MCT8 2017 Symposium
Hotel Ritz Carlton, Marina Del Ray, California

Day 1 – Wednesday 4th January, Venue: BALLROOM TERRACE

19:00 **WELCOME DRINKS**

Day 2 – Thursday 5th January, Venue: MARINA VISTA

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

Note: All presentations are 15 minutes

8:40 - 10:15 Analogues and Transporters, Chair Ulrich Schweizer

8:40 Description of AHDS and thyroid hormone analog therapy in patients with the Allan-Herndon-Dudley Syndrome (AHDS): the Triac Trial – Edward Visser (20 min)

9:00 Importance of OATP1C1 for thyroid hormone transport in human brain – Theo Visser

9:15 Testing treatment strategies for AHDS in Mct8/Oatp1c1 double ko mice, an animal model for MCT8 deficiency – Heike Heuer

9:30 Discussion

10:15 COFFEE BREAK

10:25 - 12:10 MCT8 Form and Function, Chair Clive Svendsen

10:25 The basal ganglia: a new possible therapeutic target in MCT8 deficiency – Ana Guadaño-Ferraz

10:40 The effect of T3 on neuronal differentiation and maturation – Clive Svendsen

10:55 MCT8 structure and function: update – Ulrich Schweizer

11:10 Discussion

12:00 **Lunch, Venue: THE ROSE GARDEN**

13:15 - 14:45 Future Treatments, Chair Lior Appelbaum

13:15 The potential of oligodendrocyte progenitor cells (OPCs) transplantation to treat the delayed myelination in MCT8-deficient patients – Gad Vatine

13:30 Preliminary data from intranasal delivery of thyroid hormone to wild-type and Mct8 deficient mice – María del Carmen Grijota-Martínez

13:45 Treatment of AHDS with chemical or pharmacological chaperones – Doreen Braun

14:00 Discussion

14:45 - 15:30 Gene Therapy, Chair Theo Visser

14:45 Adeno associated virus 9-based gene therapy delivers a functional MCT8 which improves thyroid hormone availability to brain of Mct8 deficient mice – Samuel Refetoff

15:00 Genetic and pharmacological treatments in *mct8*-/- zebrafish – Lior Appelbaum

15:15 Next step in gene therapy – Brian Kaspar

15:30 Discussion

16:15 **COFFEE BREAK**

16:30 - 18:00 Ask the Doctor

Panel – Edward Visser, Stephan Groeneweg, Samuel Refetoff, Amnon Zung, Davide Tonduti, Ken Holden, Rene De Coo

16:30 Phenotypic characterization of the Allan-Herndon-Dudley Syndrome: establishment of a patient registry – Edward Visser

16:45 Parent questions

18:00 End of Day 2 Sessions

19:30 **CONFERENCE DINNER, Venue: BALLROOM TERRACE**

Day 3 – Friday 6th January, Venue: MARINA VISTA

8:40 - 10:10 Neural Dynamics of MCT8 Mutation, Chair Heike Heuer

8:40 Comparative studies of *mct8*-/-, *oatp1c1*-/- and thyroid gland-ablated zebrafish - Lior Appelbaum

8:55 Preliminary proteomic profiling of the effect of MCT8 deficiency/knockout has on cellular function and maturation in patient-derived induced pluripotent stem cells – Jerran Santos

9:10 Update of the Project: Role of White Matter Tracts and Gray Matter Centres in X-Linked MCT8 Transporter Deficiency, Assessed by Dedicated Magnetic Resonance Imaging (MRI) and Clinical Correlation – Ken Holden and Gisel Matheus

9:25 Discussion

10:10 - 11:10 Summation Discussion - Samuel Refetoff

11:10 Formal end with researchers

11:10 **COFFEE BREAK**

11:30 - 13:00 Patient Empowerment

11:30 Sherman Foundation - Now and into the future - Dror Ben Ami

11:50 Patient Organizations - What are they and why do we need one? A discussion about fundraising, research, advocacy and creating supports for families within patient organizations. And how do we get started? - Veronica Popa

12:40 Supports - A look at the MCT8/AHDS support network of families past, present and future - Marisa Gasiorowski

13:00 - 14:00 Therapies That Work

An opportunity for parents to share what therapies they have had success with. Also a time to share any noteworthy equipment, supplements or other approaches to helping our boys health, wellness and development - Brenda Lenahan

14:00 End of conference

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Abstracts (by 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 Collaborators

(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 (TH) transporter MCT8 result in AHDS, which is characterized by severe intellectual and motor disability and high serum T3 levels inducing thyrotoxicity in peripheral organs. At present, no effective treatment is available, although preclinical studies suggested that the T3 analog Triac is a promising candidate to 1) normalize serum T3 levels and thus alleviate the thyrotoxicosis and 2) restore TH signaling in the brain.

Methods
We conduct a world-wide prospective interventional trial in which about 45 AHDS patients are anticipated to receive 1 year Triac treatment. The primary end-point is the reduction of serum T3 levels, and secondary end-points include normalization of heart rate (HR), improvement of body weight (BW) and serum parameters that reflect TH action in peripheral tissues. The neuro(psycho)logical phenotype is assessed before and after 1 year of Triac treatment.

Results
Currently, 34 patients (age: 1-66 yr) have been enrolled of which 12 completed 1 year of follow-up. Triac treatment effectively suppressed serum TSH levels (median [IQR]: 2.3 [1.6-3.9] to 0.9 [0.2-1.9] mU/L; $p<0.001$), resulting in a strong reduction of T3 levels (5.0 [3.9-6.5] to 1.7 [1.4-2.2] nmol/L; $p<0.001$), when comparing baseline and end-study levels in these 12 patients. Importantly, BMI, serum cholesterol, creatinine and CK levels significantly increase. No significant changes are observed in basal HR and serum SHBG levels. Some neuropsychological markers show slight improvement in individual participants.

Discussion
Triac treatment effectively results in normalization of serum T3 levels. This interim analysis suggests that Triac treatment may have beneficial effects on the peripheral phenotype of AHDS, which will be further substantiated upon completion of the 1-year follow-up period by the other participants.

Importance of OATP1C1 for thyroid hormone transport in human brain

Theo J Visser

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The organic anion transporting polypeptides (OATPs) constitute a family of homologous proteins that in humans consists of 11 members. In particular the 4 members of the human OATP1 subfamily have been shown to be capable of transporting thyroid hormone (TH) derivatives. Of these, OATP1A2 shows a ubiquitous expression, OATP1B1 and 1B3 are exclusively expressed in liver, and OATP1C1 expression is restricted to the CNS, retina and testis. In human brain, OATP1C1 appears to be localized predominantly in astrocytes and the choroid plexus, while in mouse brain Oatp1c1 is also importantly expressed in the endothelial cells of the blood-brain barrier. The SLCO1 genes coding for the OATP1 subfamily transporters are located in gene clusters (in humans on chromosome 12), suggesting that they were generated by gene duplication events. As SLCO1C1 is the only strongly conserved gene in this subfamily, it probably represents the parental member of this gene clusters. In all species, OATP1C1 shows high substrate specificity towards T4.

Optimal brain development requires the precise spatio-temporal regulation of the supply of the active hormone T3 to its nuclear receptors in target cells, in particular neurons and oligodendrocytes. This involves the transport of T3 and its precursor T4 across the endothelial cells of the blood brain barrier (BBB). In humans, both T4 and T3 are transported across the BBB predominantly by monocarboxylate transport 8 (MCT8), but in mice additional T4 transport is mediated by Oatp1c1. In both mouse and human brain, OATP1C1 is strongly expressed in astrocytes, which also express the type 2 iodothyronine deiodinase (DIO2) that converts T4 to T3. This local T3 appears more important than systemic T3 for TH action in target brain cells, and MCT8 is also involved in the uptake of T3 by neurons. Neurons also express DIO3 which catalyzes the degradation of T3 (and T4) and thus limits the action of TH in case of excess.

This presentation concerns molecular and clinical aspects of TH transport by human OATP1C1.

Testing treatment strategies for AHDS in Mct8/Oatp1c1 double ko mice, an animal model for MCT8 deficiency

Sivaraj Mohana Sundaram, Eva Salveridou, Jiesi Chen & Heike Heuer

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Mct8/Oatp1c1 double knock-out (dko) mice represent a mouse model for human MCT8 deficiency as they exhibit pronounced neural differentiation deficits, locomotor disabilities and peripherally symptoms of thyrotoxicosis. The neurological symptoms of the mice (and most likely of the patients) are due to an impaired transport of TH into the CNS and, consequently, due to a disturbed 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 previously reported on the beneficial effects of the TH analog Triac that was capable of normalizing various brain parameters (development of cerebellar Purkinje cells, myelination, differentiation of GABAergic neurons) and restoring locomotor function in Mct8/Oatp1c1 dko mice when applied to during the first three postnatal weeks (P0-P21). We further expanded these studies by initiating Triac treatment at later postnatal time points. While application of Triac between P12 and P24 had still beneficial effects on neuronal differentiation and myelination, a 12 days-treatment period initiated at P22 was only very little effective. We therefore conclude that Triac treatment is most beneficial when initiated as early as possible.

In addition to Triac, we recently started to test additional TH analogs (S-compound and Dibromo). In primary neuronal cell cultures, both compounds induced Purkinje cell dendritogenesis at similar concentrations as Triac. Currently, we are testing the efficacy of both substances *in vivo* and aim to present the first results of these experiments.

The basal ganglia: a new possible therapeutic target in MCT8 deficiency

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The Allan-Herndon-Dudley syndrome is a rare disease caused by mutations in the gene *SLC16A2*, which codifies for the specific thyroid hormone cell-membrane transporter monocarboxylate transporter 8 (MCT8).

The altered expression of MCT8 leads to a singular scenario of prenatal brain hypothyroidism that is thought to underlie a dramatic neurological postnatal phenotype of developmental retardation, hypotonia and epilepsy. These clinical features start in the early infancy. Spastic tetraplegia, intellectual disability and paroxysmal dyskinesias appear posteriorly. The mechanisms underlying these clinical manifestations are poorly understood and, subsequently, a rational basis for a targeted, symptomatic therapy is still lacking.

The severe impairment in motor control of these patients, as well as the learning problems, points to basal ganglia dysfunction. Thus, we have analyzed human paraffin-embedded brain sections taken from necropsies of MCT8-deficient and control patients and we have immunohistochemically stained them to detect the MCT8 transporter, structural cell and synaptic proteins and a series of striatal markers.

Our results provide neuroanatomical evidence for severe structural and neurochemical deficiencies that might well explain the poor regulatory control of excitatory-inhibitory balance in basal ganglia microcircuitry and the extrapyramidal signs in patients affected by MCT8 mutations. This study, and others with more cases, could be the conceptual basis for further studies with '*in vivo*' non-invasive neuroimaging techniques to facilitate a rational improvement of the current symptomatic drug therapies for this disease.

The effect of T3 on neuronal differentiation and maturation

Gad Vatine and Clive Svendsen

Cedars-Sinai Medical Centre, Regenerative Medicine Institute

In order to study the neuronal phenotype that is associated with AHDS, iPSCs from MCT8-deficient patients and healthy controls were differentiated into neural progenitors termed EZ-spheres. These progenitors were then differentiated into cultures containing neural progenitors, astrocytes and neurons. Notably, MCT8-deficient neural cells exhibited reduced TH uptake and efflux. Interestingly, MCT8-deficient cells displayed normal differentiation potential and T3-induced gene expression, suggesting that when differentiated in vitro a sufficient amount of T3 is able to penetrate MCT8-deficient neural cells. In order to test the effect of MCT8 and T3 on neuronal maturation, MCT8-deficient and control neural progenitors where differentiated in the presence or absence of T3 for 40 days, on a multielectrode array (MEA) plate. Extracellular recording of neuronal activity revealed that both MCT8-deficient and control cells showed increased spontaneous activity in response to T3. These results suggest that T3 is important for neuronal maturation. RNA-seq analysis on T3-treated control cells reveals a set of genes that are T3-regulated in human neural cells and are possibly involved in the neuronal maturation process.

MCT8 structure and function: update

Ulrich Schweizer and Doreen Braun

Institut für Biochemie und Molekularbiologie, Rheinische Friedrich-Wilhelms Universität Bonn

Monocarboxylate transporter 8 (MCT8) mediates thyroid hormone (TH) transport across the plasma membrane in many cell types. Among the first human transporter structures, sugar transporters of the GLUT family are prominent – luckily, GLUTs are sharing many features with MCTs and thus homology modelling was improved over the first model that was based on a bacterial glycerol phosphate transporter. In addition, the new homology models of MCT8 are in three conformations allowing to extrapolate the dynamics of transport in the model. Several features of the model have been probed by biochemical studies and key conclusions from the earlier model, in particular the His-Arg clamps and the central role of His415 have been supported.

Homology models are a “fast” tool to generate ideas about substrate binding or transport mechanism, but they are not experimental structures. We therefore expressed recombinant MCTs in insect cells, because their membrane lipids are more similar to human membrane than bacterial membranes. We found conditions that allowed expression and enrichment from plasma membrane of MCT10 and MCT8 in good yields. T3 uptake experiments suggested that MCT8 is functional in insect cells. A critical topic with membrane protein purification is the selection of suitable detergent conditions. A comprehensive screen identified several conditions for each protein to quantitatively solubilize the transporter. Ni-NTA affinity chromatography allowed for good purification as a second step showing that the tag is functional in folded protein. Currently, conditions are sought that maintain the transporters as monomers and select against formation of liposomes.

The potential of oligodendrocyte progenitor cells (OPCs) transplantation to treat the delayed myelination in MCT8-deficient patients

Gad Vatine and Clive Svendsen

Cedars-Sinai Medical Centre, Regenerative Medicine Institute

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 MCT8-deficient patients into oligodendrocyte progenitor cells tested their maturation in response to T3. We demonstrate that the in vitro maturation of (OPCs) is not T3 dependent. In order to test the ability of MCT8-deficient OPCs to differentiate and mature in vivo, we transplanted MCT8-deficient OPCs into an immunodeficient congenital hypomyelinated mouse model. Our results demonstrate that these OPCs survive, integrate and migrate through the mouse brain. Furthermore, OPCs are able to differentiate into mature oligodendrocytes, populate the corpus callosum and myelinate axons *in vivo*. Interestingly, it appears that MCT8-deficient OPCs are less efficiently myelinating axons in vivo. These results suggest that MCT8 is important for oligodendrocyte maturation in a cell autonomous manner 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.

Preliminary data from intranasal delivery of thyroid hormone to wild-type and Mct8 deficient mice

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Numerous studies indicate that the severe psychomotor retardation in MCT8 deficiency is likely due to impaired TH transport across the blood-brain-barrier (BBB). The nasal cavity provides a direct and non-invasive route to the brain that can be used to deliver chemical therapy. We have performed preliminary studies in mice to evaluate intranasal delivery as a potential route to administer TH directly into the brain without affecting systemic TH concentrations.
Wt and Mct8KO mice were treated with a highly concentrated solution of T4. Though several experimental data indicate an increase in brain T4, the level of TH in plasma, as well as the expression of *Dio1* in liver increased. This result indicates that intranasal administration of T4 does not deliver TH selectively to the brain and that it will actually aggravates the already existing TH excess in peripheral tissues of MCT8 deficient patients. In addition, as mice express the T4 transporter Oatp1c1 at the BBB, it is not possible to determine if the observed T4 effects on the brain are the consequence of intranasal or systemic delivery of T4.
We therefore used bovine serum albumin (BSA), that binds TH with high affinity, previously described as means to enhance substance brain delivery without reaching the systemic circulation, as well as a vasoconstrictor. Unfortunately neither of these manipulations were able to prevent or even reduce the TH reaching the systemic circulation after intranasal administration.

Treatment of AHDS with chemical or pharmacological chaperones

Doreen Braun and Ulrich Schweizer

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Mutations in the thyroid hormone transporter MCT8 prevent appropriate entry of thyroid hormones into brain cells during development and cause severe mental retardation in affected patients. Current treatment options are thyromimetic compounds that enter the brain independent of MCT8. Some MCT8 deficient patients (e.g. those carrying $MCT8^{delF501}$) are not as severely affected as most others suggesting residual activity of mutant transporters. We showed that $MCT8^{delF501}$ protein has decreased protein stability, but significant residual function once it reaches the plasma membrane. We were able to rescue protein expression and function of $MCT8^{delF501}$ in a MDCK1 cell model by application of the chemical chaperone sodium phenylbutyrate (NaPB), a drug that has been used to treat children with cystic fibrosis and urea cycle defects over extended periods of time. More recently, we were able to extend our previous study and report on the NaPB dependent rescue of a series of other pathogenic MCT8 mutants that are associated with milder patient phenotypes (e.g. $MCT8^{S194F}$, $MCT8^{S290F}$, $MCT8^{L434W}$, $MCT8^{R445C}$, $MCT8^{L492P}$ and $MCT8^{L568P}$). To our surprise, NaPB also mediated rescue of some pathogenic MCT8 mutations that lead to a severe phenotype (e.g. $MCT8^{P321L}$). We now investigate whether NaPB acts not only as a chemical chaperone preventing degradation of mutant protein, but also as an activator of protein function.

Adeno associated virus 9-based gene therapy delivers a functional MCT8 which improves thyroid hormone availability to brain of Mct8 deficient mice

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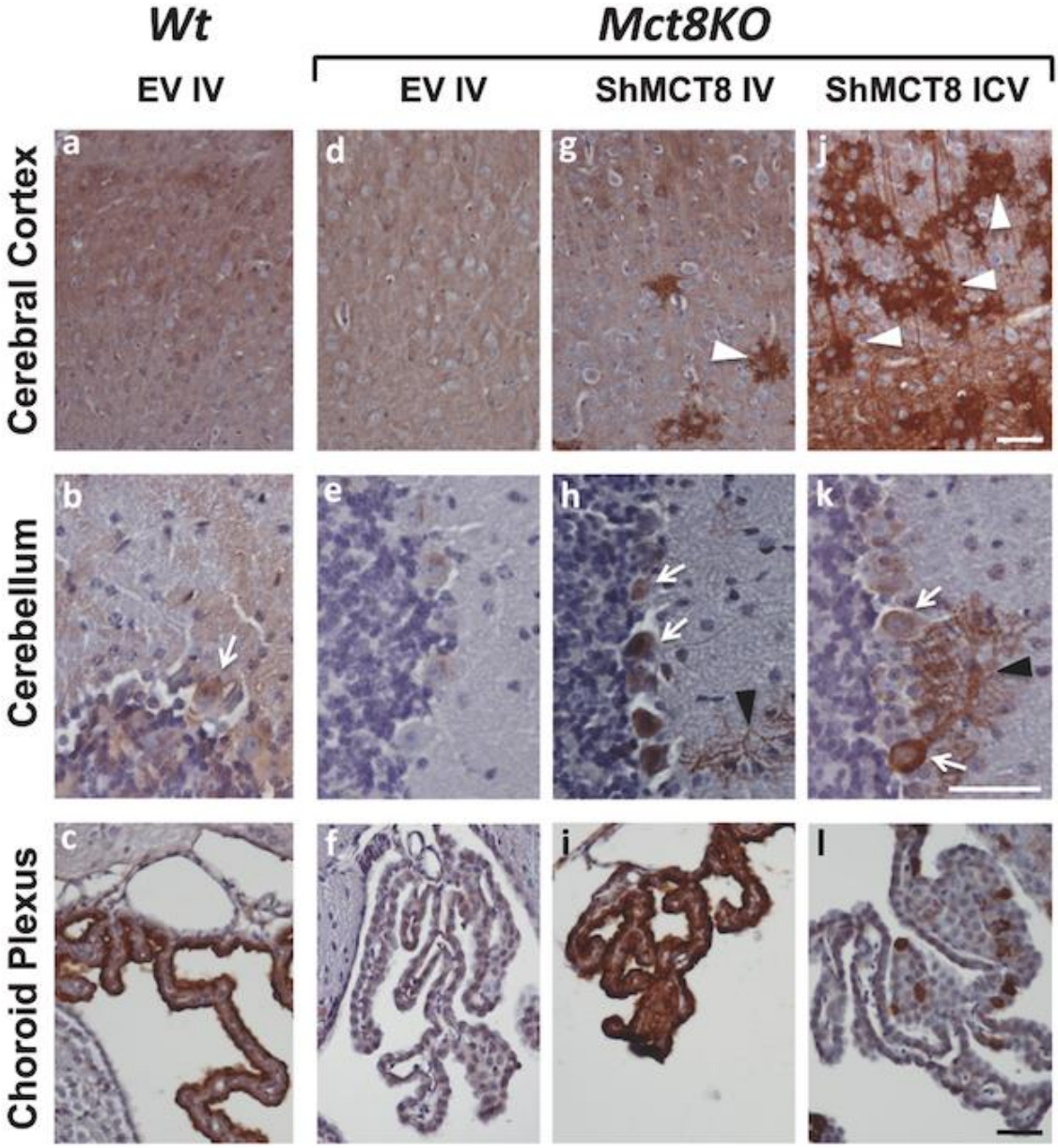
Background: *MCT8* gene mutations produce thyroid hormone (TH) deficiency in the brain, causing severe neuropsychomotor abnormalities not correctable by treatment with TH. In this proof of concept study, we examined whether transfer of human *MCT8* (*hMCT8*) cDNA using adeno-associated virus 9 (AAV9) could correct the brain defects of Mct8 knockout mice *(Mct8KO*).

Methods: AAV9 vectors delivering long and/or short hMCT8 protein isoforms or an empty vector were injected intravenously (IV) and/or intracerebroventricularly (ICV) into postnatal day 1 *Mct8KO* and wild type (*Wt*) mice. T_3 was given daily for 4 days before post-natal day 28, at which time brains were collected after perfusion to assess increase in T_3 content and effect on the T_3-responsive transcription factor, Hairless.

Results: Increased pup mortality was observed after IV injection of the *AAV9-long* hMCT8 isoform, but not after injection of AAV9-short hMCT8 isoform. Compared to IV, ICV delivery produced more hMCT8 mRNA and protein relative to the viral dose, which was present in various brain regions and localized to the cell membranes. Despite production of 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 Hairless.

Conclusions: 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. Increasing MCT8 expression on brain cells membranes, including neurons, is insufficient to produce an effect without increase in brain T_3 content. 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.



Localization of the expressed hMCT8 protein in brains of Mct8KO mice given AAV9-ShMCT8 IV and ICV compared to *Wt* and *KO* animals injected IV with empty virus. Representative images showing hMCT8 expression (in brown) detected with a specific antibody by immunohistochemistry counterstained with hematoxylin in the somatosensory region of the cerebral cortex (a,d,g,j), the cerebellar lobule 4 (b,e,h,k) and choroid plexus (c,f,i,l) of *Wt* (a-c) and *Mct8KO* (d-f) mice injected with empty vector. *Mct8KO* mice were injected with AAV9-ShMCT8 IV (g-i) or ICV)(j-l). hMCT8 was present in larger quantities in the cerebral cortex of mice injected with the virus ICV (j) than IV (g). However, much of the immunoreactivity was present in aggregates (white arrowsheads). Some Purkinje cells (white arrows) were observed in both IV and ICV-injected cerebellum (h and k) with remarkable hMCT8 expression at the dendritic arborizations (black arrowsheads). The control, *Wt* mice injected with EV, did not show the positive signal of dendritic arborizations (b). hMCT8 was abundantly present at the choroid plexus of IV-injected *KO* mice (i) and only

spottily expressed in mice given the virus ICV (l). Scale bar for each brain region is in the lower right corner of the left photograph and equals 50 μm.

Genetic and pharmacological treatments in *mct8*-/- zebrafish

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The Allan-Herndon-Dudley syndrome (AHDS) is a psychomotor retardation associated with mutations in the thyroid-hormone (TH) transporter MCT8. AHDS is characterized by severe intellectual deficiency, neuromuscular impairment, and brain hypothyroidism. In order to understand the mechanism and test potential treatments, we developed an *mct8* mutant (*mct8*-/-) zebrafish model. The transparent zebrafish model emerged as an attractive model to study AHDS due to its unique genetic and imaging toolbox, conserved structure and function of the brain, as well as its amenability to high-throughput screening. We found neurological and behavioral deficiencies in *mct8*-/- larvae; for example, we used two photon imaging of genetically encoded calcium indicators in order to visualize neuronal activity in the entire brain of live *mct8*-/- larvae. We found specific regions in the brain that demonstrate deficient activity. Since hypomyelination is a major symptom in AHDS patients, we studied myelination processes in the zebrafish model. The quantification of genetic markers for oligodendrocyte progenitor cells (OPCs) and mature oligodendrocytes revealed reduced differentiation of OPCs into oligodendrocytes. Live imaging of single glial cells showed that the number of oligodendrocytes and the length of their extensions are reduced, and the number of peripheral Schwann cells is increased in *mct8*-/- larvae. Pharmacological analysis showed that TH analogs and clemastine partially rescued the hypomyelination in the CNS of *mct8*-/- larvae. Intriguingly, triiodothyronine (T3) treatment rescued hypomyelination in *mct8*-/- embryos before the maturation of the blood-brain barrier (BBB), but did not affect hypomyelination in older larvae. Thus, we expressed Mct8-tagRFP in the endothelial cells of the vascular system and showed that even relatively weak mosaic expression completely rescued hypomyelination in *mct8*-/- larvae. These results suggest potential pharmacological treatments and BBB-targeted gene therapy that can enhance myelination and possibly behavioral performance in AHDS.

Phenotypic characterization of the Allan-Herndon-Dudley Syndrome: establishment of a patient registry

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Introduction

Although it has been known for over 12 years that mutations in MCT8 result in the Allan-Herndon-Dudley Syndrome (AHDS), systematic evaluation and follow-up of patients with this rare disorder is lacking. Due to its low prevalence, clinical expertise on AHDS is scattered throughout the world. Importantly, a detailed characterization of the natural history of AHDS in a large cohort is currently not available, complicating routine medical care and hampering the assessment of the effectiveness of any novel therapy. Therefore, we aimed to establish a uniform phenotypic characterization of AHDS through the establishment of a patient registry.

Methods and results

In a world-wide collaboration, doctors and parents of currently diagnosed AHDS patients are being approached to participate in this patient registry. This includes completing questionnaires that cover different aspects of the natural history of AHDS, including growth and development, the progress of signs and symptoms over time and historical blood test results. These questionnaires, based on expert opinion, have already been established. Moreover, historical imaging data (brain MRI) and neonatal screening cards are collected.

Discussion

Similar to other rare diseases, centralization of knowledge and expertise on AHDS will provide important clinical information that helps to better define the natural disease history and key medical problems. Together, this will improve patient care, facilitate early diagnosis and allow a better evaluation of novel therapies. In addition, a well-structured global network is essential to efficiently take potential novel therapies to the patient in multinational clinical trials.

Comparative studies of *mct8*-/-, *oatp1c1*-/- and thyroid gland-ablated zebrafish

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The X-linked psychomotor retardation Allan-Herndon-Dudley syndrome (AHDS) is associated with mutations in the thyroid hormone (TH) monocarboxylate transporter 8 (*mct8*). We utilize the zebrafish model to study the neurological mechanism and potential treatments for AHDS. In order to study the function of MCT8 and the effect of TH signaling on the brain, we developed three models: *mct8* mutant (*mct8*-/-), organic anion-transporting protein 1c1 mutant (*oatp1c1*-/-), and inducible thyroid gland-ablated zebrafish. Comparative study of their transcriptome profile and phenotypes will be presented, with an emphasis on potential treatments in adult animals.

Preliminary proteomic profiling of the effect of MCT8 mutation has on brain microvascular endothelial cells produced from patient-derived induced pluripotent stem cells

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Abstract

It is now widely known the variable mutations that can occur within the solute carrier family 16 member 2 (SLC16A2) gene results in structural and functional changes in the cell-membrane transporter protein MonoCarboxylic acid Transporter 8 (MCT8) resulting in detrimental phenotypic effects in patients. The transporter's mutation is predominantly responsible for affecting the transport of thyroid hormone T_3 across the membrane of several cell types, limiting the maturation of neuronal cells and manifesting as severe neurodevelopmental deficiency. It has been hypothesised that the brain microvascular endothelial cells, which make up the blood-brain barrier, is one of the crucial sites affected by the MCT8 transporter mutation as it limits the overall amount of T_3 thyroid hormone available in the brain tissue. This study aimed to explore the effect of MCT8 mutations on the proteome of 5 variations of brain microvascular endothelial cells (BMECs) which were generated using induced pluripotent stem cells. Protein extracts were tryptically digested and the resultant generated peptides were analysed by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). Proteomic differences were seen in the *post hoc* systems biology based analysis which reveals the large proteomic profile shifts which can be attributed to the MCT8 mutations. The interaction network analysis allowed for an in depth pathway analysis identifying changes in key interactions across cell lines.

Update of the Project: Role of White Matter Tracts and Gray Matter Centers in X-Linked MCT8 Transporter Deficiency, Assessed by Dedicated Magnetic Resonance Imaging (MRI) and Clinical Correlation.

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Abstract: In 2016 we completed the first year of our two year pilot study. A major part of our effort on this project this year was to develop a safe recruitment plan for pediatric patients with MCT8 transporter deficiency. The next step was clinical assessment of the patients combined with a dedicated brain MRI study to assess the neurological progression of their disease with special attention to the motor development/movement disorder features. The brain MRI study was created with dedicated MRI pulse sequences that acquire anatomical, iron, and tractographic images capable of providing measurable information of the brain's gray and white matter integrity. The clinical segment of the study contained a complete pediatric neurological history and physical examination by Dr. Holden (pediatric neurologist and Co-PI). A second neurologist specialized in movement disorders observed and performed a focused neurological exam to delineate the movement disorder phenotype. A total of nine patients with MCT8 deficiency and three age-matching controls have been recruited.

The acquired imaging data was of excellent quality with stable parameters to adequately proceed with several distinct quantitative analyses in the near future. These quantitative analyses will include: volume of distinct anatomical structures, delineation/assessment of the white matter tracts microstructures, assessment of the brain connectivity, and assessment of brain parenchyma iron deposition. Preliminary imaging data has already shown that the technique is adequate to infer/quantify volume of distinct anatomical structures. The clinical/neurological data has demonstrated the characteristic neurological findings including the motor component of the disease during childhood.