MCT8 2016 Symposium Schedule
Hotel Ritz Carlton, Marina Del Ray, California

Day 1 - 12th January 2016, Venue: BALLROOM TERRACE

19:00 – 21:00
Welcome Drinks & Hors d'oeuvres

Day 2 - 13th Jan, Venue: BALLROOM TERRACE

8:30-8:40
Welcome: Dror Ben-Ami and Samuel Refetoff

Note: all presentations are 10 minutes

8:45 – 9:15
Analogues and Substrates I, Chair Juan Bernal
- Thyroid hormone analogue therapy in patients with the Allan-Herndon-Dudley Syndrome (AHDS): the TRIAC trial – Edward Visser
- TRIAC treatment of Allan-Herndon-Dudley Syndrome (AHDS) due to effects in thyroid hormone transporter MCT8 – Jose C. Moreno
- Therapeutic potential of TH analogs in MCT8 deficiency – Heike Heuer

9:15 – 10:00
Discussion

10:00 – 10:15
Coffee Break

10:15 – 10:45
Analogues and Substrates II, Chair Clive N. Svendsen
- Effect of TRIAC in the treatment of Mct8 – Soledad Bárez-López
- Myelination deficiencies and pharmacological treatments in zebrafish model for AHDS – David Zada
- Export of iodotyrosines by human MCT8 and MCT19 – Theo Visser

10:45 – 11:30
Discussion

11:30 – 11:45
Coffee Break

11:45 – 12:15
Future Treatments, Chair Ken Holden
- Translational approaches to treat the AHDS delayed myelination phenotype – Gad D. Vatine
- Intranasal therapeutics to bypass the blood-brain barrier and treat MCT8 – William H. Frey II
- Chemical chaperones rescue several pathogenic MCT8 mutations *in vitro* and structure and function of MCT8 – Doreen Braun

12:15 – 13:00
Discussion

13:00 – 14:00
Lunch

14:00 – 14:30
Gene Therapy, Chair Theo Visser

- AAV9-Based gene therapy delivers a functional MCT8 transporter which improves thyroid hormone availability to brain of Mct8 deficient mice – Samuel Refetoff
- The role of Mct8 in the transport of TH across the human BBB – Clive N. Svendsen
- What's possible in gene therapy for AHDS – Brian Kaspar

14:30 – 15:15
Discussion

15:15 – 15:30
Coffee Break

15:30 – 16:10
Neural Dynamics of MCT8 Mutation, Chair Heike Heuer

- Transcriptome profiling of MCT8-deficient zebrafish: future directions – Lior Appelbaum
- Genomics of MCT8 mouse model – Juan Bernal
- Spatial and temporal expression pattern of MCT8 in the human brain– Anna Guadaño-Ferraz
- Brain MRI Imaging Findings and Clinical Correlations in MCT8 Transporter Deficiency – Gisel M. Matheus

16:10 – 17:00
Discussion

17:00 – 17:15
Coffee Break

17:15 – 18:15
Parents - Therapies that Work

19:30
Conference Dinner
Venue: BALLROOM TERRACE

Day 3 - 14th Jan, Venue: SALON 2 (on Ballroom Level)

8:30-10:30
Gene Therapy - Technical Session, Chair - Brian Kasper

- Discussion of how to move forward
- How can the various groups contribute

PARTICIPANTS

Lior Appelbaum
The Faculty of Life Sciences and
the Multidisciplinary Brain Research Center.
Bar Ilan University, Israel
Lior.Appelbaum@biu.ac.il

Soledad Barez Lopez
Instituto de Investigaciones Biomédicas CSIC-UAM
Department of Endocrine and Nervous
System Pathophysiology
Madrid, Spain
sbarez@iib.uam.es

Dror Ben-Ami
Father of Dov and Lev
Herzliya, Israel
drorba7@hotmail.com

Juan Bernal
Bernal Lab
Instituto de Investigaciones Biomédicas, CSIC-UAM
Madrid, Spain
jbernal@iib.uam.es

Doreen Braun
Institute for Experimental Endocrinology
Charit Universit tsmedizin
Berlin, Germany
Doreen.braun@uni-bonn.de

Nicola Cowan
Mother of Aaron
London UK
Diese1970@btinternet.com

Lisa Davis
Mother of Brandon
Oklahoma USA
lisadaviscnrn@gmail.com

William Frey
Center for Memory & Aging (Alzheimer's Research Center)
Neurology and Neuroscience
University of Minnesota
alzheimr@umn.edu

Hermann Garcia Castillo
Father of Sebastian
New Jersey, USA
hermann.garcia@us.henkel.com

Judy Gay
Nurse practitioner
USA

Maria Gisele Matheus
Department of Radiology and Radiological Science
Medical University of South Carolina (MUSC)
Charleston, South Carolina, USA
matheus@musc.edu

Ana Guadaño-Ferraz
Bernal Lab
Instituto de Investigaciones Biomédicas, CSIC-UAM
Madrid, Spain
aguadano@iib.uam.es

Sharon Herman
Grandparent of Mason Lewis
Missouri USA
jsherman1927@gmail.com

Joe Herman
Grandparent of Mason Lewis
Missouri USA
jsherman1927@gmail.com

Heike Heuer
Leibniz Research Institute for Environmental Medicine
Düsseldorf , Germany
hheuer@fli-leibniz.de

Ken Holden
Department of Neurosciences
The Medical University of South Carolina (MUSC)
Charleston, South Carolina, USA
holdenk@musc.edu

Roelof Jobing
Father of Ben
Capetown, South Africa
jobingr@gmail.com

Theresa Jobing
Mother of Ben
Capetown, South Africa
jobingr@gmail.com

Brian Kaspar
The Research Institute at Nationwide Children's Hospital
The Ohio State University
USA
Brian.Kaspar@nationwidechildrens.org

Penny Kostakis
Mother of child with MCT8
Melbourne, Australia
cuiccimark@yahoo.com

Brenda Lenahan and Dan Josephson
Parents of child with MCT8
Vancouver, Canada
brendalenahan@gmail.com

Xiao-Hui Liao
Department of Medicine
The University of Chicago
Chicago, USA
xliao1@uchicago.edu

Joe Ludica
Father of child with MCT8
Melbourne Australia
cuiccimark@yahoo.com

Chrissy Masco
Mother of Eli
Utah, USA
chrissy.masco@gmail.com

Bowen Masco
Father of Eli
Utah, USA
chrissy.masco@gmail.com

Bruce Milthorpe
Dean of Science
University of Technology
Sydney, Australia
bruce.milthorpe@uts.edu.au

Dr Jose Moreno
Thyroid Molecular Laboratory
Institute for Medical and Molecular Genetics (INGEMM).
La Paz University Hospital
Madrid Spain
josecarlos.moreno@salud.madrid.org

Beatriz Morte Molina
Bernal Lab
Instituto de Investigaciones Biomédicas, CSIC-UAM
Centre for Biomedical Network Research on Rare Diseases (CIBERER)
Madrid, Spain
bmorte@iib.uam.es

Andrew Nelson
HealthPartners Institute for
Education and Research
Minneapolis, USA
Andrew.F.Nelson@healthpartners.com

Heather Payne
Mother of child with MCT8
USA
ANDY.PAYNE@hcbe.net

William Ray
Institute for Applied Cancer Science
University of Texas
MD Anderson Cancer Center
Houston, Texas
WJRay@mdanderson.org

Samuel Refetoff
Departments of Medicine, Paediatrics and Genetics
The University of Chicago
Chicago, USA
srefetof@uchicago.edu

Shawn Roberts
Father of Devin
Pennsylvania, USA
sdrst17@yahoo.com

Jerran Santos
Postdoctoral Research Fellow - Advanced Tissue Regeneration & Drug Delivery Group
Technical Officer - Proteomics Core Facility
University of Technology
Sydney, Australia
jerran.santos@uts.edu.au

Barry Sechos
The Sherman Group
Sydney, Australia
bsechos@shermangroup.com.au

Steven Sherman
Department of Endocrine Neoplasia & Hormonal Disorders
Houston, Texas
sisherma@mdanderson.org

Panudda Srichomkwun
Department of Medicine
The University of Chicago
Chicago, USA
panuddas@uchicago.edu

Clive Svendsen
Cedars-Sinai Regenerative Medicine Institute
Los Angeles, USA
clive.svendsen@cshs.org

Adri Van der Walt
Pediatric Neurologist
Cape Town, South Africa
adrimed@iafrica.com

Gad Vatine
Postdoctoral Researcher
Svendsen Laboratory
Regenerative Medicine Institute
Cedars-Sinai Medical Center
Gad.Vatine@cshs.org

Theo Visser
Department of Internal Medicine
Erasmus Medical Centre
Rotterdam, Netherlands
t.j.visser@erasmusmc.nl

Edward Visser
Department of Internal Medicine
Erasmus Medical Centre
Rotterdam, Netherlands
w.e.visser@erasmusmc.nl

Roy Weiss
Department of Medicine
The University of Miami Miller School of Medicine
rweiss@med.miami.edu

David Zada
The Faculty of Life Sciences and the Multidisciplinary Brain Research Center.
Bar Ilan University, Israel
david.zada85@gmail.com

ABSTRACTS

(in order of presentation)

Thyroid hormone analog therapy in patients with the Allan-Herndon-Dudley Syndrome (AHDS): the Triac Trial

S Groeneweg, IR de Coo, IM van Beynum, C den Uil, FK Aarsen, YB de Rijke, RP Peeters, TJ Visser, WE Visser, on behalf of the Triac Trial research group

(SG, YBR, RPP, TJV, WEV) Internal Medicine, (IRC) Pediatric Neurology, (IMB) Pediatric Cardiology, (CAU) Cardiology, and (FKA) Neuropsychology, Erasmus University Medical Center, Rotterdam, The Netherlands

Introduction
Mutations in the thyroid hormone transporter MCT8 result in AHDS, characterized by severe psychomotor retardation and abnormal serum thyroid function tests (TFTs) (low-normal T4, high T3, high-normal TSH). High T3 levels cause symptoms of peripheral thyrotoxicosis. At present, no effective treatment is available. Recent findings suggest that the T3 analog Triac is a promising candidate to 1) normalize serum T3 levels and hence alleviate the thyrotoxicosis and 2) provide adequate thyromimetic effects in the brain.

Methods
We conduct a world-wide prospective interventional trial in which 30-40 AHDS patients receive Triac for 1 year. The primary end-point is the reduction of serum T3 levels, and secondary end-points are reduction of heart rate (HR), improvement of body weight (BW) and serum parameters that reflect thyroid action in peripheral tissues. In addition, the neuropsychological phenotype and motor function are assessed before and after 1 year of Triac treatment.

Results
Currently, 19 patients have been enrolled (age: 0,9-66 yr). All patients show a severely disturbed neuropsychological development, primitive reflexes, elevated tendon reflexes, dystonia and central hypotonia. Triac treatment (average duration: 6 months) effectively suppressed serum TSH levels (median [IQR]: 3.1 [1.6-5.1] to 0.9 [0.5-1.5] mU/L; p=0.003), resulting in a strong reduction of T3 levels (4.4 [3.5-5.1] to 2.0 [1.6-2.7] nmol/L; p=0.003). In addition, peripheral markers are slightly improving. Alkaline phosphatase (145 [113-188] to 207 [114-232] U/l; p=0.028), sex hormone binding globulin (179 [86-244] to 151 [86-195] nmol/L; p=0.062) and creatinine (37 [30-50] to 43 [37-62] ε mol/L; p=0.005) serum levels show (near-)significant responses to Triac treatment; whereas HR, BW and serum cholesterol levels show non-significant changes.

Discussion
Triac treatment effectively results in normalization of serum T3 levels. This interim analysis suggests that Triac treatment may have a beneficial effect on the peripheral phenotype of AHDS. A longer follow-up period and inclusion of more patients will further substantiate these findings.

Keywords: MCT8, Allan-Herndon-Dudley Syndrome, Triac, clinical trial

TRIAC TREATMENT OF ALLAN-HERNDON-DUDLEY SYNDROME (AHDS) DUE TO DEFECTS IN THYROID HORMONE TRANSPORTER *MCT8*

Ainhoa Iglesias [1], Ana L. Gómez-Gila [2], Paula Casano [3], Jesús Pozo [4], M. Carmen de Mingo [5], Natividad Pons [6], Fernando Calvo [6], Beatriz Morte [7], Maria Jesús Obregón [7], Juan Bernal [7], José C. Moreno [1].

1 Thyroid Molecular Laboratory. Institute for Medical and Molecular Genetics (INGEMM). La Paz University Hospital. Madrid. Spain
2 Paediatric Endocrinology. Virgen del Rocío University Hospital. Sevilla. Spain.
3 Paediatric Endocrinology. San Joan de Deu Hospital. Barcelona. Spain.
4 Paediatric Endocrinology. Niño Jesús Hospital. Madrid. Spain.
5 Paediatric Endocrinology. La Fé Hospital. Valencia. Spain.
6 Paediatric Endocrinology and Diabetes. Lluís Alcanyis Hospital. Xátiva, Valencia. Spain.
7 Institute for Biomedical Research (IIB). CIBERER, CSIC. Madrid, Spain.

AHDS is a complex disease caused by defects in the thyroid hormone (TH) transporter MCT8. Endocrine expression is heralded by systemic hyperthyroidism with elevated serum T3, mildly increased TSH and decreased T4. However, the brain is hypothyroid, causing severe psychomotor retardation. Therapeutic attempts with PTU+Levothyroxine or the T3-analogue DITPA did normalize TH derangements but without neurological improvement. Recently, in vitro and mouse studies support the therapeutic utility of triiodothyroacetic acid (TRIAC) in MCT8 deficiency.

Objective To investigate the hormonal effects of TRIAC in AHDS.

Patients & Methods Five children diagnosed with AHDS between 8 months and -6 years of age, harboring various defects in *MCT8* (p.P215L, p.delF230, p.V254DfsX24, p.L304_I539del, p.G401R). TRIAC compassive treatment started with 10 µg/kg/day, doubling the dose approximately every 2 weeks until normalization of TH parameters. Determination of serum TSH, FT3/T3, FT4 and SHBG by immunoassay, and TRIAC and rT3 by radioimmunoassay.

Results: At baseline, patients (9 mo-8 years old) showed high FT3 (7.3±2.4 ng/dL; n<4) or T3: (4.2±0.2 nmol/L; n< 3.8), low FT4 (0.6± 0.08 ng/dL; n>0.8), borderline-high TSH (4.4±2.6 mU/L; n< 4.5) , low rT3 (3/5) (8.6±2.8 ng/dL; n>15) and high SHBG (207.6±55.8 nmol/L; n< 100). After mean 11.4 weeks treatment and mean TRIAC dose of 33.3µg/kg/day (20-40), FT3 and T3 normalized (3.8 ± 0.6ng/dL-3/5-; T3 3.14nmol/L -1/5-) and TSH decreased (1.99± 1µU/mL), but FT4 and rT3 remained low (0.39±0.05ng/d; 7.8±4.9ng/dL,) and SHBG elevated (221± 59nmol/L). Serum TRIAC increased 2-10-fold from baseline to final dose in connection to age.

Conclusions TRIAC normalizes hyperthyroidism and hyperthyrotropinemia in AHDS, but not FT4, rT3 or SHBG. The required dose to achieve effects seems related to age, possibly due to larger distribution volume of drugs in early childhood. Effects of TRIAC on child neurodevelopment and brain myelination are being prospectively evaluated by psychometric tests and MRI in periodic follow-up investigation.

Therapeutic potential of TH analogs in MCT8 deficiency

Heike Heuer

Leibniz Research Institute for Environmental Medicine (IUF); Düsseldorf (Germany)

Patients with inactivating mutations in the thyroid hormone transporter MCT8 suffer from a severe form of psychomotor retardation and abnormal serum TH levels. The neurological symptoms are most likely due to an impaired transport of TH into the CNS and, consequently, due to a disturbed differentiation and maturation of brain cells. Treatment of patients with TH analogs that can replace TH in the brain but are not dependent on MCT8 for cellular entry have been suggested to be a promising therapeutic approach.

We recently started to test different TH analogs in Mct8/Oatp1c1 dko mice, an animal model for human MCT8 deficiency. Treatment of mice with Triac (TA3) during the first three postnatal weeks resulted in normalization of different brain parameters. In particular, TA3-treated Mct8/Oatp1c1 dko mice exhibited normal cerebellar Purkinje cell development, myelination and development of inhibitory interneurons. Moreover, electrophysiological recordings of brain sections revealed a normalization of the input-output curve indicating normal neuronal firing. Mct8/Oatp1c1 dko mice treated with TA3 for three weeks and analyzed at the age of 10 weeks displayed normal locomotor functions suggesting that a transient treatment with TA3 during the early postnatal period is sufficient to prevent locomotor deficits in Mct8/Oatp1c1 dko animals. Ongoing studies will reveal to which extent a TA3 treatment initiated later in life leads to an improvement in brain parameters as well as in locomotor performance.

Effect of TRIAC in the treatment of Mct8

Soledad Bárez-López, Maria Jesus Obregon, Raquel Martínez-de-Mena, Juan Bernal, Ana Guadaño-Ferraz & Beatriz Morte

Instituto de Investigaciones Biomédicas, CSIC-UAM and CIBERER, Madrid, Spain

Monocarboxylate transporter 8 (MCT8) is a thyroid hormone-specific cell membrane transporter. Mutations in MCT8 gene lead to profound psychomotor retardation and abnormal thyroid hormone serum levels with low thyroxine (T4) and high triiodothyronine (T3). Currently, therapeutic options for patients are limited. Triiodothyroacetic acid (TRIAC) has potential therapeutic value. The aim of this study was to evaluate the effects and efficacy of therapeutic doses of TRIAC on Mct8-deficient mice (Mct8KO).

Wild-type (Wt) and Mct8KO mice were treated with 30 ng TRIAC/g BW/day, given in drinking water, from postnatal day 21 to 30. TRIAC, T4 and T3 levels in plasma, as well as T3 and TRIAC content in the cerebral cortex and striatum were measured by specific radioimmunoassays. The activities of deiodinases 1 and 2 were measured in liver and cortex. The effect of TRIAC treatment in the expression of T3-dependent genes was measured in the heart, cerebral cortex and striatum.

Plasma TRIAC concentration were the same in Wt and Mct8KO animals after treatment. TRIAC treatment greatly decreased plasma T4 in Wt and Mct8KO mice, and reduced T3 to normal levels in the Mct8KO. Deiodinase 1 activity and gene expression in the liver increased while it did not have any effect in the expression of *Serca2a* in the heart. TRIAC treatment did not induce the expression of T3-dependent genes in the cerebral cortex or striatum but further decreased expression of *Flywch*2 in the cortex and *Aldh1a*1 and *Flywch*2 in striatum. Direct measures of TRIAC and T3 content in the cortex and striatum revealed a decrease in T3 after treatment with no significant increase in the level of endogenous TRIAC.

Therapeutic doses of TRIAC in Mct8KO mice restored plasma T3 levels but severely decreased T4 levels. TRIAC has a direct effect on deiodinase 1 in the liver and does not have an effect on the gene expression in the heart. The increase in the plasma TRIAC levels after treatment is not sufficient to increase TRIAC levels in the brain and to promote the expression of T3-dependent genes in brain cells. Instead, it leads to a state of brain hypothyroidism with reduced T3 content.

Myelination deficiencies and pharmacological treatments in zebrafish model for AHDS

David Zada, Adi Tovin, Lior Appelbaum

The Mina & Everard Goodman Faculty of Life Sciences and Leslie and Susan Gonda Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan 52900, Israel

Allan Herndon Dudley syndrome (AHDS) is a severe psychomotor retardation disorder characterized by neurological impairments and abnormal thyroid hormone (TH) levels. THs regulate metabolism, embryonic development, neurogenesis and myelination. Mutations in the TH transporter, monocarboxylate transporter 8 (MCT8), are associated with AHDS, however, the function of this gene and the mechanism of the disease are elusive. Similar to human patients, MCT8-knockout mice exhibit impaired TH levels; however, they lack neurological defects. We utilize the zebrafish as a model to study MCT8 function and psychomotor retardation. This transparent vertebrate emerged as a promising model to study neurobiology and development; because it shares the advantage of high-throughput genetics of invertebrates with mammalian-like conserved genome and brain structure including the hypothalamic-pituitary-thyroid gland axis. Importantly, MCT8-knockout zebrafish exhibit impaired neurogenesis and altered behavioural performances. Here, we studied the development of myelination in MCT8 mutant (*mct8*-/-) zebrafish. We used the *myelin basic protein* (*mbp*) as a marker for mature oligodendrocytes in the CNS. The levels of expression of *mbp* mRNA were reduced in *mct8*-/- larvae. Furthermore, time-lapse live imaging of *mbp:EGFP* transgenic fish showed a decreased number of oligodendrocytes in different brain regions of *mct8*-/- larvae during development. In order to test potential treatments, the effect of few drugs on myelination was determined during several developmental stages in live *mct8*-/- larvae. These treatments results in partial to full recovery of myelination. These results suggested that, as is the case in humans, loss of MCT8 affects the development of myelination in zebrafish larvae. It also suggest a putative pharmacological treatment for AHDS patients. The *mct8*-/- zebrafish provide a model to study the mechanism and treatment of psychomotor retardation and may help to understand other myelination disorders such as multiple sclerosis (MS).

EXPORT OF IODOTYROSINES BY HUMAN MCT8 AND MCT10

ELAINE C. LIMA DE SOUZA, CHANTAL ZEVENBERGEN, W. EDWARD VISSER, THEO J VISSER

Department of Internal Medicine and Rotterdam Thyroid Center, Erasmus University Medical Center, Wytemaweg 80, 3015 CN, Rotterdam, The Netherlands.

Introduction:

MCT8 and MCT10 are highly homologous and effective thyroid hormone (TH) transporters. Despite this, MCT10 also transports aromatic amino acids, whereas MCT8 seems to be specific for iodothyronines. The iodotyrosines, MIT and DIT, are intermediates in TH biosynthesis, showing structural resemblance with iodothyronines. Therefore, we investigated if in addition to the iodothyronines T4, T3, rT3 and 3,3ôT2 (T2), MCT8 and MCT10 may also transport MIT and DIT.

Materials and Methods:

COS1 cells were transiently transfected with hMCT8, hMCT10, hLAT1(+ CD98) or hLAT2(+ CD98), and incubated for 5-60 minutes at 37 °C with 10 nM ^{125}I-labeled T3, T4, rT3, T2, and 10µM MIT or DIT in PBS. RT-qPCR was performed for LAT1 and LAT2.

Results:

We found that MCT8 transports iodothyronines with preference for T4~T3>rT3~T2, while MCT10 shows preference for T3~rT3>T4~T2. We also showed that MCT8 and MCT10 facilitates cellular export of MIT and DIT. Export of MIT by MCT8 is inhibited by T4 and T3; however this is not observed for DIT. We found that uptake of MIT and DIT by COS1 cells is inhibited by the specific LAT inhibitor BCH, and over-expression of LAT1 or LAT2 results in stimulation of MIT and DIT uptake. However, LAT1 is expressed in COS-1 cells, but not LAT2. The time course of MIT and DIT uptake shows the involvement of different transporters. Endogenous uptake of MIT is performed by an exchanger transporter, most likely LAT1. However, DIT uptake is not dependent of the exchange with amino acids but is partially sodium dependent showing the involvement of an unknown sodium dependent transporter.

Conclusion:

We demonstrated export of MIT and DIT by MCT8 and MCT10, and uptake by LAT1 and LAT2. The physiological importance of the transport of MIT and DIT by MCT8 and MCT10 must be further analyzed, such as the involvement in the clinical phenotype of MCT8 deficient patients.

Translational approaches to treat the AHDS delayed myelination phenotype

Gad D. Vatine and Clive N. Svensen

Regenerative Medicine Institute, Cedars-Sinai Medical Center

Delayed myelination has recently been established as a prominent symptom of AHDS. To investigate the role of Mct8 in the development of myelin in the human CNS, we have differentiated iPSCs from AHDS patients and healthy controls into mature oligodendrocytes and tested their response to T3. We demonstrate that the maturation of AHDS oligodendrocyte progenitor cells (OPCs) is less sensitive to T3 when compared to healthy controls *in vitro*. In order to explore the potential of cell transplantation to treat AHDS, we transplanted AHDS and control OPCs into an immunodeficent congenital hypomyelinated mouse model. Our results demonstrate that these OPCs survive and integrate in the mouse brain. Furthermore OPCs are able to differentiate into mature oligodendrocytes and myelinate axons *in vivo*. In order to test this cell therapy approach as a potential treatment for AHDS patients we have introduced an additional mutation to the MCT8/OATP1C1 double KO (dKO) mouse rendering it immunodeficent and suitable for human cell transplantation experiments. OPCs transplantation into the triple KO mouse model is underway. In addition, we have recently initiated a study in which we test the potential of the small molecule Clemastine, to promote myelination in the dKO mouse.

Intranasal therapeutics to bypass the blood-brain barrier and treat MCT8

William H. Frey II
Center for Memory & Aging (Alzheimer's Research Center), University of Minnesota

In 1989, I discovered and patented that therapeutics administered to the upper third of the nasal cavity could be non-invasively delivered directly to the brain along the olfactory and trigeminal neural pathways, bypassing the blood-brain barrier and reducing systemic exposure and unwanted side effects. I subsequently developed the intranasal insulin treatment that has been shown to safely improve memory in both normal adults and patients with Alzheimerôs disease in multiple phase 2 clinical trials with no change in the blood levels of insulin or glucose. Preclinical studies have also demonstrated intranasal delivery and targeting of gene therapy to the brain using AAV vectors. Finally, we discovered and patented the non-invasive intranasal delivery and targeting of therapeutic cells, including stem cells, immune cells and genetically-engineered cells, to the brain to treat Parkinsonôs and other brain disorders. The ways in which intranasal delivery can be used to treat MCT8 will be discussed.

Chemical chaperones rescue several pathogenic MCT8 mutations *in vitro* structure and function of MCT8

Doreen Braun and Ulrich Schweizer

Institut für Biochemie und Molekularbiologie

The severity of the Allan-Herndon-Dudley syndrome differs among MCT8 patients. Partial activity of MCT8 can be caused either by mutations affecting the transport mechanism (e.g. substrate interaction, conformational change) or by inefficient protein expression, membrane translocation or stability. The latter MCT8 mutants may be responsive to chemical chaperones which stabilize the protein or support membrane localization. We have already shown that the application of the chemical chaperone sodium phenylbutyrate rescues expression and transport activity of pathogenic ΔPhe501 mutants *in vitro*. Furthermore we will show new mutants (e.g. L434W) which are responsive to sodium phenylbutyrate and others. Sodium phenylbutyrate is a common drug used for the treatment of urea cycle defects and cystic fibrosis. Thus, the administrations of the drug could be a new tool for the therapy of MCT8 deficiency to improve the patient's quality of life.

The already published MCT8 homology model in the inside open conformation helped to identify amino acid residues which are important for transport activity and substrate binding (His-Arg clamp). It also gave a better insight in the localization of pathogenic MCT8 mutations. Since it did not explain how substrate might bind from the extracellular side, we have generated two new MCT8 models built on the crystal structure of the partly occluded outward conformation of XylE and the outside open conformation of FucP.

Homology models are a "fast" tool to generate ideas about substrate binding or transport mechanism, but are only models, not experimental structures. In order to solve an experimental structure, which will give a far more detailed picture of MCT8, we expressed recombinant MCT8 protein in bacteria and purified the transporter. It turned out that the MCT8 protein forms aggregates in bacterial membranes which are thus not suitable for the expression of recombinant MCT8 protein. Consequently, we changed the expression system to insect cells. Thanks to the experience in the institute with this expression system, we are now expressing human MCT8 using a baculoviral system. Purification will follow the method developed with bacterial protein.

AAV9-Based Gene Therapy Delivers a Functional MCT8 Transporter which Improves Thyroid Hormone Availability to Brain of Mct8 Deficient Mice

Hideyuki Iwayama[1], Xiao-Hui Liao[1], Lyndsey Braun[4,5], Soledad Bárez-López[6,7], Brian Kaspar[4,5], Roy E Weiss[8], Alexandra M Dumitrescu[1], Ana Guadaño-Ferraz[6,7] and Samuel Refetoff[1,2,3]

Departments of [1]Medicine, [2]Pediatrics and [3]Committee on Genetics, The University of Chicago, Chicago, Illinois, USA

[4] The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA

[5] Department of Neuroscience, The Ohio State University, Columbus, Ohio, USA

[6]Institute for Biomedical Research "Alberto Sols", Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid, Spain

[7]Center for Biomedical Research on Rare Diseases (Ciberer), Unit 708, Instituto de Salud Carlos III, Madrid, Spain

[8]Department of Medicine, University of Miami, Miami Florida, USA

MCT8 gene mutations produce thyroid hormone (TH) deficiency in brain, causing severe neuropsychomotor abnormalities not correctable by TH treatment. We examined whether transfer of human *MCT8* (*hMCT8*) cDNA using adeno-associated virus 9 (AAV9) could correct the brain defects of Mct8 knockout mice *(Mct8KO*). AAV9-hMCT8 or empty vector were injected intraventricularly (IV) and/or intracerebroventricularly (ICV) into postnatal day 1 *Mct8KO* mice and the active TH, T_3, was given for 4 days before tissue collection at post-natal day 28. Compared to IV, ICV delivery produced more hMCT8 mRNA and protein, which was present in various brain regions and localized to the cell membranes. Despite more abundant hMCT8 mRNA and protein with ICV delivery, only IV delivered AAV9-hMCT8 targeted the choroid plexus and significantly increased brain T_3 content and expression of the TH-regulated transcription factor, Hairless. These results indicate that MCT8 delivery to brain barriers by IV but not ICV injection is crucial for its proper function. MCT8 has no constitutive activity but acts through an increase in T_3 entering brain tissue. The correct hMCT8 isoform along with an optimized delivery method are critical for an effective gene therapy to provide functional MCT8 in the brain of patients with MCT8 mutations.

The role of Mct8 in the transport of TH across the human BBB

Clive N. Svendsen and Gad D. Vatine

Regenerative Medicine Institute, Cedars-Sinai Medical Center

The recent emergence of technologies to reprogram mature human cells back to a pluripotent stage, and the development of specific protocols to differentiate these induced pluripotent stem cells (iPSCs) into cells of the human brain provide an unprecedented opportunity to human disease-in-a-dish models. Using iPSC derived brain microvascular endothelial cells (BMECs) on a Transwell system we investigate the role of Mct8 in the transport of THs across the human BBB. We demonstrate that T3 does not cross the AHDS BBB and as a result, the AHDS brain is starved from T3. Using this system we demonstrate that the Mct8-mediated transport of T3 is unidirectional, and developed a mass spectrometry approach to test whether TH analogues are crossing the human BBB. In addition, we have developed a novel and more advanced approach to model the human BBB on a microfluidic device. The human BBB-on-Chip offers physiologically relevant features that more closely recapitulate the human BBB, thus enabling further investigation of the role of Mct8 in this neurovascular unit.

Transcriptome profiling of MCT8-deficient zebrafish: future directions

Adi Tovin, David Zada, Lior Appelbaum

The Mina & Everard Goodman Faculty of Life Sciences and Leslie and Susan Gonda Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan 52900, Israel

The X-linked psychomotor retardation Allan-Herndon-Dudley syndrome (AHDS) is associated with mutations in the thyroid hormone (TH) monocarboxylate transporter 8 (*mct8*). AHDS is characterized by severe intellectual deficiency, neuromuscular impairment, and high serum TH levels. We utilize the zebrafish model to study the neurological mechanism and potential treatments of AHDS. The zebrafish combines a transparent brain with conserved structure and function of the Hypothalamic–pituitary–thyroid axis. We generated an *mct8* mutant (*mct8*-/-) zebrafish, which mimics pathological conditions of AHDS patients. Two-photons live imaging of single oligodendrocytes, neurons and synapses, and video-tracking of behavior revealed deficiencies in axon branching, myelination and synaptic density, which are associated with altered locomotor activity. TH analogs partially restored these deficiencies in *mct8*-/- larvae. These results uncover novel mechanisms for TH-dependent developmental psychomotor retardation and suggest potential pharmacological treatment that can specifically reduce neurological damage. In this work, we will show present and future studies including brain mapping and deficient neuronal activity as well as sequencing of the transcriptome, which revealed hundreds of differentially expressed genes in in *mct8*-/- larvae.

Genomics of Mct8 mouse model

Juan Bernal, Pilar Gil-Ibañez, and Beatriz Morte

Instituto de Investigaciones Biomédicas, CSIC-UAM and CIBERER, Madrid

Analyzing the role of thyroid hormone transporters in thyroid hormone action, and their homeostatic relationship with other factors such as deiodinases, is essential to understand the pathogenesis of MCT8 transport defect. In the mouse, Mct8 inactivation causes few alterations in the expression of thyroid hormone-dependent genes. T3 transport through the blood-brain barrier (BBB) is impaired in Mct8 deficiency, but enough T3 is formed locally in the brain from T4 through Dio2 activity. Accordingly, the combined inactivation of Mct8 and Dio2 results in gene expression patterns that are more similar to hypothyroid conditions. Surprisingly, even in this situation, many thyroid hormone-dependent genes are still normally expressed despite that the entry of T3 to the brain and its local generation from T4 are presumably blocked. Oatp1c1 is a T4 transporter present in the BBB and the astrocyte end-feet that facilitates transport of T4 from the blood to the astrocytes, the cells expressing Dio2. We found that genes expressed normally in the double Mct8 and Dio2 KO mice are in the hypothyroid range in the cerebral cortex and the striatum in the combined inactivation of Mct8 and Oatp1c1. This observation is difficult to explain, because deprivation of the T4 substrate, as in Oatp1c1 KO should be equivalent to inactivation of the enzyme as in the Dio2 KO. We believe that this observation is important because it does not fit within the current models of thyroid hormone distribution and action in the brain, and several mechanistic possibilities will be discussed.

Spatial and temporal expression pattern of MCT8 in the human brain

Daniela López-Espíndola, Juan Bernal and Ana Guadaño-Ferraz

Instituto de Investigaciones Biomédicas, CSIC-UAM and CIBERER, Madrid, Spain

Histopathological data from MCT8-deficient human brains strongly suggest that brain hypothyroidism is an important pathophysiological condition involved in the development of the neurological signs and symptoms. Several therapeutic pharmacological strategies have been specifically developed for the treatment of MCT8-deficient patients but to date none has been effective in palliating and/or normalizing the neurological alterations of the patients.
This fact along with previous studies with animal models strongly suggest that the replacement of the mutated gene by a normal one in the MCT8 expressing cells, by means of gene therapy, could be a good approach to treat the neurological syndrome. For all these reasons, it is fundamental to know the spatial and temporal pattern of MCT8 expression in the human brain.

We have studied the prenatal brain expression of MCT8 by immunohistochemistry in autopsy brain tissue from 14, 16, 20, 25, 27, 30 and 38 gestational weeks fetuses. Paraffin tissue sections were analyzed histologically by hematoxylin-eosin staining and by immunohistochemistry using specific polyclonal antibodies for MCT8.
MCT8 is detected prenatally in all brain barrier structures (intraparenchymal and meningeal blood vessels, leptomeningeal cells, choroid plexuses epithelial cells and ependymocytes and tanycytes at cerebral ventricles), and at the membrane of some neurons (including Cajal Retzius cells) and glial cells. Interestingly MCT8 is expressed in radial glial processes in the cortical plate.

Our data indicate a complex spatial and temporal expression pattern of MCT8, probably due to important regulatory mechanisms involved in gene expression and protein processing. The significant and constant expression of MCT8 in all the brain barriers points to the cells forming and participating in these barriers as the crucial targets for gene therapy to overcome MCT8-deficiency and brain hypothyroidism by improving the availability of thyroid hormones inside the human brain.
We consider important to continue getting insight into MCT8 brain expression by analyzing its normal expression at earlier stages in the developing human brain and also its subcellular localization in neural cells.

Brain MRI Imaging Findings and Clinical Correlations in MCT8 Transporter Deficiency

Gisel M. Matheus and Ken Holden
Department of Radiology and Radiological Science, Medical University of South Carolina, Charleston, SC

Abstract: In 2015 we accomplished our goal regarding brain MRI imaging findings and clinical motor phenotype in patients with MCT8 transporter deficiency (MCT8). We reassessed the brain MRI and neurological phenotype of 6 pediatric MCT8 patients. The preliminary results were presented in January 2015 to the MCT8 group and published in the Journal of Child Neurology (October 2015). These preliminary results revealed more specifically which areas were predominantly affected on brain MRI images and how the findings change over time. Tractographic MRI images were able to show subtle changes in the white matter tracts. Neurological exams of our cohort demonstrated that the dystonic component of this pediatric population was stronger than the spastic component, and that hypotonia was the major neurologic motor finding.

Advanced brain MRI studies have increased the capability to assess the microstructure integrity of white matter tracts and gray matter centers and to analyze their connections (connectivity studies). These studies reveal the alterations imprinted in the brain connections by several diseases beyond the macromorphological assessment allowed by conventional MRI. A better understanding of how diseases affect the brain can now potentially be correlated with clinical findings, provide a biomarker for future treatment trials, and influence rehabilitation strategies. Our MCT8 study group is now familiar with the possibilities and pitfalls that can occur with these new imaging methods. We are now ready and capable to start recruitment to analyze the connectivity of patients with MCT8 and to correlate this with their neurological exams.

Reference: Matheus, MG et al. J Child Neurol 2015 Oct;30(12):1664-8.