributes therapeutic solutions focused on patients’ needs. Sanofi has core strengths in the field of healthcare with seven growth platforms: diabetes solutions, human vaccines, innovative drugs, rare diseases, consumer healthcare, emerging markets, and animal health. Sanofi is listed in Paris (EURONEXT: SAN) and in New York (NYSE: SNY).

Booth No.2 MERCK SERONO

Website: www.merck.co.kr

E-mail: minhee.kang@merckgroup.com

Tel: 82-2-2185-3323
Fax: 82-2-2185-3930

A unique force in the pharmaceutical industry MerckSerono combines its complementary expertise in new chemical entities(NCEs) and new biological entities(NBEs) to offer innovative prescription drugs of either origin.

With comprehensive skills in all areas from research and development to manufacturing, marketing and sales, the division offers its world-class products in over 150 countries worldwide. Our locally positioned workforce of 8,000 sales and marketing employees ensures commercial coverage in the U.S. and Canada, Latin America, Europe, Asia-Pacific and Japan.

The division has leading brands serving patients in its therapeutic areas of focus: Endocrinology, CardioMetabolic care, Oncology, Neurodegenerative Diseases, Fertility, etc.

Saizen® (Somatropin for injection) is a recombinant human growth hormone made by DNA technology which is
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For nearly 90 years, Novo Nordisk has combined drug discovery with technology to turn science into solutions for people with diabetes. We also provide treatments for people with haemophilia and growth hormone deficiency and for women experiencing symptoms of menopause. We leverage our expertise with protein molecules, chronic disease management and device technology to provide innovative treatments that make a difference in quality of care.

Novo Nordisk has more than 32,000 employees in 75 countries and markets products in more than 190 countries. Our B shares are listed on NASDAQ OMX Copenhagen and our ADRs are listed on the New York Stock Exchange under the symbol NVO.

Novo Nordisk markets Norditropin® (generic name: somatropin), a human growth hormone for the treatment of growth hormone deficiency in children and in adults, Turner Syndrome, Chronic Renal Insufficiency, Small for Gestational age, Noonan Syndrome and other indications. Norditropin® is the world’s first liquid, pre-mixed human growth hormone in a dedicated pen system.

▼ The key benefits of Norditropin® include:

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- The only liquid growth hormone product that can be kept at room temperature (2–25°C) after first use. No refrigeration necessary once opened
- Ready to use, no mixing required.

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Dong-A Pharmaceutical Co., Ltd is a Korea-based manufacturer of pharmaceutical products. The Company's offerings include prescription medicines, over the counter (OTC) drugs, biological products, consumer products, energy drinks and active pharmaceutical ingredient (API) drugs. Its product portfolio of prescription medicines and OTC drugs consists of acne treatments, emollients, preparations for hair, antibiotics, digestives, anti-inflammatory enzymes, treatments of analgesia, blood circulation activators, dietary functional foods, vitamins and others. Its consumer products include condoms, hair dyes, body washes and disposable plasters, among the others. Its energy drinks include fruit-flavor energy drinks and energy booster drinks. The API drugs include antihypertensives, antituberculosis drugs, anticancer drugs, antiasthmatics, antidiabetics, analgesics and others. The Company operates four domestic factories.

Growtropin® is a bioengineered growth hormone drug, the first such drug developed in Korea. Clinically applied for the treatment of stunted growth resulting from pituitary hormone deficiency, this drug comes in many convenient dosages, including 4IU, 12IU, 16IU and 30IU forms. In particular, the 30IU pen cartridge form, released in 2008, was well received by both doctors and patients.
Useful Information

Climate
Korea's climate is regarded as a continental climate from a temperate standpoint and a monsoon climate from a precipitation standpoint. The climate of Korea is characterized by four distinct seasons: spring, summer, fall, and winter. April is in the early spring. The average temperature range in early April when the meeting will be held is between 8°C and 18°C. A light jacket is recommended early in the morning and night.

Passport & Visa
Any foreign visitor wishing to enter the Republic of Korea to attend the Meeting should possess a valid passport, and a Korea visa, if required.

Visitors with roundtrip tickets from countries who have a special agreement with Korea may be exempt from visa requirement and can stay in Korea visa-free for periods up to 30 days or 90 days, depending on the type of agreement between the two countries. For more information, please contact the local Korean consulate or embassy in your country or refer to the website at www.immigration.go.kr. A Korean embassy or consulate can issue two types of visas: a short-term visa for visitors who want to stay up to 90 days and a special long-term visa. Special long-term visa holders are required to submit an Alien Registration Form to the Immigration Office in Korea within 90 days of arrival.

Korean Food
Eating out is one of the great pleasures of visiting Korea, a country famous for its diverse native dishes. Korean cuisine is nutritious, well balanced and low-cal as it involves a wide variety of vegetable and fermented foods. Bulgogi (marinated, barbecued beef) and Bibimbap (Boiled rice mixed with vegetables) are the most famous. When having a meal, try to keep pace with others and do not hold the bowl in the hand. After a meal, put spoon and chopsticks on the spot where they were originally placed.

Currency & Exchange
The unit of Korean currency is the Won(￦). Specifically, various notes and coins are used: Notes include 1,000 won, 5,000 won, 10,000 won and 50,000 won denominations, while coins include 10 won, 50 won, 100 won, and 500 won denominations. As of November 2011, the exchange rate is approximately US$1 to KRW 1,100. Foreign bank notes and traveller's checks can be converted into the Korean won at foreign exchange banks and other authorized money exchange outlets. The exchange rate is subject to market fluctuation.

Credit Card
Diners Club, Visa, American Express, and Master Card are widely accepted at major hotels, shops and restaurants in the larger cities. Check with your credit card company for details of merchant acceptability and
other services which may be available.

**Time**

The Korean time is 9 hours ahead of Greenwich Mean Time (GMT +9).

**Telephone Call**

For international calls, dial the international dialing code (001, 002, or 008), country code, area code, and the individual number. Domestic and international phone cards are available for sale at most convenient stores, hotels, and airports.

**Mobile Phone**

Mobile phone can be rented at the airport or at major hotels.

To apply for a mobile phone rental service, you will need an identification card (passport) and a credit card.

**Business Hours**

Government office hours are usually from 9:30 to 16:30 on weekdays and closed on Saturday and Sunday. Major stores are open every day from 10:30 to 20:00 including Sundays.

**Electricity**

The standard electricity supply is 220-volts AC/60 cycles.

Most hotels have 220-volt writing installed, but hotels may provide outlet converters for 110 and 220 volts.

**Tip & Tax**

**Tip** Tipping is not a regular practice in Korea. Service charges are included in your bill for rooms, meals, and other services at hotels and upscale restaurants. Koreans occasionally do tip when they are especially pleased with the service they receive.

**Tax** Upon request, visitors can receive nearly 10 percent VAT refunds for purchase at Duty Free shopping outlets. Goods must be taken out of Korea within three months of purchase to be eligible for a tax refund. Only foreign tourists in Korea apply for this tax refund. Visitors can receive a refund on a receipt for a minimum purchase of KRW 50,000 (around US$45). To receive this refund, present refund receipts with purchased goods at the customs desk at Incheon International Airport when departing. These receipts can be cashed in at the Cash Refund Office in front of the Airport Duty Free Shop.

**Emergency Call**
International SOS Korea provides a 24-hour emergency service for participants, for a fee, acting as a link between the patient and the Korean hospitals. For Medical Emergencies dial 1339.

119 Emergencies for fire / rescue & hospital services

112 Police

129 First Aid patient

Useful Website

Korea National Tourism Organization

www.etourkorea.com

Incheon International Airport

www.airport.kr

Seoul Metropolitan Government

http://english.seoul.go.kr

Visa & Immigration

www.immigration.go.kr/indeximmeng.html

Seoul Call Center: DASAN 120

http://120.seoul.go.kr

Tel. 02-120 (+9)

Important Notice

The Organizing Committee of the 2nd ACIMD & 12th AEWIEM Meeting shall not be responsible for and shall be exempt from all liability in respect of any loss, damage, injury, accident, delay or inconvenience to any person, or his/her luggage or any property for any reason whatsoever, for any tourist services provided. Personal travel and health insurance are recommended.
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