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Welcome

I would like to welcome you on behalf of the Organizing and Scientific Committees of INFORM and the ICIEM for your participation in the 4th Annual International Network for Fatty Acid Oxidation Research and Management symposium.

This year we have over 120 attendees representing 25 countries and are very thankful to be working in concert with the 13th ICIEM in this beautiful location and host city of Rio de Janeiro.

Our Network will provide a collaborative framework for ongoing communication and research. The main objective over the next two days is to make you familiar with recent advances in the diagnosis and treatment of disorders of fatty acid oxidation and of the carnitine cycle presented in a very open and interactive symposium format.

I am delighted that you have chosen to participate in INFORM 2017!

Jerry Vockley

Thank you for joining us!
Organizing Committee

Jerry Vockley, MD, PhD
Co-Chairman
University of Pittsburgh School of Medicine

Ute Spiekerköetter, MD
Co-Chairman
Department of Pediatrics and Adolescent Medicine,
University Children’s Hospital, Freiburg

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Daniela Karall, MD
Department for Child and Adolescent Medicine,
Medical University of Innsbruck

Melanie Gillingham, PhD, RD
Molecular and Medical Genetics Department,
Oregon Health & Science University
**Sunday September 3, 2017**

**SESSION 1: WELCOME AND KEYNOTE SPEAKER**

15:30-16:30 Registration

16:30-16:45 Welcome and Overview to INFORM 2017 & Keynote Speaker Introduction  
Jerry Vockley, Pittsburgh PA, USA

16:45-17:45 Keynote, The Impact of NBS of FAODs  
Nicola Longo, Salt Lake City, Utah, USA

17:45-18:15 Discussion

19:00-21:30 Networking Reception and Opening Dinner

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**Monday Morning, September 4, 2017**

**SESSION 2: BASIC SCIENCE**

7:30-8:30 Registration / Breakfast

8:30-9:00 Myotubes from CPT2 Deficient Patients as a Disease Model  
Fatima Djouadi, Paris, France

9:00-9:30 Free Fatty Acid Receptors and the Gut Microbome: Questions in relation to FAO Defects  
Niels Gregersen, Aarhus Denmark

9:30-9:45 Endoplasmic reticulum-mitochondria crosstalk and redox homeostasis disruption in very long-chain acyl-CoA dehydrogenase deficient fibroblasts  
Bianca Seminotti, Porto Alegre, Brazil

9:45-10:00 Development and characterization of patient-specific iPSC-derived retinal pigmentary epithelia (RPE)-like cells as a model of LCHAD-associated retinopathy  
Tiffany DeVine, Portland, Oregon, USA

10:00-10:30 Break
10:30-11:00 NAD+Therapy Improves Cardiac Function and Bioenergetics in a Mouse Model of Heart Failure  
Matt Hirschey, Duke University, USA

11:00-11:30 Lessons Learned from Animal Models of VLCAD Deficiency  
Ute Spiekerkoeter, Freiburg, Germany

11:30-11:45 VLCAD deficiency related chronic inflammation pattern is suggestive of systemic mediators  
Megan Beck, Memphis, TN, USA

11:45-12:00 Experimental evidence that fatty acids accumulating in VLCAD deficiency disrupt mitochondrial respiration in heart, liver and brain of young rats  
Cristiane Cecatto, Porto Alegre, RS, Brazil

12:00-12:30 Panel Discussion

12:30-14:00 Lunch

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**Monday Afternoon September 4, 2017**

**SESSION 3: CLINICAL SESSION**

14:00-14:30 Nutritional Ketosis  
Kieran Clarke, Oxford, UK

14:30-15:00 Ketone Body Treatment in FAODs  
Gepke Visser, Utrecht/Amsterdam, Netherlands

15:00-15:15 3-hydroxybutyrate (3-HB) treatment in multiple acyl-CoA dehydrogenase deficiency: a systematic literature review and international retrospective cohort study  
Willemijn J. van Rijt, Groningen, the Netherlands

15:15-15:30 Results from a 78-week Single-arm, Open-label Phase 2 Study to Evaluate UX007 in Pediatric and Adult Patients with Moderate to Severe Long-Chain Fatty Acid Oxidation Disorders (LC-FAOD)  
Jerry Vockley, Pittsburgh, PA, USA

15:30-15:45 Break
**Agenda**

**Monday Afternoon September 4, 2017, continued**

15:45-16:15  An overview of FAODs and unmet needs in Latin America  
*Ida Schwartz, Porto Alegre, Brazil*

16:15-16:30  Health-related Quality of Life in Patients with long-chain Fatty Acid Oxidation Disorders  
*Suzan JG Knottnerus, Amsterdam, The Netherlands*

16:30-16:45  Intravenous Sources of Medium Chain Triglycerides for Critically Ill Patients with Fatty Acid Oxidation Disorders  
*Heather Bausell, Chicago, IL, USA*

16:45-17:15  Panel Discussion

17:15-17:30  Summary / Closing remarks

17:30-18:45  Cocktail Reception & Poster Session with Oral Presentations for Jr. Investigators interested in presenting

19:00  INFORM Advisory Committee Meeting

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**INFORM Symposium at ICIEM / September 7, 2017**

**FATTY ACID OXIDATION DISORDERS**

Chair: Mike Bennett (Philadelphia, PA, USA) / Co-Chair: Eric Goetzman (Pittsburgh, PA, USA)

- Anti-oxidant therapy as an adjunct for treatment of long chain fatty acid oxidation disorders: *Guilhian Leipnitz, Brazil*

- Novel mechanisms of pathogenesis in mitochondrial trifunctional protein deficiency: implications for clinical outcome and treatment: *Areeg El-Gharbawy, USA*

- Rhabdomyolysis and inflammation in metabolic muscle disease: *Yamina Hamel, France*
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Our mission is to reduce the impact of extremely rare and devastating diseases by providing urgently needed therapies.

We work side-by-side with rare disease communities to increase awareness, improve diagnosis and expand availability of treatments for people with rare diseases.
The following Abstracts were selected to be presented at INFORM 2017:

Endoplasmic reticulum-mitochondria crosstalk and redox homeostasis disruption in very long-chain acyl-CoA dehydrogenase deficient fibroblasts
Bianca Seminotti, Porto Alegre, RS, Brazil

Development and characterization of patient-specific iPSC-derived retinal pigmentary epithelia (RPE)-like cells as a model of LCHAD-associated retinopathy
Tiffany DeVine, Portland, Oregon, USA

VLCAD deficiency related chronic inflammation pattern is suggestive of systemic mediators
Megan Beck, Memphis, TN, USA

Experimental evidence that fatty acids accumulating in VLCAD deficiency disrupt mitochondrial respiration in heart, liver and brain of young rats
Cristiane Cecatto, Porto Alegre, RS, Brazil

3-hydroxybutyrate (3-HB) treatment in multiple acyl-CoA dehydrogenase deficiency: a systematic literature review and international retrospective cohort study
Willemijn J. van Rijt, Groningen, Groningen, the Netherlands

Health-related Quality of Life in Patients with long-chain Fatty Acid Oxidation Disorders
Suzan JG Knottnerus, Amsterdam, The Netherlands

Intravenous Sources of Medium Chain Triglycerides for Critically Ill Patients with Fatty Acid Oxidation Disorders
Heather Bausell, Chicago, IL, USA

Results from a 78-week Single-arm, Open-label Phase 2 Study to Evaluate UX007 in Pediatric and Adult Patients with Moderate to Severe Long-Chain Fatty Acid Oxidation Disorders (LC-FAOD)
Jerry Vockley, University of Pittsburgh, Pittsburgh, PA, USA
Fatty acid oxidation defects (FAOD) inherited as autosomal pattern are rare inborn errors of fatty acid metabolism with one of which is carnitine transporter deficiency (CTD). Prior to mitochondrial entry, fatty acids are conjugated with coenzyme A in the form of fatty acyl-CoA in cytosol which then can pass mitochondria through carnitine shuttle. In mitochondria, fatty acids are metabolized in various steps of oxidation yielding energy in the form of ATP, used directly as fuel by heart, skeletal muscle and gut. Deficiency of carnitine transporter leads to accumulation of fatty acids in blood which can cause serious health problems. People with CTD cannot metabolize fat for energy purpose. Symptoms of CTD appear either in infancy or in childhood. In infancy, symptoms are vomiting, diarrhea, nausea, irritable mood if untreated then enlarged heart, enlarged liver, swelling in brain, muscle weakness, if left untreated coma sometimes leading to death. Symptoms in children appear from age of one year to seven year with enlarged heart, muscle weakness if left untreated death may occur due to heart failure. The main treatment of persons with CTD is lifelong use of L-carnitine with low fat high carbohydrate diet. For both diet and medication, monitoring with blood tests is recommended. Careful management of CTD leads to healthy lives and reversal of cardiomegaly and hypotonia/ muscle weakness.

**Keywords**: FAOD, CTD, L-carnitine, Cardiomegaly, Hypotonia

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**NAD+ THERAPY IMPROVES CARDIAC FUNCTION AND BIOENERGETICS IN A MOUSE MODEL OF HEART FAILURE**

Angelical S. Martin1,2, Dennis M. Abraham2, Kathleen A. Hershberger1,2, Lan Mao3, Huaxia Cui1, Juan Liu2, Xiaojing Liu2, Michael J. Muehlbauer1, Jason W. Locasale1,2, R. Mark Payne4, and Matthew D. Hirschey1,2,5

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Hypertrophic cardiomyopathy and heart failure are common human pathologies stemming from inborn errors in fatty acid metabolism. Increasing NAD+ levels by supplementing with the precursor nicotinamide mononucleotide (NMN) improves cardiac function in multiple mouse models of heart failure. While NAD+ influences several aspects of mitochondrial metabolism, the molecular mechanisms by which increased NAD+ enhances cardiac function are poorly understood. A putative mechanism of NAD+ therapeutic action is via activation of the mitochondrial NAD+-dependent protein deacetylase sirtuin 3 (SIRT3). Therefore, we set-out to assess the therapeutic efficacy of NMN and the role of SIRT3 in a genetic mouse model of cardiomyopathy in Friedreich’s Ataxia (FXNKO). At baseline, the FXNKO heart has mitochondrial protein hyperacetylation, reduced SIRT3 mRNA expression, and increased demand for NAD+. Remarkably, NMN administered to FXNKO mice restored cardiac function to levels near normal. To determine whether SIRT3 is required for NMN therapeutic efficacy, we generated SIRT3KO and SIRT3KO/FXNKO (dKO) knockout models. The improvement in cardiac function upon NMN treatment in the FXNKO is lost in the dKO model, demonstrating that the effects of NMN are dependent upon cardiac SIRT3. These results demonstrate that NAD+ therapy leads to improvements in both cardiac and extra-cardiac metabolic function and energy metabolism. Furthermore, we find a key role for SIRT3 in mediating these cardioprotective effects. Taken together, these results serve as important preclinical data for NMN supplementation or SIRT3 activator therapy in patients with inborn errors in fatty acid metabolism and cardiomyopathy.

**Financial Support:** We thank the Friedreich’s Ataxia Research Alliance (FARA)
3-HYDROXYBUTYRATE (3-HB) TREATMENT IN MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY: A SYSTEMATIC LITERATURE REVIEW AND INTERNATIONAL RETROSPECTIVE COHORT STUDY

Willemijn J. van Rijt, BSca, Emmalie A. Jager, BSca, Carolyn J. Ellaway, MD PhD, Sabine Scholl-Bürgi, MD PhD, Matthias Gautschi, MD PhD, François-Guillaume Debray, MD PhD, Michel C. Tchan, MD PhD, Manuel Schiff, MD PhD, David Gil-Ortega, MD PhD, A. Çiğdem Aktuğlu Zeybek, MD PhD, Austin A. Larson, MD, Johan L. Van Hove, MD PhD and Terry G.J. Derks, MD PhD.

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Multiple acyl-CoA dehydrogenase deficiency (MADD; glutaric aciduria type II) is a rare disorder of both mitochondrial fatty acid oxidation and amino acid metabolism. Few case reports describe successful experimental sodium-D,L-3-hydroxybutyrate (3-HB) treatment in severely affected MADD-patients. After 1) an extensive systematic literature review, we are currently performing 2) an international, retrospective questionnaire study on clinical presentation, 3-HB treatment method and (long-term) outcome in MADD(-like)-patients to investigate the efficacy and safety of 3-HB treatment in MADD.

Our systematic review identified 14 MADD(-like)-patients treated with 3-HB. To date, first results of our questionnaire study summarize 13 patients, including four previously reported cases. Median age at first clinical presentation was 5 months (range: 0 days – 26 years), six patients were also identified via abnormal population newborn screening results. Molecular analysis identified five patients with homozygous ETFA (c.1-40G>A; c.797C>T) and ETFDH (c.463A>G; c.1106G>C; c.1141G>C) mutations and three patients with compound heterozygous ETFA (c.200T>C and c.854A>T), ETFDH (c.896T>C and c.1842C>A) and SLC52A3 (c.49T>C and c.639C>G) mutations. Surprisingly, one patient demonstrated two ETFA (c.365G>A and c.809-811delTAG) and ETFB (c.217-4G>T and c.438+20C>T) mutations. Two other patients carry only one ETFDH (c.1774T>C ) and SLC52A3 (c.678-680del) mutation, respectively.

Median age at start of 3-HB treatment was 9 months (2 months – 26 years). Prescribed dosages ranged between 130 and 2600 mg/kg/day in three to six times per day, four patients received 3-HB continuously during the night. Administration methods included oral or nasogastric solution, solution via gastrostomy, oral powder and oral capsules, mostly combined with nutrition. Reported treatment rationales (TR) and clinical improvements (CI) included leukodystrophy (TR n=4; CI n=3), cardiac pathology (TR n=2; CI n=2), muscle pathology (TR n=10; CI n=6 + CI in two additional patients without this original TR), liver pathology (TR n=4; CI n=3), neuropathy (TR n=1; CI n=0) and to prevent complications (TR n=2). Overall, CI was reported in eight patients. Most frequently reported side effects included dehydration, vomiting/nausea, constipation and abdominal pain. Treatment was terminated in six patients due to (a combination of) clinical improvement un-necessitating further 3-HB treatment (n=2), no clinical improvement (n=2), death (n=1), side effects (n=1) or costs (n=1). Median age at termination of 3-HB treatment was 5 years (8 months – 26 years). Median duration of 3-HB treatment was 2 years (1 month – 8 years).

This is the largest international, collaborative cohort study on 3-HB treatment in MADD (-like)-patients. Based on our preliminary retrospective data, 3-HB can be efficacious and safe in selected MADD-patients. To improve patient monitoring, a clinical severity score for MADD is warranted.
INHIBITING LONG-CHAIN 3-KETOACYL-COA THIOLASE: A NOVEL STRATEGY FOR TREATING MITOCHONDRIAL FATTY ACIDS OXIDATION DISORDERS

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Background: Mitochondrial fatty acid oxidation (MFAO) has two major components, a transport component comprised of CPTI, CACT, and CPTII, and a catabolic β-oxidation pathway-spiral component, comprised of the acyl-CoA dehydrogenases (SCAD, MCAD and VLCAD) and the mitochondrial trifunctional protein (MTFP). MTFP is an α4β4-octamer containing the enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase functions for long-chain substrates. Channeling of intermediates in such structural arrangement for these two major components is expected. Binding of substrates/products to the catalytic sites of proteins often provides stability as has been reported for MCAD. Missense unstable variants have been reported for these MFAO proteins in patients with clinical deficiencies. In this study, trimetazidine, an inhibitor of LCKAT approved as ischemic heart disease therapy in >90 countries, was used to induce accumulation of MFAO intermediates and investigate their effect on upstream MFAO proteins stability.

Methods: Fibroblasts from patients with VLCAD, MCAD, LCHAD, and TFP deficiencies were treated with trimetazidine up to 10 μM in culture. Protein variants presence was monitored using in situ immunostaining, western blotting and/or enzyme assay. Acylcarnitines were monitored in one VLCAD deficient cell line.

Results: In all deficient fibroblasts the level of the mutant protein increased significantly in a dose dependent fashion. The increase varied with mutation and correlated with the relative stability of the protein. Acylcarnitines in one culture VLCAD deficient cell line decreased with up to 0.25 μM trimetazidine supplementation, then increased in the presence of higher concentrations of drug.

Conclusion: Trimetazidine could provide therapeutic benefit for patients with MFAO disorders.

MITOCHONDRIAL DYSFUNCTION CAUSED BY FATTY ACIDS ACCUMULATING IN VLCAD DEFICIENCY

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Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is clinically characterized by episodes of metabolic decompensation, hypoketotic hypoglycemia, liver dysfunction and cardiomyopathy, whose pathophysiology is poorly understood. We investigated the effects of the major fatty acids accumulating in biological fluids of the affected patients (myristic - Myr and cis-5-tetradecenoic - Cis-5 acids) on important mitochondrial functions in heart, liver and brain mitochondria from young rats. Myr markedly decreased mitochondrial membrane potential (Δψm) in all tissues, whereas Cis-5 mildly dissipated Δψm. Furthermore, Myr markedly decreased NAD(P)H content in the heart, and to a lesser degree in liver and brain. These effects were enhanced after Ca2+ addition, particularly in the heart. In addition, Ca2+-induced brain mitochondrial swelling was elicited by Myr but not by Cis-5. Noteworthy, Myr induced decrease of Δψm and swelling was abolished by cyclosporine A (CsA) plus ADP or ruthenium red (RR) in heart, liver and brain implying the involvement of mitochondrial permeability transition (mPT). Cis-5 induced decrease of Δψm was also prevented by CsA plus ADP and RR in the heart, implying mPT induction. Finally, we found that Ca2+ retention capacity was reduced by Myr and Cis-5 in heart and liver mitochondria, but not in the brain. These data suggest that predominant fatty acids accumulating in VLCAD deficiency, especially Myr, disturb mitochondrial functions with a higher toxicity directed towards the heart and liver. It is proposed that disturbance of mitochondrial homeostasis may be involved in the liver dysfunction and cardiomyopathy characteristic of VLCAD deficient patients.

Financial support: We thank PROPESQ/UFRGS, FAPERGS and CNPq.

Keywords: very-long-chain acyl-CoA dehydrogenase deficiency; cardiomyopathy; hepatopathy; mitochondrial functions.
MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY DUE TO A NOVEL HOMOZYGOUS AND COMPOUND HETEROZYGOUS MUTATION IN THE ETFDH GENE IN THREE SOUTH AFRICAN PATIENTS

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Introduction and objectives: Multiple acyl-CoA dehydrogenase deficiency (MADD, OMIM: 231680) is an autosomal recessive metabolic disorder due to mutations in the ETFA, ETFB and ETFDH genes resulting in the deficient function of alpha or beta subunits of the electron transfer protein (ETF) or electron transferring flavoprotein dehydrogenase (ETFDH), respectively. Dysfunction in either of these two flavoproteins leads to compromised fatty acid and amino acid oxidation as well as choline metabolism. We report on clinical, biochemical and genetic findings of three South African patients with ETFDH deficiency.

Patients and results: These patients were found to be homozygous (patient 1) for a novel c.1067G>A (p.Gly356Glu) or compound heterozygous (patient 2 and 3) for the described novel and known c.1448C>T (p.Pro483Leu) mutations in the ETFDH gene, respectively. Functional studies in muscle and fibroblasts confirmed the deleterious effects of compromised ETFDH expression on the respiratory chain function and fatty acid oxidation. The clinical-biochemical presentation for patient 1 included severe neonatal onset with congenital abnormalities, hypoglycaemia, metabolic acidosis, hyperammonemia as well as a characteristic metabolite profile of accumulating mono- and dicarboxylic acids, N-acylglycines and acylcarnitines commonly associated with MADD. Delayed onset was noted for patients 2 and 3 which also presented with the characteristic MADD metabolite profile as well as episodic metabolic acidosis, non-ketotic hypoglycaemia, mild hyperammonemia, progressive myopathy and hepatosplenomegaly. Early death in patient 1 occurred whereas the compound heterozygotes responded to L-carnitine and riboflavin treatment. Progressive muscle weakness and severe migraine-like episodes occurred in patient 2 and 3. Patient 3 died at the age of 23 years after a stroke. Conclusion: A clear genotype-phenotype correlation was confirmed for the novel mutation present in homozygous and/or compound heterozygous state. These findings may potentially predict the prognosis of MADD due to ETFDH deficiency in the affected South African population.
A MITOCHONDRIAL-TARGETED ELECTRON SCAVENGER AND A CARDIOLIPIN BINDING PEPTIDE DECREASE SUPEROXIDE GENERATION AND IMPROVE MITOCHONDRIAL RESPIRATION IN ACAD9-DEFICIENT FIBROBLASTS

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Background: Acyl-CoA dehydrogenase 9 (ACAD9) is a flavoprotein that catalyzes the first step in long-chain fatty acid β-oxidation and acts as an assembly factor for mitochondrial respiratory chain complex I. Individuals with ACAD9 deficiency present with progressive encephalomyopathy, recurrent Reye syndrome, and cardiomyopathy that can be fatal. Although some patients are responsive to riboflavin therapy, there are limited treatment options for this disorder.

Objective: Evaluating the effect of potential protective compounds on superoxide generation and mitochondrial respiration in fibroblasts of an ACAD9-deficient patient.

Methods: Patient fibroblasts were cultured in medium without glucose for 48-72 hr to assess the ability of ACAD9-deficient cells to accommodate the shift of energy source from glucose, and the effect of JP4-039, a mitochondrial targeting free radical scavenger, as well as a novel cardiolipin targeting peptide on superoxide production and oxygen consumption.

Results: Superoxide generation was increased, whereas basal respiration and reserve capacity were decreased in ACAD9-deficient cells, compared to normal cells. While either JP4-039 or the cardiolipin targeting peptide decreased superoxide levels, the antioxidants N-acetylcysteine, trolox, resveratrol, and mitoQ, and the pan-PPAR agonist bezafibrate did not reduce superoxide levels. JP4-039 and the peptide increased basal respiration and reserve capacity in deficient cells as well.

Conclusion: These findings suggest that some of the presumed damaging biochemical abnormalities caused by ACAD9 deficiency can be alleviated by JP4-039 and the novel cardiolipin targeting peptide, in addition to improving bioenergetics in ACAD9-deficient fibroblasts. This provides the impetus for further evaluation of these molecules as potential therapeutics for this disorder.

HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH LONG-CHAIN FATTY ACID OXIDATION DISORDERS

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Background: Patients with long-chain fatty acid oxidation disorder (FAO) are at risk to develop clinical symptoms as hypoketotic hypoglycemia, rhabdomyolysis and cardiomyopathy. There is still limited knowledge about the impact of these symptoms on the quality of life (QoL) in FAO patients and families compared to healthy peers. Parents of a chronically ill child are at risk of a lower health-related QoL, experience more posttraumatic stress symptoms, and report higher levels of distress than parents of healthy children. In addition, studies show that parental psychosocial problems influence the well-being of the child.

Over the past decade, patient reported outcome measures (PRO) targeted at the QoL, have become crucial for the assessment of new treatment options. Systematically monitoring QoL in FAO patients will therefore be of great value in the evaluation of upcoming treatment options and furthermore will provide more insight in the burden of the disorders.

Objective: to investigate whether it is feasible to systematically monitor Health Related QoL and psychosocial functioning of patients with FAO in daily practice. How QoL is compared to healthy peers.

Method: Implementation of a web-based program KLK (Quality of Life in Clinical Practice) in the national Dutch FAO centre. All patients were invited to join the KLK program before they visited the outpatient clinic for their regular check-up. After registration digital questionnaires (Basic, LTO, PedsQL, PedsQL fatigue) are available for both patients and parents, which have to be filled in before each visit to the clinic. Data are collected on physical, emotional and social wellbeing. Individual outcome on each domain is compared with a healthy norm score.

(Continued on next page)
MOLECULAR DIAGNOSIS FOR TARGET METABOLIC DISEASES OF NEWBORN SCREENING USING A GENE PANEL IN JAPAN

Hideo Sasai M.D., Ph.D., Hiroki Otsuka M.D., Ryoji Fujiki Ph.D., Osamu Ohara Ph.D., Yoko Nakajima M.D., Ph.D., Tetsuya Ito M.D., Ph.D., Masahisa Kobayashi M.D., Ph.D., Go Tajima M.D., Ph.D., Osamu Sakamoto M.D., Ph.D., Shiro Matsumoto M.D., Ph.D., Kimitoshi Nakamura M.D., Ph.D., Takashi Hamazaki M.D., Ph.D., Hisanori Kobayashi M.D., Ph.D., Yuki Hasegawa M.D., Ph.D., Toshiyuki Fukao M.D., Ph.D.

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Newborn screening (NBS) using tandem mass spectrometry has been performed since 2014 in all over Japan and the target metabolic diseases (TMDs) increased from 6 to at least 19 diseases. Molecular diagnosis for TMDs is not commercially available in Japan and until recently such molecular analyses were mainly performed by pediatricians with “volunteer spirits”. To change the situation, we designed and conducted molecular diagnosis for TMDs using a gene panel.

We designed a gene panel which consists of more than 60 genes covering the TMDs and the related diseases. This research was financially supported by Japan Agency for Medical Research and Development. DNA was purified from patients’ blood at Gifu University and the gene panel analysis was performed at Kazusa DNA Research Institute using the MiSeq or NextSeq (Illumina®). Sanger sequencing was performed to confirm the detected mutations.

Pediatric coauthors in this study are experts responsible to make mutation reports. We analyzed 138 patients who were positively screened during three years (January 2014 to March 2017) and 44 patients who were diagnosed before that period. The number of patients with TMDs detected by NBS were as follows: Propionic acidemia (35), Hyperphenylalaninemia (19), Methylmalonic acidemia (17), VLCAD deficiency (15), Maple syrup urine disease (13), Methylcrotonylglycinuria (13), Galactosemia (10), primary systemic carnitine deficiency (9), MCAD deficiency (8), Citrullinemia type 1 (7), Glutaric acidemia type 1 (6), CPT2 deficiency (4), Glutaric acidemia type 2 (4), CPS1 deficiency (4), OTC deficiency (4), Multiple carboxylase deficiency (3), Others (11). In most cases, we could find the gene mutations in their corresponding genes and found some common mutations for some TMDs in a Japanese population. Clinical course and severity may differ among patients in some TMDs. One major factor to determine clinical phenotype is of course genotype. Hence, it is important to follow up mutation-defined patients to evaluate efficacy of treatment and management. We will individualize clinical guidelines by genotypes in some TMDs in the near future.

Results: Currently 15 patients have participated. Preliminary results show that only a very limited amount of patients and parents continue to fill in the questionnaires. In collaboration with the designers of the program we have adapted the method and now allow patients to fill in the questionnaires when they are in the clinic for their evaluation. The most evident finding thus far in LTO is, as expected, the increased anxiety which parents experience. Additional effort is needed to validate the test for patients above the age of 30.

Conclusion: Systematically monitoring Health Related QoL in patients with FAO is feasible but not yet routine and additional support is needed to collect the baseline information before the data can be used as PRO.
ENDOPLASMIC RETICULUM-MITOCHONDRIA CROSSTALK AND REDOX HOMEOSTASIS DISRUPTION IN VERY LONG-CHAIN ACYL-COA DEHYDROGENASE DEFICIENT FIBROBLASTS

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Very long-chain acyl-CoA dehydrogenase (VLCAD) enzyme deficiency is the most common mitochondrial β-oxidation (FAO) defect of long-chain fatty acids. The clinical phenotype is heterogeneous, including hepatomegaly, ardiomyopathy and hypoketotic hypoglycemia, frequently induced by prolonged fasting and infectious illnesses. Skeletal myopathy associated with rhabdomyolysis may also be occasioned. Nevertheless, the cellular mechanisms related to the pathophysiology remain unclear.

VLCAD deficient fibroblasts were cultured in medium without glucose for 48 h to shift cellular metabolism to fatty acids as energy source. We evaluated reactive oxygen species production, the immunocontent of proteins involved in endoplasmic reticulum-mitochondria crosstalk and function (DDIT3, IP3, Grp75, Grp78 and VDAC1) and the transcription factors Nrf2 and NF-kB.

Superoxide production and DCFH oxidation were higher in VLCAD deficient fibroblasts. JP4-039 and XJB-5-131, mitochondrial targeted free radical scavengers, were able to decrease the levels of reactive oxygen species.

We also observed increased Nrf2 and NF-kB antigens in VLCAD deficient cells, which were slightly decreased by JP4-039, as compared to control cells. Finally, we observed increased DDIT3 antigen, and decreased IP3 receptor, Grp75 and VDAC1 antigens, but no changes in Grp78 and Mfn2 antigen signal.

Our results showed increased reactive oxygen species levels that were improved by the treatment with the antioxidants JP4-039 and XJB-5-131. The expression of transcription factors related to oxidative stress and inflammatory signaling pathways in VLCAD deficient fibroblasts was altered, and partially restored by JP4-039. Finally, alterations in the proteins involved in the endoplasmic reticulum-mitochondria crosstalk were observed, indicating a disturbance in this process and its function.

INTRANOVENOUS SOURCES OF MEDIUM CHAIN TRIGLYCERIDES FOR CRITICALLY ILL PATIENTS WITH FATTY ACID OXIDATION DISORDERS

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Objective: Treatment of critically ill patients with long chain fatty acid oxidation disorders (FAOD) with intravenous (IV) sources of medium chain triglycerides (MCT).

Methods: Three critically ill patients with long chain FAODs were admitted in metabolic crisis unable to receive enteral nutrition. Patient A was a 19 month old female with a presumed long chain FAOD admitted in metabolic crisis with cardiomyopathy and ventricular tachycardia. The patient had an affected older female sibling that was deceased. The patient was later found to have TANGO2 gene variants. Patient B was a 10 month old female with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency admitted in metabolic crisis and heart failure secondary to dilated cardiomyopathy. Patients A and B had worsening cardiac function and were considered for cardiac transplantation. Patient C was an 18 year old male with trifunctional protein (TFP) deficiency, status post heart transplantation, admitted in metabolic crisis with severe rhabdomyolysis associated with intercurrent illness. Based on the critical nature of their condition and inability to tolerate enteral nutrition, all three patients received Lipofundin® MCT/LCT 20%, an IV source of MCT available in Europe. Lipofundin was obtained with approval of an emergency Investigational New Drug application (eIND) from the Food and Drug Administration (FDA) and Institutional Review Board (IRB). Lipofundin® provided 50% MCT and 50% long chain triglycerides (LCT). During a subsequent admission, Patient C developed a bowel obstruction preventing enteral feeds. Smoflipid®, an IV source of MCT approved by the FDA in 2016, was administered to Patient C. Smoflipid® provided 30% MCT and 70% LCT. Lipofundin® and Smoflipid® were administered with the goal of <10% of calories from LCT until enteral feeds were tolerated.

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INFANT MORTALITY ASSOCIATED WITH INBORN ERRORS OF METABOLISM: A STUDY BASED ON SUDDEN DEATH

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Abstract: Sudden unexpected death in infancy (SUDI) is one of the most frequent causes of death during the first year of life after the neonatal period. Literature points out that 0.9% to 6% of children who die unexpectedly may suffer from some metabolic disorder. A recent systematic review showed that at least 43 inborn errors of metabolism (IEM) are associated with sudden death, with the most commonly associated IEM being medium chain acyl-CoA dehydrogenase deficiency (MCADD). Fatty acid oxidation defects (FAOD) are not included in the Brazilian National Neonatal Program. Despite the decline in Brazil, infant mortality remains a major concern in Public Health. The infant mortality rate in Brazil (14.1:1,000 live births) is considered high and incompatible with a developed country.

Objectives: To estimate and characterize infant mortality associated with IEM in Brazil through data analysis from the Mortality Information System (SIM), a vital information system managed by the Ministry of Health.

Methodology: Cross-sectional population-based study with information from SIM. From 2002 and 2014, all death records of children under one year of age, in which the cause of death, coded by ICD10 (International Classification of Disease and Related Health Problems), was: ICD10-E70 (disorders of amino acid metabolism), ICD10-E71 (disorders of branched-chain amino acid metabolism and fatty acid metabolism), ICD10-E72 (other disorders of amino acid metabolism), and ICD10-E74 (other disorders of carbohydrates metabolism) were selected.

Results: From 2002 to 2014, 199 children under one year of age died from IEM (median of 17 deaths per year). Of those, 18 (9.0%) occurred in the North region of Brazil, 43 (21.6%) in the Northeast, 80 (40.2%) in the Southeast, 46 (23.1%) in the South, and 12 (6.0%) in the Central-West region. In all regions, death by ICD10-E74 was the most frequent (80 cases; 32.2% of registries). On the other hand, ICD10-E71 was the less frequent. The national infant death rate for selected IEM in the period was 0.067 per 1,000 live births, with the South region rate being 0.12; the Southeast 0.068; the Central-West 0.049; the Northeast 0.052; and the North 0.056 per 1,000 live births.

Conclusions: This is the first study to evaluate the relationship between sudden death and IEM in Brazil. The low death rate found may not suggest the rarity of IEM, but the underreporting of cases. Although 1 to 3% of sudden deaths in neonates are associated with FAOD, ICD10-E71 was the less frequent. This may demonstrate the underdiagnosis of this group of diseases. Studies on infant mortality rate are fundamental for the development of health surveillance actions and for the decision-making process in order to subsidize the formulation procedure of public policies and to evaluate their results and impacts.

Results: Lipofundin® and Smoflipid® were well tolerated with no observed adverse reactions. Patients improved or remained metabolically stable while receiving these products. The initial shipments of Lipofundin® were held by United States Customs, delaying use for several weeks in critically ill patients. Smoflipid® was readily available and did not require eIND or IRB applications. The limitation of Smoflipid® was the 30% MCT content compared to 50% MCT content of Lipofundin®.

Conclusion: Use of Lipofundin® and Smoflipid® in long chain FAOD patients was well tolerated with no adverse effects.
THE BIOCHEMICAL BASIS FOR OVERLAP OF CLINICAL FEATURES OF LCHAD/TFP DEFICIENCY WITH MITOCHONDRIAL CHAIN DEFECTS: IMPLICATIONS FOR NEW THERAPEUTIC APPROACHES

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**Introduction:** Patients with long chain 3- hydroxy-acyl CoA dehydrogenase (LCHAD) and Trifunctional Protein (TFP; a 4x4β heterotrimer that includes LCHAD function) deficiencies, respiratory chain complex 1 defects (CI), and Barth syndrome (BS) share overlapping features including cardiomyopathy, fatigue, exercise intolerance, hypoglycemia and lactic acidosis during metabolic decompensation. This underscores the need to understand the functional relationship among mitochondrial bioenergetic pathways. While a multifunctional fatty acid oxidation (FAO) complex in which TFP physically interacts with CI in supercomplexes has been described, a link between FAO and cardiolipin remodeling through TFPα and monolysocardiolipin acetyl transferase (MLCLAT) has also been reported. Cardiolipin plays an important role in sustaining the integrity of the electron transport chain (ETC) by maintaining supercomplex stability in the inner mitochondrial membrane.

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CARNITINE PALMITOYL TRANSFERASE 1A DEFICIENCY: HOW TO SCREEN AND TREAT?

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Background: Carnitine palmitoyl transferase 1A (CPT1A) deficiency does exist in Finland. Patients with CPT1A deficiency have died of decompensation episodes triggered by infections. Hence it is a significant clinical problem. In order to decide whether it should be included in the national newborn screening program (NBS), we wanted to ensure that there is a good screening test available and that we can provide clinically effective treatment. Therefore, we studied global screening data and data of our patients. Prior to the NBS, CPT1A deficiency diagnosis was usually made after a decompensation episode of the patient or one of his relatives.

Methods: Finnish and global screening data from Region4 project were used to determine best parameters and cut-offs. This was achieved by comparing values of known cases to those suffering from other metabolic diseases and to those of healthy controls. In addition, based on experience elsewhere, we decided to treat one child diagnosed at the age of four immediately after diagnosis with a diet with normal fat content, but with frequent meals and uncooked corn starch in the evening. Earlier we have used a low-fat diet, but positive results encouraged us to transfer four patients treated first with a low-fat diet to a free diet. The dietary follow-up included assessment of food diaries or dietary review and recommendations about the diet, fasting tolerance and dietary supplements. Patients have been followed up in a metabolic clinic by a physician and a dietitian at least annually, and clinical assessment has been complemented by laboratory tests.

Results: Dried blood spot samples are used in the Finnish NBS, and acylcarnitine analysis is performed for detection of several fatty acid oxidation and carnitine metabolism disorders. We found that both C0, C0/(C16+C18) and C0/C16 were good at separating CPT1A deficiency patients from other patients and controls. C0/C16 was very sensitive and had a good specificity with cut-off at around 50. In rare cases when C16 is extremely low (<0.5) using C0 with a cut-off at around 100 is better.

All patients have grown and developed normally. There have been no complications related to CPT1A deficiency. Laboratory values show normal blood count, liver and renal function, muscle enzymes, bone markers etc. Acylcarnitine profile remains abnormal with elevated C0 and low long-chain acylcarnitines.

We have occasionally observed low vitamin levels if patients have omitted their recommended supplements. The one child who has never been on a low-fat diet has followed the recommendations and taken his supplements, and thus has not had any vitamin deficiencies. The other patients have liberalized their diets variably. Some still follow a relatively low-fat diet whereas others have adopted a relatively high-fat diet.

Conclusions: CPT1A is a significant clinical problem in Finland. There is a good laboratory test available for the newborn screening. The treatment is effective, safe and easy to administer. Therefore CPT1A deficiency was included in the expended newborn screening program in Finland. Based on our experience, it seems safe to treat patients at least from four years onwards with a diet with no reduction in the fat content. Fasting tolerance is nevertheless reduced and patients require an emergency regime for inter-current illness.

Dietary follow-up is recommended to ensure a balanced diet.

Abstracts

We hypothesize that mutations in TFP disrupt its interaction with CI, and is associated with alterations in MLCLAT activity and cardiolipin, leading to supercomplex instability, and increased reactive oxygen species (ROS). We also hypothesize that defective cardiolipin in BS alters the TFP-CI interaction due to supercomplex instability.

Methods: Cells and tissues from patients with confirmed LCHAD, and TFP deficiencies, as well as BS were assessed for cardiolipin, ETC, and TFP. Mitochondrial extracts were subjected to blue native PAGE followed by SDS-PAGE and western blotting. Flow cytometry was used to measure ROS (Mito Sox Red) and mitochondrial proliferation (Mito Tracker Green). Oxygen consumption studies were performed using a Seahorse XF®96 Analyzer. Cardiolipin was studied using liquid chromatography mass spectrometry. TFPa and MLCLAT were quantitated using SDS-PAGE followed by western blotting, and measurement of MLCLAT activity.

Results: Fibroblasts from patients with LCHAD deficiency and BS both had a reduction in MLCLAT activity compared to control cells. There was evidence of altered cardiolipin content, destabilization of TFPa interaction with supercomplexes, increased ROS production, and mitochondrial proliferation compared to control.

Discussion: These findings suggest that studying alterations in the FAO-ETC-Cardiolipin interaction increase our understanding of the pathophysiology of these disorders, providing impetus for development of new therapeutic approaches.


**RESULTS FROM A 78-WEEK SINGLE-ARM, OPEN-LABEL PHASE 2 STUDY TO EVALUATE UX007 IN PEDIATRIC AND ADULT PATIENTS WITH MODERATE TO SEVERE LONG-CHAIN FATTY ACID OXIDATION DISORDERS (LC-FAOD)**

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**Conclusion**

UX007 treatment significantly reduced the number and duration of major clinical events. The overall mean annualized event rates decreased from 1.69 to 0.88 events/year (p=0.0208) and the mean annualized duration rate decreased from 5.96 to 2.96 days/year (p=0.0284) following UX007 initiation. Hospitalizations due to rhabdomyolysis, the predominant major clinical event, also decreased from 1.03 to 0.63 events/year (p=0.1044) following UX007 initiation. Initiation of UX007 eliminated hypoglycemia events leading to hospitalization (0.30 vs 0 hospitalization events/year; p=0.0667) and ICU care (0.05 vs 0 ICU events/year; p=0.1609). Adult subjects reported significant improvements in the SF-12 Physical Component Summary Scale (n=5, p=0.0354), while pediatric subjects reported significant improvements in the SF-10 Physical Summary Scores (n=3, p=0.0001). Finally, UX007 treatment reduced cardiomyopathy events by 69.6% (0.07 vs 0.02 events/year; p=0.3090). The most common related treatment-emergent adverse events (TEAEs) were diarrhea, abdominal or gastrointestinal pain, vomiting, and acne, with most mild to moderate in severity.

**Conclusion**

Major clinical events were significantly reduced following UX007 treatment in contrast to pre-treatment, during which most subjects were treated with MCT.
EXPERIMENTAL EVIDENCE THAT FATTY ACIDS ACCUMULATING IN VLCAD DEFICIENCY DISRUPT MITOCHONDRIAL RESPIRATION IN HEART, LIVER AND BRAIN OF YOUNG RATS
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Deficiency of very long-chain acyl-CoA dehydrogenase (VLCAD) is the most frequent disease of β-oxidation of very long-chain fatty acids (14 to 20 carbons). The disorder is biochemically characterized by predominant tissue accumulation of myristic (Myr - C14:0) and cis-5-tetradecenoic acids (Cis-5 - C14:1). Affected patients usually manifest cardiomyopathy, hepatopathy and rhabdomyolysis, especially during catabolic situations. Considering that the pathophysiology of this disorder is poorly established, we investigated the effects of Myr and Cis-5 on important parameters of mitochondrial respiration measured by oxygen consumption in organelles from heart, liver and brain of young rats. Myr markedly increased state 4 respiration in mitochondria from all tissues at a similar magnitude, whereas Cis-5 only altered this parameter in the brain, indicating an uncoupling behavior for these fatty acids. Atractyloside was not able to prevent these alterations, making unlikely the involvement of the adenine nucleotide translocator. In addition, Myr markedly decreased state 3 and uncoupled respiration in mitochondria from all tissues, indicating a metabolic inhibition, with no effect of Cis-5. We also observed that Myr-induced inhibition of oxidative phosphorylation involved complex I activity, since it was more evident with NADH-linked substrates (pyruvate, malate and glutamate) in rat heart mitochondria. Taken together, our results indicate that Myr is more toxic to mitochondrial respiratory activity as compared to Cis-5, behaving as uncoupler and metabolic inhibitor of oxidative phosphorylation. It may be therefore presumed that lipotoxicity contributes to mitochondrial bioenergetics dysfunction and this may represent an important pathomechanism in VLCAD deficient patients.

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Keywords: very long-chain acyl-CoA dehydrogenase deficiency; myristic acid; cis-5- tetradecenoic acid; mitochondrial respiration.

DEVELOPMENT OF A CLINICAL SEVERITY SCORE FOR MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY
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Classically, multiple acyl-CoA dehydrogenase deficiency (MADD; glutaric aciduria type II) patients are phenotypically divided into three types. However, individual phenotypes and treatment response can vary extremely. To improve assessment of disease severity and to facilitate patient monitoring, we propose to develop an MADD-clinical severity score (CSS) at INFORM 2017.

The MADD-CSS will be designed according to a three-step method: 1) a systematic literature review and meta-analysis to identify disease symptoms and domains; 2) prioritization of disease symptoms and domains by clinical experts; 3) assembly of the MADD-CSS according to the previous steps.

Preliminary data of step 1 identified the following disease domains in 427 published patients (57 neonatal-onset, 369 later-onset and 1 unreported onset-age): CARDIAC (34/427;8.0%), CENTRAL NERVOUS SYSTEM (14/427;3.3%), PERIPHERAL NERVOUS SYSTEM (32/427;7.5%), RESPIRATORY SYSTEM (66/427;15.5%), LIVER (139/427;32.6%) and MUSCLE (372/427;87.1%).

We propose to introduce step 2 at INFORM 2017 and subsequently develop the score. The MADD-CSS can be used for a detailed patient classification and standardized assessment of disease activity and treatment efficacy, to support decision-making.
Abstracts

DEVELOPMENT AND CHARACTERIZATION OF PATIENT-SPECIFIC IPSC-DERIVED RETINAL PIGMENTARY EPITHElia (RPE)-LIKE CELLS AS A MODEL OF LCHAD-ASSOCIATED RETINOPATHY

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Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) is one of three enzymatic domains found within the trifunctional protein (TFP) complex that mediates long chain fatty acid oxidation (FAO) in mitochondria. Unlike other FAO disorders, patients with LCHAD deficiency develop vision loss from progressive chorioretinopathy. Currently, there is no animal model available to study LCHAD associated chorioretinopathy. While the mechanism of retinal pathogenesis in LCHAD deficient patients remains poorly understood, there is evidence to suggest that the initial physiologic perturbations begin in retinal pigment epithelial (RPE) cells and progress to other retinal cell layers. We sought to develop an in-vitro RPE cell model as a tool to uncover pathogenic mechanisms caused by loss of LCHAD. Additionally, this model can be used to explore novel therapies for the treatment of LCHAD associated retinopathy. In this study we reprogrammed patient fibroblasts harboring the HADHA (G1528C) mutation into induced pluripotent stem cells (iPSC). These cells, along with wild type control iPSCs were subsequently differentiated into retinal pigment epithelium (RPE) through a directed method. Using immunofluorescence and RNA expression analysis, we have confirmed that iPSC derived RPE are histologically similar to primary human RPE despite remaining homozygous for the G1528C mutation. We have also confirmed that several fatty acid oxidation proteins (HADHA, HADHB, VLCAD, CPT1) are expressed in both control and HADHA mutant RPE. Cellular energetics were evaluated in both wildtype and mutant RPE cells using the Seahorse Biosciences XFe analyzer. Our results indicate that wild type RPE exposed to 200uM BSA-palmitate + carnitine in glucose limited media show a steady increase in the oxygen consumption rate (OCR), a 1.5-fold increase over 45 minutes, when compared to cells treated with BSA alone.

In contrast, LCHAD deficient cells showed no change in OCR when exposed to palmitate, suggesting that their ability to utilize long-chain fatty acids as an energy source is impaired. LCHAD deficient RPE that were fed BSA-palmitate (200uM) + carnitine showed a dramatic increase in C16-OH acylcarnitine in the media after 48 hours (.31uM) compared to LCHAD deficient RPE cells that were exposed to BSA alone (.02uM). Media collected from wildtype RPE under the same conditions showed no change either in the presence of palmitate (.002uM) or in the BSA control (.002uM). These results demonstrate that LCHAD deficient RPE derived from patient iPSCs may be an effective model to study the pathophysiology of LCHAD-associated retinopathy and for the evaluation of potential novel therapies to treat this severely disabling disease.

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ASSESSMENT OF THE OUTCOME AND BIOCHEMICAL CHARACTERISTICS OF INFANTS WITH ABNORMAL NBS FOR SYSTEMIC PRIMARY CARNITINE DEFICIENCY AND ITS THE CARRIER FREQUENCY IN THE US

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Systemic primary carnitine deficiency (CDSP) is an autosomal recessive condition caused by mutations in the SLC22A5 gene that encodes the high-affinity carnitine transporter in various tissues. CDSP can be treated with L-carnitine but it results in hypoketotic hypoglycemia, skeletal myopathy, cardiomyopathy or heart failure if untreated, while many patients with CDSP remain asymptomatic even without treatment unless they are under metabolic stress. Recent introduction of the expanded newborn screening (NBS) has been successful to identify CDSP patients who are at risk for life-threatening heart failure. A previous study indicated that NBS detects not only infants with CDSP but also carriers of this condition or affected mothers. To assess the outcome biochemical characteristics of infants with abnormal NBS suspecting CDSP, we performed a single-center retrospective analysis of the patients who were referred to the Program for Inherited Metabolic Diseases of Mount Sinai Hospital from the New York State NBS Program between Jan 2012–May 2017. We also evaluated carrier frequency of CDSP in the US using an NGS carrier screening test performed at the Mount Sinai Genetic Testing Laboratory.

A total of 32 patients were evaluated for low C0 levels detected by the NBS program. Patients who persistently required L-carnitine supplement to maintain normal free carnitine levels and/or who had two pathogenic or likely pathogenic variants in SLC22A5 were diagnosed with CDSP. Among those 32 patients, nine patients had CDSP (28%). The etiologies of the remaining patients included eleven cases of premature birth earlier than 30 weeks gestational age (PB) (35%), ten cases of maternal carnitine deficiency (MD) (31%), and two others. There was no significant difference in initial NBS C0 levels between the groups.

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Plasma free carnitine levels at the initial evaluation were significantly lower in the CDSP group compared to the other groups (CDSP: 7.6±2.89, MD: 14.8±6.44, PB: 50.0±17.8µM (mean±SD), CDSP vs MD p<0.01, CDSP vs PB p<0.0001). Urine free carnitine level in CDSP at the initial evaluation was significantly higher than that of MD (p<0.0001) but lower than that of PB (CDSP: 765.1±302.99, MD: 23.2±24.42, PB: 2853.4±2503.1µM (mean±SD)).

 Sequencing of SLC22A5 was performed for the patients in the CDSP (5/9 cases) and MD (8/10 cases) groups. In all five cases of CDSP, two variants were identified, including two novel variants, c.761G>A, p.R254Q and c.1088T>A, p.L363H. Of interest, 50% of cases from the MD group that were sequenced (4/8 cases) were found to be carriers of CDSP, and one of the mothers of these carriers was confirmed to have CDSP.

Among the 32,908 individuals screened, we detected 42 different pathogenic or likely pathogenic variants in SLC22A5 in 243 heterozygous and one compound heterozygous individuals. Based on these findings, we estimated a carrier frequency of 1/135 for CDSP in an unselected US population. The four most frequent variants identified were c.641C>T, p.A214V (18.4%), c.136C>T, p.P46S (17.6%), c.1345T>G, p.Y449D (13.5%), and c.1400C>G, p.S467C (8.2%).

Our findings suggested that high urine free carnitine excretion in a setting of very low plasma free carnitine at the initial evaluation is a strong predictor for CDSP among NBS positive infants. It was also confirmed that NBS is a tool to identify carriers of CDSP or mothers who have asymptomatic CDSP as previously described. According to the estimated carrier frequency, CDSP may be underdiagnosed, particularly in populations who were born before introduction of expanded NBS.

THE COMPARISON OF THE DIGESTION AND ABSORPTION OF TRIOCTANOIN (C8) AND TRIHEPTANOIN (C7) IN PATIENTS WITH LONG-CHAIN FATTY ACID OXIDATION DISORDERS.

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Objective: To determine if there is a difference in the appearance rates of triheptanoin or trioctanoin in peripheral circulation after a mixed meal or a single nutrient bolus.

Methods: This is a secondary analysis of Phase 2 Study of Triheptanoin to treat long-chain fatty acid oxidation disorders. Subjects with CPT2, VLCAD, or LCHAD/TFP were randomly assigned to consume trioctanoin or triheptanoin in a mixed breakfast meal. Blood was drawn fasting and at 1, 2, and 4 hours after the meal. Separately, samples were drawn 20 minutes after an oral bolus of oil alone at 0.3mg/kg lean body mass and again after 45 minutes of exercise. Blood samples were analyzed for free fatty acids, quantitative total fatty acid profiles, triglycerides and Apolipoprotein B48 (apoB48, the lipoprotein found in chylomicrons).

Results: Blood samples were available for a total of 30 subjects, n=15 in each group. Serum total free fatty acid concentrations were higher at fasting due to fasting-induced mobilization of endogenous fatty acid stores and then decreased after feeding and increased with exercise as expected. After the trioctanoin or triheptanoin mixed meal, we observed a gradual rise in total C8 in subjects receiving C8 and C7 (in subjects receiving C7) in the quantitative fatty acid profile (free and bound fatty acids) and both peaked at four hours. However, the total amount of C7 (104±25µmol/L) measured in plasma at 4 hours after a mixed meal was less than that of C8 (177±88µmol/L), after comparable intakes. The rises in plasma C8 and C7 corresponded with increased plasma total triglycerides and apoB48 suggesting that at least some of the C8 or C7 oil consumed as part of a mixed meal had been incorporated into chylomicrons. Total plasma C7 (166±37µmol/L) or C8 (343±191µmol/L) concentrations were higher after the single nutrient oral bolus than after the mixed meal; total plasma triglycerides did not increase after the single nutrient bolus suggesting that C7 and C8 had been absorbed via the portal circulation rather than as chylomicrons.

Conclusions: We had expected C8 and C7 fatty acids to be rapidly absorbed through portal circulation and to peak in peripheral blood after 1 hour. However, levels of both C8 and C7 peaked only at 4 hours, suggesting that they may have been incorporated into chylomicrons after a mixed meal that also contained long-chain fat. The greater rise in C8 plasma levels was also unexpected which suggests there is a difference in the digestion, absorption or clearance of C8 and C7. Both fatty acids rose after the single nutrient oral bolus and peaked at the final blood sample following exercise suggesting a more rapid and dramatic rise in circulating C8 or C7 after a single nutrient bolus in comparison to a mixed meal. The appearance of medium chain fatty acids in peripheral circulation differs when the oil is fed as a mixed meal versus a single nutrient bolus.

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Abstracts

FATTY ACID OXIDATION DISORDERS: CASE SERIES OF A TERTIARY TEACHING HOSPITAL OF SOUTHERN BRAZIL

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Mitochondrial fatty acid β-oxidation disorders (FAOD) are a heterogeneous group of defects in fatty acid transport chain and mitochondrial β-oxidation, affecting energy homeostasis mainly in the liver, heart and skeletal muscles and often present with multi-system involvement, including several life-threatening manifestations. FAOD are inherited as autosomal recessive disorders and have a widely varied presentation, with either neonatal or late onset. In south Brazil, there are no studies on the clinical characteristics of FAOD patients until today.

Objective: To describe the main clinical and demographic features from a series of patients with FAOD managed at an outpatient referral clinic of these inborn errors of metabolism in a tertiary teaching Hospital of Southern Brazil.

Method: This is a retrospective study of FAOD patients followed at an outpatient clinic for treatment of inborn errors of metabolism in Medical Genetics service of Hospital de Clínicas, Porto Alegre, Brazil. Based on a convenience sample, we analyzed demographic and clinical data of eight patients with clinical, biochemical and/or molecular diagnosis of FAOD who are regularly seen at our outpatient unit.

Results: The diagnosis were Very long chain acyl-coA dehydrogenase deficiency (n=1); Long chain acyl-coA dehydrogenase deficiency (n=2); Multiple acyl-coA dehydrogenase deficiency (n=2); Carnitine palmitoyltransferase II deficiency (n=2) and Carnitine palmitoyltransferase II deficiency (n=1). The group comprised five women and four men, with ages ranging from three months to 39 years (median: 10 years; average: 11.07 years) and disease onset between 18 hours of life and 14 years old (median: 1 month; average: 22.12 months). Median age at diagnosis was 8 months (range: 20 days to 36 years of age). The diagnosis was confirmed by molecular tests in five patients. All patients had nonspecific presenting symptoms: seven out of the eight patients had hypoglycemia reported as the initial symptoms began. Seven had neurological symptoms which included seizure (n=3), developmental delay (n=1), hypotonia (n=6) and feeding difficulties (n=1).

Three patients had hepatomegaly and one had dehydration. The patient diagnosed at age 36 presented initially with lower limb weakness and pain, rhabdomyolysis, hypoglycemia and deep vein thrombosis. The patient with the earlier age of onset (18 hours) had hypotonia, feeding difficulties and hypothermia. Of these 8 patients, one had cardiomyopathy and arrhythmia. Unspecific dysmorphism was present in 4 patients. Two patients have a global developmental delay and one has a psychodiagnosis of moderate intellectual disability. Three patients had a previous sibling with unexplained death and one had consanguineous parents.

Conclusion: Throughout this descriptive study, we can emphasize the multisystemic, severe and life-threatening character of FAOD. Therefore, this group of pathologies requires integrated and multidisciplinary management. Although it is the most prevalent disorder in other series, no case of Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency was observed in this sample. We do not have newborn screening for such diseases at the national level, thus some patients arrive late to the reference centers for diagnosis and management. Greater awareness of this disorder among clinicians and pediatricians should aid their search for an etiological diagnosis in cases of severe hypoglycemia, hypotonia, hepatic and cardiac manifestations that might otherwise be improperly managed. Adequately treated patients can lead intellectually and socially satisfying lives with no severe limitations. Larger studies should be done to better understand those symptoms in Brazilian patients, as well as unified ones in a national basis.

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Abstracts

DIFFICULT MANAGEMENT OF AN 8 YEAR OLD PATIENT
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Introduction
Very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD) is an autosomal recessive metabolic disorder that affects mitochondrial fatty acid β-oxidation, that can impacts in heart, liver and muscle function. We aimed to explore the clinical, biochemical and nutritional findings of a challenging case.

Case report
A male was born at term with birth weight of 3100 grams by vaginal delivery to a 35 year-old mother. Pregnancy, prenatal labs and ultrasound were unremarkable. Delivery was uncomplicated. Infant was discharged home on third day of life. The child’s family history showed two death siblings before the diagnosis was made. Cause or death was reported as mitochondrial cardiomyopathy. The diagnosis was made by acylcarnitines analysis performed in dried blood spots that were saved.

At two weeks of life, the baby became lethargic with poor feeding and dehydration. The infant was admitted to the intensive care unit for rehydration and sepsis evaluation. Biochemical findings showed hypoglycemia, hyperlactacidemia and acidosis. Cardiac evaluations showed mild hypertrophy cardiomyopathy. Because of family history and following symptoms we analyzed baby’s acylcarnitines profile and organic acids. Initial plasma acylcarnitine concentrations were indicative of a VLCADD diagnosis and decreased with treatment. It showed C14:1 and C14 elevations: C14 15, 1 µmol/L (1,6-16), C14:1 0.51 µmol/L (<0,24) high ratio C14:1/C2 0,19 (<0,05) and dicarboxylic aciduria. Molecular DNA testing for ACADVL is still pending.

Persist. Sometimes there is no trigger during events. Other therapeutic strategies should be taken into account to improve their quality of life.

Nutritional treatment was sustained on MCT based formula with additional MCT oil 1grams/kilo/day. Total calories intakes were around 2000-2500 kcal/day and the distribution of energy was: 18% as protein, 58% as carbon hydrates and 24% as fat. The fat’ distribution was 18% medium chain and 6% long chain. Supplementation with L-caritnine is subject to plasma levels.

The numbers of admissions were increased with years: varied from 1 to 4 episodes, with an average of 3 per year. Before 2 years, biochemical findings were hypoglycemia and acidosis. After two years old, the causes of admissions were rhabdomyolysis sometimes with renal impairment. He presented with severe rhabdomyolysis during exercise or increased of catabolism. Most of them without a known trigger. Once he required renal replacement with hemodialysis. He required gastrostomy placement and porth cath due to frequents admissions.

Nowadays, he’s 8 years old and his cardiac function is normal. He tolerates 3 hours of fasting during the day and continues gastric feeding at night. He’s restricted in his physical activity. Its anthropometric measurements reveal obesity (+5,6DS) and normal height (+2 DS). He has normal cognitive outcome.

Discussion
The importance of early diagnosis of undiagnosed cases of fatty acid oxidation defects might avoid symptomatic hypoglycemia or sudden unexplained death. The family of our reported case had lost two previous children due to VLCAD deficiency; in this report, the patient’s condition was fortunate to have been recognized early. In Argentina neonatal screening for fatty acid oxidation disorders is not available.

We saw rapid response to high concentration of MCT-based feeding formula. With growing he developed a myopathic form with a high risk of renal failure/ chronic disease.

Despite adequate dietary treatment and exercise restriction, episodes of rhabdomyolysis persist. Sometimes there is no trigger during events. Other therapeutic strategies should be taken into account to improve their quality of life.
UNUSUAL TRIPLE TROUBLES OF GENETIC DISORDERS LEAD TO COMPLEX CLINICAL PRESENTATIONS

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Background: Inborn errors of metabolism (IEMs) are rarely caused by copy number variations. We identified triple troubles in a two and half year old girl born through in vitro fertilization. Initial visit revealed that she had history of failure to thrive, hypotonia, and development delay. Other clinical features included microcephaly and dysmorphic face. Brain MRI at age of one-year-old showed that her frontal lobes size was slightly atrophy. Mass spectrometry results from her blood and urine suggested 3-methylcrotonyl-CoA carboxylase deficiency (3-MCCD). Close examination revealed that she had 20 teeth with small size and yellow discoloration.

Methods: Medical Exome Sequencing covered with 4000 known disease genes that could cause clinically genetic diseases was used to screen suspected genetic causes on DNA extracted from her peripheral blood samples and parental samples for Trio analysis. Sanger sequencing and PCR were used to confirm the results.

Results: Three genetic abnormalities were identified in the case. A homozygous c.1630delA (p.R544Dfs*2) of MCCC1 gene was found. Her mother was confirmed to be heterozygous for this mutation. No point mutation of MCCC1 gene was detected on the parental chromosome. A de novo 1.36 Mb deletion of 3q27.1 encompassing MCCC1 gene was identified. This de novo deletion presumably occurs on the paternal chromosome. Compound heterozygous mutations of c.536C>G (p.S179*) and c.2686C>T (p.R896*) on WDR72 gene were identified. Her father is a carrier of c.536C>G (p.S179*) and her mother is a carrier of c.2686C>T (p.R896*).

Conclusion: A 1.36Mb deletion on 3q27.1 has never been reported before. However, 3 cases on the similar region had been reported to have development delay, microcephaly and dysmorphic features. The identified de novo deleted region harboring MCCC1 gene and one mutation of MCCC1 inherited from her mother together with consistent biochemical findings lead to the confirmed diagnosis of 3-MCCD. The diagnosis of amelogenesis imperfecta II A3 caused by WDR72 gene was also established after closely clinical examination. The delineation of these mutational mechanisms provides additional insight for the diagnosis of IEMs. The triple troubles identified here present the advantage of NGS that could provide both CNV and SNV in single assay. The extra layer complexity has improved our understanding of the pathogenesis of complex diseases with unexplained clinical symptoms.

Key Words: Triple troubles, Inborn Errors of Metabolism, 3q27.1 deletion, 3-methylcrotonyl-CoA carboxylase deficiency, amelogenesis imperfecta II A3, genetic causes, complex presentations.

THE MIXED OXIDASE/DEHYDROGENASE ACTIVITIES OF HUMAN LONG-CHAIN ACYL-COA DEHYDROGENASE (LCAD)

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The first step of mitochondrial fatty acid β-oxidation (FAO) is conducted by a family of acyl-CoA dehydrogenase flavoproteins. Three of these, long-chain acyl-CoA dehydrogenase (LCAD), very long-chain acyl-CoA dehydrogenase (VLCAD), and acyl-CoA dehydrogenase-9 (ACAD9) have largely overlapping substrate specificities in vitro, catalyzing the dehydrogenation of long-chain acyl-CoA species between 12 and 20 carbons in length. Determining the relative roles of these enzymes in human metabolism is important for understanding the physiology of FAO and the pathophysiology of genetic disorders thereof. VLCAD is the dominant long-chain enzyme in human heart and muscle, and ACAD9 has a moonlighting role as an assembly factor for Complex I of the respiratory chain.

LCAD, in contrast, is poorly understood. LCAD is expressed in human tissues not normally thought to rely upon FAO for energy such as the lung, thyroid, breast, and prostate. In mice LCAD is widely expressed and appears to fulfill the role that VLCAD does in humans. These differences suggest that human LCAD may have alternative functions.

In the present studies, we observed that recombinant human LCAD has mixed dehydrogenase/oxidase activities. In the absence of the physiological electron acceptor electron transferring flavoprotein (ETF), LCAD directly reduced oxygen to hydrogen peroxide (H2O2). Human LCAD's oxidase activity was 12-to-25-fold greater than

(Continued on next page)
that of recombinant human VLCAD depending upon the substrate used (palmitoyl versus stearoyl-CoA), albeit still 35-fold lower than the activity of the peroxisomal long-chain acyl-CoA oxidase-1 (ACOX1), a pure oxidase that will not pass electrons to ETF. Recombinant ACAD9, in comparison, had negligible oxidase activity and was not further studied.

When recombinant human LCAD and VLCAD were mixed in the oxidase assay at a ratio approximating that which we observed in human liver, using palmitoyl-CoA as substrate, the oxidase activity of LCAD was suppressed. This suggested that VLCAD has a higher affinity for acyl-CoA substrate than LCAD. This was confirmed by anaerobic substrate titrations with the two enzymes mixed at various ratios.

Finally, we compared recombinant human LCAD to recombinant mouse LCAD and observed that the human enzyme has several-fold lower activity as a dehydrogenase but several-fold higher activity as an oxidase. Our data suggest that in human tissues where LCAD expression dominates over VLCAD, such as lung, thyroid, and prostate, the oxidase activity of LCAD could conceivably be a considerable source of H2O2. In human liver, where our data show that LCAD and VLCAD are co-expressed, VLCAD would be expected to outcompete LCAD for acyl-CoA substrate and H2O2 generation would be low. However, rising acyl-CoA concentrations or insufficient VLCAD activity (i.e., VLCAD deficiency) could result in elevated H2O2 production. Lastly, our data showing higher oxidase/lower dehydrogenase activity for human LCAD compared to the mouse enzyme helps to explain observed inter-species differences with regards to the physiological role of LCAD.

VLCAD DEFICIENCY RELATED CHRONIC INFLAMMATION PATTERN IS SUGGESTIVE OF SYSTEMIC MEDIATORS

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Background and Objectives: Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is a life-threatening disorder of mitochondrial fatty acid oxidation. Newborn screening with early intervention provides the best opportunity to prevent morbidity and mortality. Anaplerotic energy supplementation therapy has been shown to be effective in treating hypoglycemia; however, rhabdomyolysis episodes and atypical chronic inflammation often persist. We hypothesize that rhabdomyolysis susceptibility is associated with maladaptive systemic inflammation that is independent of energy deficiency.

Materials and Methods: We analyzed pathways with Ingenuity and Pathway Commons programs to correlate previously observed cytokines and proteins that link inflammation and VLCAD deficiency phenotype. Bone marrow was collected from C57BL/6 and VLCAD-/- mice at 10 weeks of life to create in vitro monocytes/macrophages (Mf) and dendritic cells (DCs). We then stimulated inflammatory pathways in half of the cells with lipopolysaccharide (LPS). Spent media was collected, and cytokine profiles were analyzed by a custom 17-Plex (CRP, IFN-g, IL-1a, IL-1β, IL-4, IL-12p70, IL-13, IL-17A, IL-23p19, IL-33, MCP-1, M-CSF, MIP-1α, MIP-1β, S100A8, S100A9, and TNFa) Luminex Assay (R&D Systems, LXSAMSM17).

Results: In LPS treated VLCAD-/- Mf, IL-12p70 and IL-23p19 were significantly elevated compared to wild type (WT). Otherwise VLCAD-/- cytokine levels were lower or not significantly different from WT.

Discussion/Conclusion: Our results show that in vitro inflammatory changes occur in Mf and DCs from VLCAD-/- mice that distinguish them from WT controls. Increased production of IL-12p70 and IL-23p19 by LPS-stimulated VLCAD-/- Mf cells as compared to WT further supports proposed monocyte-activation related mechanism. Additional differences may be explained by cell-specific production and age-dependent increase of cytokines. Current results were found in primary cell culture from 10-week-old mice in comparison to prior data on 6-month-old mouse plasma. We will explore these postulates through time-course measurements of cytokines in mouse plasma. Considering the hypothesis that inflammation contributes to episodes of rhabdomyolysis; clearly chronic inflammation does play a role in VLCAD deficiency and offers a therapeutic target with optimized immune modulators (e.g. infliximab) that could significantly improve patient quality of life.
A NOVEL SMALL-MOLECULE PPARδ MODULATOR FOR THE TREATMENT OF FATTY ACID OXIDATION DISORDERS

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Fatty acid oxidation disorders (FAODs) are a heterogeneous group of inborn errors of metabolism that are characterized by reduced metabolic flexibility leading to hypoglycemia, reduced exercise tolerance, and multi-organ dysfunction. They are caused by mutations in metabolism-related genes, including those coding for key enzymes of the fatty acid β-oxidation cycle, which lead to reduced fatty acid metabolism. MA-0211 (a.k.a. MTB-1) is a novel, orally-available, small molecule currently in a Phase 1 clinical study that modulates PPARδ, a key nuclear hormone receptor which regulates cellular metabolic flexibility. Administration of MA-0211 in multiple animal models has demonstrated significant improvements in several FAOD related manifestations, such as increasing exercise endurance, protecting against cardiac dysfunction and acute kidney injury. In the present report, we evaluated whether MA-0211 can improve fatty acid oxidation in fibroblasts derived from patients with very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency.

The results show that MA-0211 increases VLCAD mRNA, protein levels, and enzymatic activity in a dose responsive manner. MA-0211 increases utilization of palmitate and changes the acyl-carnitine profile in a manner consistent with increased long-chain fatty acid oxidation in patient cells that are expected to have some residual enzymatic activity based on their mutation. Additionally, improvements in fatty acid oxidation were observed in patient fibroblasts derived from long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency and mitochondrial trifunctional protein (TFP) deficiency. In conclusion, the significant improvements seen in FAOD patient fibroblasts together with previous demonstrations of in vivo pharmacological activity, support studying MA-0211 in patients with FAODs.
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