University of Technology Sydney and Sherman Foundation MCT8 Symposium 2015

12th-14th of January, 2015

The Ritz Carlton, Marina Del Rey, Los Angeles

4375 Admiralty Way Marina del Rey, CA 90292, United States, Tel: (310) 823-1700

Monday 12th January – MARINA VISTA

19:00 Welcome drinks

Day 1

Tuesday 13th January – MARINA VISTA

8:40 Brian Sherman and Dror Ben-Ami welcome

8:55 Samuel Refetoff welcome

New approaches, Chair – Theo Visser

9:00 Theo Visser - What is new for thyroid hormone transporters

9:15 Heike Huer- Analysis of neuromuscular functions in Mct8/Oatp1c1 dko mice

9:30 Ulrich Schweizer - Structure and function of MCT8

9:45 Discussion

10:30 Coffee break

Transport, Chair – Juan Bernal

10:45 Juan Bernal - Thyroid hormone (TH) transport routes in the fetal brain

11:00 Hideyuki Iwayama - Thyroid Hormone Transport Across the Blood Brain Barrier Is MCT8 Dependent: Therapeutic Implications

11:15 Theo Visser - In search of alternative substrates for MCT8

11:30 Discussion

12:15 Lunch

Treatment I, Chair – Ken Holden

14:00 Samuel Refetoff – Placenta passage of the thyroid hormone analogue DITPA to wild-type and Mct8 deficient mice

14:15 Heike Heuer - Therapeutic potential of the TH metabolite Triac (TA3) in MCT8 deficiency

14:30 Discussion

15:00 Coffee

Treatment II, Chair – Lior Appelbaum

15:15 Edward Visser - Thyroid hormone analog therapy in patients with the Allan-Herndon-Dudley Syndrome: The Triac Trial

15:30 Jose Moreno - TRIAC treatment of a 9-month-old child with Allan-Herndon-Dudley Syndrome: effects on Iodothyronine concentrations in serum and cerebro-spinal fluid

15:45 Ken Holden - Redefining the clinical pediatric phenotype of psychomotor disabilities with X-Linked MCT8 Disease: Implications for improved therapies

16:00 Discussion

16:45 Coffee

Parents Forum, Chair - Ondine Sherman

17:00 Videos

17:20 Parents observations & questions

18:00 End Day 1

19:00 Dinner – MARINA VISTA

Day 2

Wednesday 14th JANUARY – MARINA VISTA

Neural development, Chair – Clive Svendsen

8:30	Gad Vatine - Neuronal abnormalities in induced pluripotent stem cells (iPSCs) Derived neurons from AHDS patients
8:45	Lior Appelbaum - Neurological and behavioural deficiencies in a zebrafish model for Allan-Herndon Dudley syndrome
9:00	Gisele Matheus - Brain MRI microstructural abnormalities of white matter tracts in X-Linked MCT8 Deficiency: Implications for earlier diagnosis and treatment
9:15	Ana Guadaño-Ferraz - From neuroanatomy to mechanisms of disability and treatment
9:30	Discussion
10:15	Coffee

Future directions, Chair – Ulrich Schweitzer

10:30	Clive Svendsen – Studying the role of Monocarboxylate transporter 8 (Mct8) in the transport of thyroid hormones (THs) across the human blood brain barrier (BBB) using an induced pluripotent stem cell (iPSC)-based system
10:45	Lior Appelbaum - Elucidating the mechanism and treatment of AHDS using mct8 mutant zebrafish –future directions
11:00	Dror Ben-Ami – Discussion of MCT8 website
11:15	Discussion
12:00	Lunch

Summation and Next Steps, Chair - Samuel Refetoff

12:30	Discussion
14.00	End of Symposium

For those that are interested:

Thursday 15th JANUARY

Tour of the Cedars-Sinai Regenerative Medicine Institute – Clive Svendsen/Gad Vatine

10:00 We will meet at Cedars-Sinai, 8700 Beverly Blvd., Advanced Health Sciences Pavilion, 8th Floor, Los Angeles, CA 90048

* Transport arrangements from the hotel will be discussed at the conference the day before.

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What is new for thyroid hormone transporters

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The Netherlands

Multiple transporters have been identified which are capable of transporting thyroid hormone (TH). Among these, MCT8, MCT10 and OATP1C1 have been shown to be the most specific and active TH transporters. However, uptake of TH by different cell types appears to be mediated by a L-type amino acid transporter (LAT). Previous studies have demonstrated significant TH transport by the heterodimeric transporters LAT1 and LAT2, which consist of a specific light chain (SLC7A5, SLC7A8) and a common heavy chain (SLC3A2). More recently, the monomeric transporters LAT3-5 (SLC43A1-3) have been characterized, but their role in TH transport has not been determined. Therefore, we decided to investigate the transport of iodothyronines and iodotyrosines by LAT1-5.

COS1 cells were transiently transfected with FLAG-tagged constructs of LAT1-5, and cellular uptake of ^{125}I-labeled T4, T3, rT3, 3,3'-diiodothyronine (T2), and 3-iodotyrosine (MIT) was determined. In addition, the metabolism of iodothyronines was studied in cells co-transfected with each of these transporters and either the type 1 (D1) or type 3 (D3) deiodinase.

LAT1 facilitated uptake of all substrates, and LAT2 showed net uptake of T3, T2 and MIT. Expression of LAT3 or LAT4 did not affect transport of T4 and T3 but resulted in decreased cellular accumulation of T2 and MIT. LAT5 did not facilitate transport of any substrate. Co-transfection with LAT3 or LAT4 strongly diminished cellular accumulation of T2 and MIT by LAT1 and LAT2. These findings were confirmed by the metabolism studies.

LAT1 and LAT2 show distinct preferences for uptake of the different iodocompounds, whereas LAT3 and LAT4 specifically facilitate T2 and MIT efflux. Together, our findings suggest that different sets of transporters with specific influx or efflux capacities may cooperate to regulate cellular thyroid hormone levels.

Analysis of neuromuscular functions in Mct8/Oatp1c1 dko mice

Steffen Mayerl[1], Reinhard Bauer[2], Julia von Maltzahn[1], & Heike Heuer[1,3]

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[3]Leibniz Research Institute for Environmental Medicine (IUF); Düsseldorf

Patients with inactivating mutations in the thyroid hormone (TH) transporting MCT8 suffer from severe form of psychomotor retardation including muscle hypoplasia and spastic paraplegia. Likewise, Mct8/Oatp1c1 double ko (dko) mice, an animal model for human MCT8 deficiency, display severe locomotor deficits as well. These abnormalities may be partly explained by a disturbed neuronal differentiation and an impaired myelination in the CNS that are caused by the pronounced TH deficiency in the brain. However, ubiquitous deletion of Mct8 and/or Oatp1c1 may also have a direct impact on muscle physiology possibly contributing to the neuromuscular phenotype.

Electrical stimulation of the sciatic nerve highlighted normal compound motor action potentials and nerve conduction velocity in TH transporter deficient mice pointing to normal muscle innervation and function of the sciatic nerve. Immunohistochemical analysis of different skeletal muscles revealed an increase in fast and a reduction of slow muscle fibers in adult Mct8 ko and Mct8/Oatp1c1dko mice pointing to a hyperthyroid state of these tissues. Interestingly, analysis of floating EDL muscle fiber cultures demonstrated the presence of Mct8 and Oatp1c1 in Pax7 positive satellite cells. Moreover, in Mct8/Oatp1c1 dko mice the number of MyoD positive cells was significantly reduced after culture indicating that the differentiation of satellite cells is impaired. In agreement with these in vitro findings, analyses of the tibialis muscle after injury revealed a reduced muscle fiber feret together with increased numbers of Pax7 positive satellite cells only in TH-transporter deficient mice. Altogether, our data point to a hitherto unknown role of Mct8 and Oatp1c1 in muscle stem cell differentiation.

Structure and function of MCT8

Ulrich Schweizer, Doreen Braun, Dorothea Bayer

We aim to understand the structure of MCT8. To this end, we follow two lines of investigations:

We study biochemically the function of MCT8 mutants which are designed according to our MCT8 homology model. Here, we mainly focus on mutations affecting substrate recognition. In related work, we have been able to demonstrate massive activation of pathogenic MCT8 mutants by the use of chemical chaperones.

Along the other line of investigation, we are expressing human MCT8 in *E. coli* for recombinant production and for structure determination by X-ray crystallography. We have found conditions under which MCT8 can be expressed at reasonable yield. We identified a detergent able to extract MCT8 from the bacterial membrane, and purified the soluble protein by chromatography. Since the yield is still moderate, we are now optimizing several characteristics of the gene, truncate termini, and test fusion proteins. We are collaborating with Dr. Werner Kühlbrandt from the Max Planck Institute for Biophysics in Frankfurt who guides our efforts and in whose laboratory the preparative purification of MCT8 will be performed.

We will introduce a model of MCT8 in the outside-open conformation, which was established in a collaboration with Dr. Krause from FMP Berlin, and briefly present our understanding of T3 transport from a structural point of view.

At last year's meeting we learned of the MCT8 R388Q mutation in a patient presented by Dr. Roy Weiss. We have investigated position R388 by targeted mutations replacing it with glutamine (as in the patient), glutamate (isosteric to glutamine, but negatively charged), lysine (positively charged, but smaller than arginine), and methionine (similar size, but no charge). MDCK1 cells stably expressing each of these mutants were tested for T3 uptake in vitro. Michaelis constants remained between 3.9 and 6.4 µM, close to the wildtype value. Our experiments did not reveal an important role of R388 for T3 import. The patient's mild phenotype may be unrelated to his R388Q mutation in MCT8, or the mutation affects an unknown property of MCT8 unrelated to T3 transport.

Dorothea Bayer's work on recombinant expression and crystallization of MCT8 is financed by the Sherman family.

Thyroid hormone (TH) transport routes in the fetal brain

J. Bernal, D Lopez-Espindola, B. Morte, and A. Guadaño-Ferraz

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

In the 2013 MCT8 symposium we presented data on the pathology of MCT8-deficient brain, showing histological alterations compatible with impaired TH action during neural development. Mechanisms of TH transport and their interaction with deiodinases in the developing human brain are poorly understood. In rodents Mct8 is present in the BBB, the neural cell membranes, and the choroid plexus, and Oatp1c1 is present in the BBB, the astrocytes, the choroid plexus and the ventricular surface. D2 is present in glial cells and D3 in neurons. Understanding how all these factors interact is essential to understand the pathophysiological mechanisms in MCT8 deficiency. In this presentation we will show that in the human fetus MCT8 is expressed in brain vessels, and also in the ependimocytes and the radial glia. D3 immunoreactivity is also present in the radial glia. DIO2 mRNA is expressed in the developing cortex, probably in the radial glia, but this awaits confirmation. The data suggest additional transport routes whereby the radial glia regulates the amounts of T4 and T3 available to the developing cortex. Future directions will include the analysis of this process in more detail by correlating the expression of transporters and deiodinases in the developing human brain at different stages of maturation, and looking for its correlate in the mouse brain.

Thyroid Hormone Transport Across the Blood Brain Barrier Is MCT8 Dependent

Hideyuki Iwayama[1], Xiao-Hui Liao, BS[1], Alfonso Massimiliano Ferrara[1], Lyndsey Braun[2], Brian Kaspar[2], Roy E Weiss[1], Alexandra M Dumitrescu[1] and Samuel Refetoff[1],

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Background: MCT8 is a specific T_3 and T_4 membrane transporter. MCT8 gene mutations produce thyroid hormone (TH) deficiency in brain, leading to a severe neuropsychiatric disorder that cannot be corrected with physiological doses of T_3 or T_4.

Aim: We examined the efficacy of the human MCT8 cDNA transferred by an AAV9 viral vector (AAV9-hMCT8) to increase brain T_3 uptake and action on TH controlled genes (Hr, D3 and Cbr2) of Mct8 knockout (Mct8KO) mice.

Method: AAV9-hMCT8 or Empty Vector (EV) was injected into 1 day old (P1) mice intravenously (IV, $2x10^{11}$ particles/mouse) or intracerebroventricularly (ICV, $3x10^{10}$). T_3 (5µg/100g body weight·day) was injected at P25-28 and tissues were obtained at P28. Result: Human MCT8 mRNA content in brain was higher in ICV injected mice compared to IV injected mice. However the opposite profile was observed in liver where IV injected mice had a higher concentration of hMCT8 mRNA than ICV injected mice. Mice injected with AAV9-hMCT8 IV had higher brain T_3 content than those injected IV with EV (23.6±2.1 vs 12.2±1.6 pg/mg·protein, $p<0.01$). This was not the case in ICV injected mice despite higher content in brain of hMCT8 mRNA (measured by qPCR) and protein (detected by Western blotting). IV but not ICV injected AAV9-hMCT8 significantly increased the TH-regulated genes in brain: Hr 2-fold ($p<0.001$), D3 5-fold ($p<0.001$) and Cbr2 2-fold ($p<0.01$). T_3, T_4 in serum before (P25) and after T_3 injection (P28), and T_3 in liver were not significantly different in AAV9-hMCT8 and EV injected mice. The activity of AAV9-hMCT8 was confirmed in vitro by transient transfection in human fibroblast and JEG3 cells.

Conclusion: These results indicate that intravascular injection of MCT8 is important in the delivery of T_3 across the blood brain barrier (BBB) in Mct8 deficient mice. This suggests that localization of Mct8 in BBB cells achieved by IV injection is not accomplished by ICV injection. Furthermore, an increase of MCT8 expression in brain is insufficient to correct the defective gene expression in brain of Mct8KO mice without the provision of T_3. Therefore, strategies that target the endothelial cells with MCT8 as delivered by intravascular administration of AAV9 are important for translational development.

In search of alternative substrates for MCT8

Chantal Zevenbergen, Elaine Lima de Souza, Edward Visser, Barbara Kloeckener*, Theo Visser

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The Allan-Herndon-Dudley syndrome (AHDS) is caused by mutations in the MCT8 thyroid hormone (TH) transporter. The pathogenic mechanism involves defects in TH transport in the brain during critical stages of brain development. However, it has not been excluded that MCT8 also transports biologically substrates other than TH. This may also seem likely in view of the characteristics of the highly homologous MCT10 transporter, which also transports in particular aromatic amino acids in addition to iodothyronines. Therefore, we re-investigated the possible transport of amino acids by MCT8.

In contrast to the marked efflux of the aromatic amino acids Trp, Phe and Tyr facilitated by MCT10, no significant transport was observed of these and other amino acids by MCT8. However, both MCT8 and MCT10 effectively facilitate cellular efflux of 3-iodotyrosine (MIT) and 3,5-diiodotyrosine (DIT). MIT and DIT are intermediates in the biosynthesis of TH. Normally, excess MIT and DIT are deiodinated within the thyroid gland by the enzyme iodotyrosine dehalogenase (DEHAL) to allow re-utilization of the iodide for TH synthesis. Some MIT and DIT may escape from the thyroid gland to be deiodinated by DEHAL expressed in other tissues such as the liver and kidney.

Interestingly, MIT also appears to be a potent inhibitor of tyrosine hydroxylase, an important enzyme for the biosynthesis of dopamine. MIT is taken up by cells through the L-type amino acid transporters LAT1 and LAT2, which are highly expressed in the blood-brain barrier (BBB) and central neurons. If the efflux from neurons is impaired by MCT8 mutations, the consequent increase in MIT accumulation may result in a significant inhibition of dopamine synthesis. Therefore, further studies should be done to determine the effects of MCT8 mutations on dopamine homeostasis in the brain.

Our studies clearly indicate that MCT8 also transports other substrates than iodothyronines. We now use a metabolomic approach in combination with the *Xenopus* oocyte expression system to identify potentially important additional MCT8 substrates.

Placenta passage of the thyroid hormone analogue DITPA to wild-type and Mct8 deficient mice

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MCT8 deficiency causes severe X-linked intellectual and neuropsychological impairment associated with abnormal thyroid function tests (TFTs) producing thyroid hormone (TH) deprivation in brain and excess in peripheral tissues. The TH analogue diiodothyropropionic acid (DITPA) corrected the TFTs abnormalities and hypermetabolism of MCT8 deficient children but did not improve the neurological phenotype. The latter result was attributed to the late initiation of treatment. Therefore, we gave DITPA to pregnant mice carrying Mct8 deficient embryos, in order to determine whether DITPA, when given prenatally, crosses the placenta, and affects the serum TFTs and cerebral cortex of embryos.

After depletion of the endogenous TH, Mct8 heterozygous pregnant dams, carrying both wild-type (*Wt*) and Mct8 deficient (*Mct8KO*) male embryos were given DITPA. Effects were compared to those treated with L-T_4. With DITPA treatment, serum DITPA concentration was not different in the two genotypes, which produced equal effect on serum TSH levels in both groups of pups. In contrast, with L-T_4 treatment, TSH did not normalize in *Mct8KO* pups while it did in the *Wt* littermates and dams despite higher concentration of serum T_4. Finally, both treatments similarly modulated the expression of the TH-dependent genes *Shh*, *Klf9* and *Aldh1a3* in brain. Thus, the ability of DITPA to cross the placenta, its thyromimetic action on the expression of TH dependent genes in brain and its better accessibility to the pituitary than L-T_4, as assessed by serum TSH, make DITPA a candidate for the prenatal treatment of MCT8 deficiency.

Therapeutic potential of the TH metabolite Triac (TA3) in MCT8 deficiency

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[2]Leibniz Research Institute for Environmental Medicine (IUF); Düsseldorf (Germany),

The monocarboxylate transporter 8 (MCT8) plays a critical role in mediating the uptake of thyroid hormones (TH) into the brain. In patients, inactivating mutations in the MCT8 genes are associated with a severe form of psychomotor retardation and abnormal serum TH levels (Allan-Herndon-Dudley Syndrome (AHDS)). The neurological symptoms are most likely caused by an insufficient transport of TH into the brain that in turn compromises neural differentiation. Here, we investigated the therapeutic potential of the biologically active T3 metabolite triiodothyroacetic acid (TA3) as TA3 can exert thyromimetic actions by binding to TH receptors but is not a substrate for MCT8.

For that purpose, we treated Mct8/Oatp1c1 dko mice, a recently developed mouse model for human MCT8 deficiency, after birth with different doses of TA3 and studied the effects of this treatment on cerebellar development, myelination and differentiation of Parvalbumin expressing cortical interneurons. For comparison, we included also animals in our study that we treated with different doses of the TH analog Ditpa. We indeed could show that TA3 treatment resulted in a normal cerebellar Purkinje cell maturation and myelination and also restored normal cortical parvalbumin expression whereas Ditpa treatment was less effective. Ongoing analysis will reveal whether this treatment also leads to an improvement of locomotor deficiencies as well as learning and memory deficits.

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

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Introduction: Mutations in MCT8 result in the Allan-Herndon-Dudley Syndrome (AHDS), characterized by severe psychomotor retardation and abnormal thyroid function tests (TFTs) (low-normal T4, high T3, high-normal TSH) associated with low body weight (BW) in males. At present, no effective treatment is available. Recent findings suggest that the T3 analog Triac is a promising candidate to 1) restore the abnormal TFTs and reduce the harmful effects of the high T3 levels and 2) provide adequate thyromimetic effects in TH deprived tissues.

Methods: We conduct a world-wide trial in which over 40 AHDS patients receive Triac for 1 year. The primary aim is to normalize abnormal TFTs and alleviate thyrotoxicosis in peripheral tissues. This will be assessed with clinical characteristics (BW, heart rate), bone mineral density (BMD; by DEXA) and biochemical assays, including TFTs and serum markers reflecting thyroid state in different tissues. The neurocognitive phenotype will be assessed by neuropsychological tests.

Results: Currently, Triac treatment has commenced in 3 patients (mean age 18 yr). Baseline analysis showed expected TFTs and peripheral TH effector markers (low cholesterol, high SHBG), low body weight , low BMD and tachycardia . Psychomotor development was severely impaired (developmental age of motor scales: 1-2 mo, cognitive scales 3-4 mo). Results of the first control visits are pending, but will be available in January.

Discussion: These baseline findings illustrate the severity of the AHDS phenotype and the urgent need to develop effective treatment strategies. Our study is the first therapeutic trial for AHDS patients. Additionally, we will extensively describe the AHDS phenotype to further understand the mechanisms of disease and optimize patient care.

TRIAC treatment of a 9-month-old child with Allan-Herndon-Dudley Syndrome: effects on iodothyronine concentrations in serum and cerebro-spinal fluid.

Ainhoa Iglesias [1], Olga Esther Alonso [2], Ana Lucía Gómez-Gila [3], Juan A. Campos-Cerveró [4], María Palomares [5], Eduvigis Contreras [6], Beatriz Morte [7], M. Jesús Obregón [7], Juan Bernal [7], José C. Moreno [1].

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Allan-Herndon-Dudley Syndrome (AHDS) is a devastating disease with severe neurological and metabolic consequences caused by defects in the transporter of thyroid hormones MCT8. A typical systemic hyperthyroidism is heralded by elevated serum T3 levels (with mildly increased TSH and decreased T4), probably involved in the cachexia and reduced life span of patients. In contrast, the brain is hypothyroid, causing incapacitating psychomotor retardation associated with spastic paraparesia, epilepsy and hypomyelination. In the past, therapeutic attempts in AHDS aimed to inhibit thyroid hormone production and hepatic T4 activation (PTU) beside tailored levo-thyroxine substitution, or to correct thyroid hormone abnormalities with the T3-analogue DITPA. Both approaches achieved correction of serum thyroid hormone derangements, without neurological improvement. This discouraging outcome can be consistent with a state of persistent hypothyroidism in the brain despite efficient correction of serum thyroid parameters, or due to late start of treatment. Recently, administration of TETRAC to *Mct8* KO mice was show to correct thyroid hormone abnormalities in this model for human AHDS.

Objetive To determine the effect of TRIAC (a rapidly-generated metabolite of TETRAC) in a child with early-diagnosed AHDS on his thyroid hormone metabolism and neurological development.

Patient & Methods An 8-month old male with severe axial hypotonia, psychomotor retardation and apparent hypothyroidism (TSH 7.7 µU/mL, FT4 0.48 ng/dL) but elevated FT3 (8.86 pg/mL) was clinically suspicious of AHDS. PCR-direct sequence of *MCT8*, thyroid-targeted CGH array (Thyroarray©). Treatment of patient´s cultured fibroblasts with 1-10 nM T3, TRIAC, TETRAC or DITPA and quantification of T3-dependent gene expression (*RCNA2, HR, BTEB1*). Compassionate treatment with increasing doses of TRIAC (10-40 µg/kg/day) for 1 year. Follow-up with thyroid hormone profiles, brain MRIs and psychometric tests (Brunet-Lezine) every 3 months. Measurement of T4, T3 and TRIAC in cerebrospinal fluid (CSF) before and after TRIAC treatment (40 µg/kg/day) using radioimmunoassays.

Results An undescribed *de novo* deletion in *MCT8* was identified from mid-exon 3 downstream, spanning 25.04 Kb and reaching the 3´-intergenic region. TRIAC was taken-up by patient´s fibroblasts *in vitro*, showing equal potency to T3 on gene expression. TETRAC and DITPA showed no significant effects on gene expression. In the boy, treatment with TRIAC corrected FT3 and TSH to normal levels (4.1 pg/mL y 1.2 mU/L) but further reduced FT4 (0.3 ng/dL), requiring additional L-T4 substitution at dose 1.5 µg/kg/day. No brain myelination was detectable at 5-mo of age. At trial start (9-mo age) he showed the "typical 3-mo pattern" on MRI, and at 16-mo he reached "typical 8-mo levels". Initial improvement of motor (from 18 to 40%) and social-language (sustained at 70-72%) areas after 3 months treatment was followed by slowing-down in the achievement of new developmental milestones, as reflected by decreasing percentages compared to normal children (30 and 55% at 12 months treatment). TRIAC was detectable in patient´s CSF (5 ng/dl, N: 1.4-4.7) before trial´s start and increased to 8.4 ng/dl after receiving the highest TRIAC dose. Compared to 12 age-matched controls, T4 in CSF was low (50.2 ng/dl; N:65-166) and further decreased under TRIAC (19.2 ng/dl), reflecting similar trend to that of serum FT4. Under estimation of 50% cross-reactivity of TRIAC on the T3 determination, T3 in CSF was also low, both before (0.7 ng/dL; N: 1.9-2.85) and after TRIAC treatment (1,06 ng/dl ; N: 1.9-2,85).

Conclusions This is the first report of an AHDS patient treated with TRIAC. TRIAC normalizes serum FT3 and TSH, but decreased FT4 in our patient, requiring L-T4 substitution. TRIAC may have helped brain myelination timing. After initial improvement of milestone acquisition, developmental deceleration occurred. Concentrations of iodothyronines in CSF are consistently low in our AHDS patient irrespective of the treatment. Shortage of T4 and T3 in CSF indicate that Blood-CSF barrier transport of iodothyronines is restricted in AHDS, consistent with the presence of MCT8 in the choroid plexus. The pathogenic significance of decreased T4 and T3 in CSF is currently unknown, and should be investigated. If it is relevant, recent concepts that CSF-flow can access the brain parenchyma may open novel and exciting therapeutic avenues involving the intrathecal administration of drugs in AHDS.

Redefining the clinical pediatric phenotype of psychomotor disabilities with XLinked MCT8 Disease: Implications for improved therapies.

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INTRODUCTION: Monocarboxylate Transporter 8 (MCT8) is a thyroid hormone transporter expressed in various human organs, including brain tissue. Loss-of-function mutations in the MCT8 gene, located on the X chromosome, manifest as mild-tomoderate intellectual disability and moderate-to-severe psychomotor impairment in young boys with pleasant dispositions and elevated serum T3 levels. While affected boys present with marked axial hypotonia and quadriparesis, they have surprisingly little spasticity early in their disease course. Rather, their hypotonia tends to be accompanied by subtle involuntary movements, primarily dystonia. The lack of spasticity represents a challenge for their rehabilitation and explains why spasticity-directed therapies have produced suboptimal responses. Our goal was to better define the spectrum of motor disabilities in MCT8 deficiency in order to improve the rehabilitation results.

METHODS: To accomplish our goal, we evaluated a multi-national cohort of pediatric patients with MCT8 mutations. Direct clinical evaluations, along with clinical video recordings (n=7), were reviewed by our pediatric neurogeneticist and pediatric movement disorder specialist.

RESULTS: Pediatric evaluations and videos reviewed on our MCT8 deficiency patients revealed a common pattern of hypotonic quadriparesis with superimposed dystonia.

CONCLUSION/DISCUSSION: This multi-national pediatric pilot study has better characterized the motor impairments associated with MCT8 deficiency. It is anticipated that this information will allow physicians and therapists to focus their treatment efforts on the hyperkinetic elements of MCT8 deficiency, rather than suspected spasticity.

Neuronal abnormalities in induced pluripotent stem cells (iPSCs) Derived neurons from AHDS patients

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The discrepancies between AHDS patients and the developed animal models emphasize the need for a human model to study this devastating neurological disorder. However, human research has limitations, including that, until recently access to human brain cells was limited mainly to post-mortem tissues and surgical excisions. The emergence of methods to not only reprogram human adult cells back into a pluripotent state but also to subsequently differentiate these pluripotent cells into various cell types has initiated a new era in research for neurology and heritable disease modeling.

We have generated iPSCs from AHDS patients and associated controls and differentiated them into neural cells, including neurons, astrocytes and oligodendrocytes. Intriguingly, although neural cells from AHDS patients display impaired uptake and efflux of THs, TH-dependent gene expression was not impaired in AHDS-neural cells. Moreover, the differentiation potential of control- and AHDS-iPSCs into Gfap-expressing astrocytes, βIII tubulin-expressing neurons and O4-expressing oligodendrocytes *in vivo* were similar. Interestingly, differences in neuronal maturation were observed when cells were cultured for longer time periods as displayed by expression of the mature neuronal marker MAP2ab. Furthermore, MAP2ab cells displayed impaired neurite outgrowth as pronounced by decreased number of branches and length of neuronal processes. Intriguingly, this Mct8-dependent neuronal maturation was independent of TH administration during differentiation, suggesting that in addition to THs Mct8 may transport additional yet unknown molecules.

Neurological and behavioral deficiencies in a zebrafish model for Allan Herndon Dudley syndrome

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A major challenge in the neuroscience research is to uncover the mechanism of brain disorders and provide treatment, which is capable of preventing brain damage. AllanHerndon-Dudley syndrome (AHDS) is a severe psychomotor retardation characterized by intellectual disabilities, neurological impairment and abnormal thyroid hormone (TH) levels. Mutations in the TH transporter MCT8 are associated with AHDS. Mice that lack the MCT8 protein exhibited impaired TH levels, as is the case in human patients; however, they lack neurological defects.Wwe generated an mct8 mutant (mct8-/-) zebrafish, which exhibited neurological and behavioral deficiencies and mimics pathological conditions of AHDS patients. The zebrafish is a simple transparent vertebrate and its nervous system is conserved with mammals. Time-lapse live imaging of single axons and synapses, and video-tracking of behavior revealed deficiencies in neural circuit assembly, which are associated with disturbed sleep and altered locomotor activity. These findings suggest a mechanism by which MCT8 regulates neural circuit assembly, ultimately mediating sensory and motor control of behavioral performance.

Brain MRI microstructural abnormalities of white matter tracts in X-Linked MCT8 Deficiency: Implications for earlier diagnosis and treatment.

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INTRODUCTION: X-linked Monocarboxylate Transporter 8 (MCT8) loss-of-function gene mutations result in thyroid hormone cell transporter deficiency. Affected children have moderate-severe neurodevelopmental disabilities. Previously published brain MRI data suggests these children have abnormal myelination without significant structural abnormalities. We hypothesized that MCT8 deficiency affects the complex cortical networks, specifically regional subcortical connections, which can be assessed with MRI.

METHODS: We reviewed thirteen brain MRIs from a multinational cohort of six MCT8 patients less than 8 years old. Longitudinal conventional MRI data and diffusion tensor imaging (DTI) were evaluated for regional micro-structural abnormalities.

RESULTS: MRIs revealed subtle delayed myelination by 8 months of age which increased over time. By 5 years, supratentorial white matter tracts showed abnormalities in peritrigonal regions and subcortical U-fibers. DTI demonstrated foci of reduced fractional anisotropy in the supratentorial white matter suggesting subtle microstructural abnormalities.

CONCLUSION/DISCUSSION: Brain white matter tract abnormalities on MRI in MCT8 patients reflect disruptions in cortical-subcortical connectivity which could help guide future therapies as an important tool to assess motor disabilities. DTI or Kurtosis imaging can have a robust statistical power if correctly applied. Our future goal is to prospectively assess MCT8 patients on treatment trials with age-matching controls at one international center, monitor for intrinsic variables, quantify white matter tract abnormalities involved by MCT8, and monitor how this responds to therapies over two-years-time.

From neuroanatomy to mechanisms of disability and treatment

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Mechanisms underlying motor dysfunctions in Allan Herndon Dudley Syndrome remain largely unknown. Neuroanatomical studies performed in postmortem human brain tissue obtained from control versus MCT8-deficient patients provide a suitable strategy to further understand underlying alterations of motor system circuitries.

Our recent findings indicate that normal MCT8 expression is tightly regulated, both spatially and temporally, during development of the normal motor system. Particularly, MCT8 expression increases in white matter tracts during myelination. We have also found that the motor system of MCT8 deficient brains is affected of combined gray and white matter abnormalities that can be found either in prenatal and postnatal stages, and that reveal the impairment of several key developmental processes such as neuronal differentiation, myelination and synaptogenesis.

Our observations in motor cortices, cerebellum and main motor descending tracts confirm the importance of the modulation of thyroid hormones availability during motor system ontogeny.

These results try to outline the intervening driver molecular processes that lead this rare disease in order to support the refining of current therapeutic approaches and to pursue therapeutic targets in the future. Our results also point to the relevance of persevering on translational research towards plausible prenatal diagnosis and treatment for these patients.

Studying the role of Monocarboxylate transporter 8 (Mct8) in the transport of thyroid hormones (THs) across the human blood brain barrier (BBB) using an induced pluripotent stem cell (iPSC)-based system

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The BBB is a neuro-vascular unit that shields the brain from toxins that flow in the blood. Tight-junctions that are formed between brain microvascular endothelial cells (BMECs) limit the intracellular passage of solutes from the blood flow into the CNS. In order to reach the CNS, molecules such as THs need to be actively transported from the blood flow across BMECs into the 'brain side'.

Evidence from the mouse models suggest that MCT8 plays an important role in the transport of THs across the BBB, however, major discrepancies between the mouse and animal models question the relevance of findings from the mice models.

Using iPSCs from AHDS patients we generated an *in vitro* model to investigate the role of Mct8 in the transport of THs across the human BBB. Gene expression and protein analyses reveal that Mct8 is expressed in human BMECs. Functional analyses of iPS-BMECs (iBMECs) suggest that AHDS-iPSCs are able to form proper cellular and physical BBB properties and develop normally. Notably, functional transport assays reveal that the TH transport across AHDS-iBMECs is severely impaired, suggesting that the main obstacle for TH to reach the brain lays in the BBB. Research is underway to investigate the ability of TH-analogs to cross the human BBB in the absence of a functional Mct8.

Elucidating the mechanism and treatment of AHDS using mct8 mutant zebrafish – future directions

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Thyroid hormones (THs) are key regulators of development, metabolism, and growth. Hypothyroidism in the brain is a key symptom of the psychomotor retardation Allan-HerndonDudley syndrome (AHDS), which is linked to mutations in the thyroid hormone monocarboxylate transporter 8 (mct8). AHDS is characterized by severe intellectual deficiency, neuromuscular impairment, and low TH concentration in the brain. We intend to utilize zebrafish to study the neurological mechanism of AHDS, and to evaluate potential treatments. The zebrafish model combines a transparent brain with the conserved structure of neuronal circuits, and is suitable for high-throughput genetic and chemical screens. To achieve these goals, we developed mct8 mutant (mct8-/-) zebrafish as a model for AHDS. In current and future work, we will characterize the transcriptome of specific neural circuits in mct8-/- larvae and determine which molecular pathways are altered in neurons and glial cells. In addition, the role of MCT8 in regulating cellular metabolism will be determined by imaging mitochondria in the neurons and glial cells of live zebrafish models. Furthermore, we will comprise live-imaging experiments designed to identify potential defects in neurogenesis, myelination, and structural synaptogenesis in mct8-/- larvae during embryonic development. In addition, since the mct8-/- larvae provides a high throughput system, these experiments will provide a platform for a pharmacological screen that is designed to lead to future treatment of AHDS. Taken together, this research will combine tools that are unique to zebrafish in order to uncover the mechanisms and treatment of AHDS.