MCT8 2018 Symposium in Memory of Theo Visser

Landgoed Zonheuvel Hotel, Doorn, Netherlands

Monday 10th September

13:45 Welcome: Dror Ben-Ami and Samuel Refetoff

13:55 **Treatment and research of patients, Chair Lior Appelbaum**

14:00 Thyroid hormone analog therapy in patients with MCT8 deficiency: the Triac Trial

Stefan Groeneweg, I. René de Coo, Ingrid M. van Beynum, Femke K. Aarsen, Marieke M. van der Knoop, Yolanda B. de Rijke, Robin P. Peeters, Theo J. Visser, W. Edward Visser on behalf of the Triac Trial research group

14:15 Establishing a natural history on MCT8 deficiency

S. Groeneweg, M. Stals, A. Dolcetta, R.P. Peeters, I.F.M. de Coo, W.E. Visser, on behalf of the MCT8 deficiency Working Group.

14:30 Prenatal treatment of MCT8 deficiency: A preliminary report

Stephen LaFranchi, Theodora Pappa, Karen Hansen, Meredith Williams, Maria Matheus, Alexandra Dumitrescu, Roy E. Weiss, Juan Bernal, and Samuel Refetoff

14:45 Discussion

15:25 **Non-analogue therapies, Chair Heike Hueur**

15:30 AAV9 gene therapy approach for MCT8 deficiency

Clive Svendsen and Gad Vatine

15:45 Adeno-associated virus-based delivery of functional Mct8 to the brain endothelial cells of *Mct8*/*Oatp1c1* DKO mice

Hannes Köpke, Sivaraj Mohana Sundaram and Markus Schwaninger

16:00 Sodium phenylbutyrate increases the transport function of pathogenic P□□□L mutation in monocarboxylate transporter 8

Doreen Braun and Ulrich Schweizer

16:15 Discussion

17:00 Coffee Break

17:10 **Analogs and transporters, Chair Edward Visser**

17:15 Elucidating the therapeutic potential of TH analogs in MCT8 deficiency

Eva Salveridou, Jiesi Chen and Heike Heuer

17:30 Searching for strategies to overcome the lack of MCT8 in the brain

Soledad Bárez-López, Carmen Grijota-Martínez, Meredith D. Hartley, Thomas S. Scanlan and Ana Guadaño-Ferraz

17:45 Thyroid hormone transporters in zebrafish: deficiencies and therapies

Inbal Admati, David Zada, Adi Tovin, Tali Lerer-Goldshtein, Lior Appelbaum

18:00 Discussion

18:45 End first day

19:30 Dinner

Tuesday 11th September

8:55 **Research for treatment, Chair Anna Guadaño-Feraz**

9:00 Using induced Pluripotent Stem Cells derived oligodendrocyte lineages as model for the neurological phenotype in MCT8 deficiency

Nilhan Gunhanlar, Stefan Groeneweg, Robin Peeters, Steven Kushner and W. Edward Visser

9:15 Drug rediscovery strategies for the treatment of AHDS

Jerran Santos and Bruce Milthorpe

9:30 Modeling AHDS associated delayed myelination using MCT8-deficient iPSCs

Gad Vatine and Clive Svendsen

9:45 Discussion

10:30 Coffee break

10:40 **Form and function of the brain, Chair Clive Svendsen**

10:45 On the pathogenesis of MCT8 defect

Beatriz Morte, Pilar Gil-Ibáñez, Heike Heuer and Juan Bernal

11:00 Desynchronized activity of neurons and glial cells in *mct8* mutant zebrafish

Rotem Rozenblat, David Zada, Anirudh Kulkarni, Tali Lerer-Goldshtein, German Sumbre and Lior Appelbaum

11:15 Correlation of brain MRI images with childhood MCT8 phenotypes at different ages

M. Gisele Matheus, Leonardo Bonilha, and Kenton Holden

11:30 Discussion

12:15 **Summation and discussion about next steps, led by Samuel Refetoff**

13:00 End of MCT8 Symposium

List of Professional Attendees 2017 MCT8

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Thyroid hormone analog therapy in patients with MCT8 deficiency: the Triac Trial

Stefan Groeneweg[1], I. René de Coo[2], Ingrid M. van Beynum[3], Femke K. Aarsen[4], Marieke M. van der Knoop[4], Yolanda B. de Rijke[5], Robin P. Peeters[1], Theo J. Visser[1], W. Edward Visser[1] on behalf of the Triac Trial research group

Departments of [1] Internal Medicine, Academic Center for Thyroid Diseases, [2] Pediatric Neurology, [3] Pediatric Cardiology, [4] Neuropsychology, and [5] Clinical Chemistry, Erasmus University Medical Center, MC, Rotterdam, The Netherlands

Introduction

Mutations in the thyroid hormone (TH) transporter MCT8 result in MCT8 deficiency, which is characterized by severe intellectual and motor disability and high serum T3 concentrations inducing thyrotoxicity in peripheral tissues. At present, no effective treatment is available, although preclinical studies suggest 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.

Objective

To study the effect of Triac on serum T3 concentrations and signs of thyrotoxicosis in patients with MCT8 deficiency.

Methods

We conduct a world-wide prospective interventional trial in which 46 patients with MCT8 deficiency receive Triac treatment for 1 year. The primary end-point is the reduction of serum T3 concentrations, 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, all patients (age: 1-66 yr) have been enrolled of which 35 completed 1 year of follow-up. Triac treatment effectively reduced serum TSH concentrations (mean ± SD: 2.9 ± 1.6 to 1.0 ± 1.0 mU/L; $p<0.001$), resulting in a strong reduction of T3 concentrations (5.2 ± 1.4 to 1.8 ± 0.8 nmol/L; $p<0.001$), when comparing baseline and end-study measurements in these 35 patients. Importantly, the age-specific SD scores for BW (-3.1 ± 1.9 to -2.7 ± 1.8, $p<0.05$) and BMI (-2.8 ± 2.6 to -2.2 ± 2.6, $p<0.05$) significantly increased, whereas basal HR (102 ±13 to 93±8 bpm, $p<0.01$) significantly decreased. Moreover, serum markers that reflect tissue thyroid state improved such as SHBG (222 ± 88 to 186 ± 76 nmol/L, $p<0.005$) and Creatinine (31.5 ± 10.3 to 36.1 ± 13.0 µmol/L, $p<0.005$). The youngest patients had some improvement in neuropsychological markers. No (severe) adverse reactions to Triac occurred.

Conclusions

This interim analysis indicates that Triac treatment effectively normalizes serum T3 concentrations in patients with MCT8 deficiency. Both clinical outcomes (BMI and HR) and biochemical markers representing thyroid state in different tissues improved on Triac treatment. Future studies should aim to evaluate the effect of Triac on the neurocognitive phenotype once treatment is installed early after birth.

Establishing a natural history on MCT8 deficiency

S. Groeneweg, M. Stals, A. Dolcetta, R.P. Peeters, I.F.M. de Coo, W.E. Visser, on behalf of the MCT8 deficiency Working Group.

Background: Mutations in the thyroid hormone (TH) transporter monocarboxylate transporter (MCT) 8 result in MCT8 deficiency, characterized by severe intellectual and motor disability and abnormal serum TH concentrations (high T3, low T4 and a high-normal TSH). The neurocognitive phenotype has been attributed to the local hypothyroid state in the brain, whereas the high serum T3 concentrations result in a local hyperthyroid state in tissues that do not rely on MCT8 for their TH supply (peripheral phenotype). A detailed natural history on MCT8 deficiency is currently lacking and data on the peripheral phenotype are limited. Therefore, we aim to establish a uniform phenotypic description of patients with MCT8 deficiency, focusing on neuro-developmental aspects and the peripheral phenotype.

Methods: Our cohort consists of patients with genetically confirmed mutations in the *MCT8* gene that have been enrolled in the Triac Trial (NTC02060474), or one of the named patient Triac treatment programs, or came to our attention through medical consultations. Patients were not receiving any thyroid hormone (analogue) therapy at time of evaluation. Detailed clinical and biochemical evaluations were available on the majority of patients. Historical (imaging) studies are currently being retrieved.

Results: In total, 94 subjects with over 50 different underlying mutations in *MCT8* have currently been included in our cohort. Most patients presented the characteristic thyroid function tests, with serum T3 concentrations being well above the normal range, had a low body weight, a relatively high heart rate, and alterations in serum markers that reflect thyroid state in several target tissues. In line with previous studies, hypotonia, dystonia and a variable degree of spasticity were present in all patients. Neuropsychological evaluations of over 30 subjects showed a severe delay in cognitive and motor development with only little heterogeneity in those subjects harboring a severe pathogenic mutation. A web-based MCT8 deficiency patient registry is being launched to further extent the current cohort.

Conclusion: this study allows establishing a natural history on MCT8 deficiency using uniform and quantitative measures on different clinical outcome measures. These data are of great value as a reference cohort for future clinical trials to any therapeutic intervention.

Prenatal treatment of MCT8 deficiency: A preliminary report

Dr. Stephen LaFranchi: referring pediatric endocrinologist who took care of the infant together with Dr. Nazaneen Eshragh.

Dr. Theodora Pappa identified the MCT8 gene mutation.

Ms. Karen Hansen: prenatal genetic counseling of this patient

Dr. Meredith Williams performed the amniocentesis and administered the L-T4.

Dr. Maria Matheus analyzed the brain MRIs.

Drs. Alexandra Dumitrescu, Roy E. Weiss and Juan Bernal helped in planning the treatment protocol and interpretation of the results.

Dr. Samuel Refetoff conceived and organized the protocol, interpreted the results and will write the first draft of the manuscript.

Treatment of MCT8 deficient infants and children with thyroid hormone analogues (DITPA and TRIAC) or a combination of PTU and T4 can decrease the T3-induced hypermetabolic state but do not improve the neuropsychomotor defect. This is likely because thyroid hormone deprivation in MCT8 deficiency causes damage to brain development in embryonic life as shown by A. Guadaño-Ferraz. A carrier woman who was pregnant with a second male embryo found to be affected, based on analysis of DNA obtained by amniocentesis, elected to carry the pregnancy to term. Her first child born with MCT8 deficiency had classic severe psychomotor delay as seen in other children with MCT8 deficiency. As prenatal treatment with thyroid analogues was not approved, the fetus was given L-T4 by weekly intra-amniotic instillations at a dose previously used in the treatment of fetal goiter. Seventeen instillations were performed from gestational age of 18 weeks to spontaneous delivery at 35 weeks. One week after the first L-T4 instillation the intra-amniotic T4 rose by 50-fold, T3 by 6-fold and rT3 by 140-fold. The values gradually declined as the fetus grew. Heart rate remained normal (132-161 bpm) and fetal growth proceeded normally (64-68%). Maternal thyroid hormone levels, including TSH remained stable and normal throughout the treatment. At 3 days of life the infant's serumT4 was 16.7 µg/dl (values in MCT8 deficient newborn being <5 µg/dl), T3 112 ng/dl , rT3 220 ng/dl and TSH <0.005 mU/L. The latter value indicated that the L-T4 treatment reached the fetal tissues. Compliance with the recommended postnatal dosing of L-T4 and PTU was inconsistent, such that goal serum T4 and T3 levels were reached intermittently. At 2 months the infant could raise his head, at 6 months brain MRI, read blindly, showed near normal myelination, much advanced compared to his untreated brother at the same age. At 10 months he could turn from back to belly, reach for and grasp objects and transfer them from hand to hand, but he still had truncal hypotonia and could not sit by himself. Based on studies of Dr. J Bernal showing that the thyroid hormone receptor is detected in human fetal brain at 10 weeks of gestation, reaching a peak at 16 weeks, prenatal treatment should begin earlier. Accordingly, future aims include prenatal diagnosis by CVS, following gender determination, and treatment using thyroid hormone analogues that cross the placenta and are concentrated in fetal tissues.

A gene therapy approach for MCT8-deficiency

Clive Svendsen and Gad Vatine

Accumulating evidence indicate that MCT8-deficiency results in impaired transport of TH across the blood-brain barrier (BBB) and consequently, in insufficient TH supply to the CNS. Replacement of the malfunctioning protein is appealing since AHDS is a monogenic disease and the targeted gene is well known. Moreover, expressing a correct MCT8 protein is expected to result not only in the repair of the well characterized role of MCT8 in transporting THs, but also of putative alternative molecules that can also act as MCT8 substrates.

In order to restore MCT8-mediated transport into the CNS we tested the potential of a gene therapy approach in the Mct8/Oatp1c1 double KO (dKO) mouse model. Specifically, we compared the efficiency of AAV9-MCT8 delivery through both, ICV and IV routes. Our results suggest that both delivery routes resulted in broad re-expression of MCT8 in the dKO brain, however, only IV delivery resulted in MCT8 expression in endothelial cells of the BBB. Notably, IV delivery partially rescued a deficiency in parvalbumin+ cortical interneuron expression in the dKO mice. Further, IV delivery resulted in an increase in T3-brain content as well as in T3-induced gene expression. Research is underway to determine whether this approach can rescue the myelination and behavioural deficits in the dKO mice, and thereby provide the pre-clinical basis that is required for future clinical trials.

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 T3 entering the brain tissue. Increasing MCT8 expression in brain cell membranes, including neurons, is insufficient to produce an effect without an increase in brain T3 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.

Adeno-associated virus-based delivery of functional Mct8 to the brain endothelial cells of *Mct8*/*Oatp1c1* DKO mice

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Since current therapeutics fail to significantly improve the neurological symptoms of AHDS an alternative approach is aiming to deliver the functional hMCT8 protein to the brain of patients via recombinant adeno-associated virus (rAAV) vectors. A first proof of concept study with an AAV9 vector in mice by Refetoff et al. in 2016 implied that the T3 deprivation in neurons is caused by a restricted transport of thyroid hormone (TH) over the blood-brain barrier (BBB). Therefore, this study aims to answer the question, if the delivery of the *Mct8* gene to the brain endothelial cells (BECs) of *Mct8*/*Oatp1c1* double knockout (DKO) mice via a BEC-specific rAAV2 can rescue cellular and functional hallmarks of the disease.
Therefore, we successfully generated rAAV-BR1-*Mct8* in Sf9 cells and verified the expression of *Mct8* in transduced primary mouse BECs from *Mct8*/*Oatp1c1* DKO mice by a specific Mct8 antibody. The functionality of expressed Mct8 was proven via T3 uptake assay which utilizes the specific Mct8 inhibitor silychristin as a control. In addition, we currently investigate the effect of an intravenous injection of a low and high dose of rAAV-BR1-*Mct8* into P0 *Mct8*/*Oatp1c1* DKO mice on the differentiation of parvalbumin-positive GABAergic interneurons, myelinogenesis in the cerebral cortex and the development of cerebellar Purkinje cells at P12 and P21.

Sodium phenylbutyrate increases the transport function of pathogenic P???L mutation in monocarboxylate transporter 8

Doreen Braun and Ulrich Schweizer

Introduction: Mutations in the highly specific thyroid hormone transporter MCT8 (monocarboxylate transporter) lead to the Allan-Herndon-Dudley syndrome (AHDS), a severe mental retardation disease caused by a disturbed thyroid hormone uptake into different cells (e.g. brain) during critical stages of embryonic development. Symptoms of AHDS include neurological deficits, muscle weakness, absent of speech and inability to walk. Most MCT8 mutations lead to a complete loss of transporter function while other mutants show residual transport activity. We have already demonstrated that mutations with residual activity underlie increased protein degradation of an otherwise functional MCT8 protein. These mutants can be rescued with the chemical chaperone sodium phenylbutyrate (PB). Here we report about new findings found for the pathogenic P321L mutant treated with PB.

Methods: Mutation of P321L was introduced into human MCT8 by site directed mutagenesis and stably transfected into MDCK1 (Madin-Darby canine kidney) cells. The cells were treated with sodium phenylbutyrate for two days. In addition, human derived iPS cells of MCT8 patients carrying the P321L mutation were differentiated into brain microvascular endothelial cells (BMECs) and treated with phenylbutyrate for one day.

Western blotting, mRNA analysis and radioactive thyroid hormone-uptake experiments were performed to analyze chaperone effects.

Results/Discussion: Substitution of proline to leucine at position 321 completely abolishes the MCT8 mediated T_3 uptake when expressed in MDCK1 cells and shows a decreased T_3 uptake activity in patient-derived BMECs compared to their isogenic control. Treatment with PB leads to a rescue of protein expression associated with an increase in mRNA stability and an alteration of endogenous chaperone expression. The rescue of protein expression enhances T_3 uptake to a level found in control cells.

Elucidating the therapeutic potential of TH analogs in MCT8 deficiency

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TH analogs that exert TH action in the brain but are not dependent on MCT8 have been suggested as a promising therapeutic options for patients with MCT8 mutations. Our group has tested and compared the action of the TH analogs Triac and Ditpa in Mct8/Oatp1c1 dko mice, a mouse model for human MCT8 deficiency. Indeed, both substances were able to partially normalize brain development with Triac being more efficient than Ditpa. In particular, treatment of M/O dko mice during the first postnatal weeks resulted in normal i. cerebellar development, ii. maturation of GABAergic neurons, iii. myelination and iv. locomotor function. However, initiation of Triac treatment at later postnatal time points exerted less beneficial effects as no significant improvement in myelination could be achieved by treating M/O dko mice between postnatal week 3 and 6.

Here, we tested the efficacy of four novel TH-analogs (provided by Tom Scanlan) for the treatment of AHDS. These compounds are different prodrugs of sobetirome (GC-1) with beneficial pharmacokinetic properties. Most importantly, these substances accumulate in the CNS and, therefore, affect to a much lesser extent peripheral tissues.

In a first approach, we tested these compounds in murine primary cerebellar cultures and analyzed their TH-like effects. For this task, we monitored cerebellar Purkinje cell outgrowth and quantified the number of Parvalbumin positive neurons as both parameters are strongly dependent on the presence of T3. While 10 nM Triac and 10 nM sobetirome were as effective as 1 nM T3 in promoting neuronal differentiation, significantly lower doses (0.1 nM) were needed for two of the tested prodrugs indicating a strong thyromimetic action. Currently, we are testing these compounds in M/O dko mice and study their impact on neuronal differentiation, myelination and T3-regulated gene expression. Our first results clearly indicate pronounced TH-like effects in the CNS at significantly lower doses compared to Triac. Ongoing studies will reveal whether these compounds can be considered as a treatment option for AHDS.

Searching for strategies to overcome the lack of MCT8 in the brain

Soledad Bárez-López, Carmen Grijota-Martínez, Meredith D. Hartley, Thomas S. Scanlan and Ana Guadaño-Ferraz

Loss of function mutations in the thyroid hormone (TH)-specific cell membrane transporter, the monocarboxylate transporter 8 (MCT8), lead to profound psychomotor retardation and abnormal TH serum levels, with low thyroxine (T4) and high triiodothyronine (T3). Several studies point to impaired TH transport across brain barriers as a crucial pathophysiological mechanism resulting in cerebral hypothyroidism. Treatment options for MCT8-deficient patients are limited and are focusing on overcoming the brain barriers. Our aim is to evaluate the potential use of pharmacological treatments with TH metabolites and analogues to access the brain and exert thyromimetic actions in the absence of Mct8.

We have obtained encouraging results in experimental animals indicating that systemically administrated Sobetirome and its amide prodrug Sob-AM2 can access the brain in the absence of Mct8 and exert thyromimetic actions modulating the expression of T3-dependent genes in the brain.

We are also evaluating the ability of intracerebroventricularly administered TRIAC to access the brain and exert thyromimetic actions in Mct8-deficient mice. So far, we have found that intraventricular administration of therapeutic doses of TRIAC does not further decrease T3 content and is enough to increase TRIAC content in the cerebral cortex, however, it does not modulate the expression of the T3-dependent genes. As the lack of effect on gene expression could be due to the sensitivity of the model, we are currently further assessing the ability of intracerebroventricularly administered TRIAC to modulate gene expression by increasing the dosage of TRIAC and by using mice lacking both Mct8 Dio2 which present more severe brain hypothyroidism.

Thyroid hormone transporters in zebrafish: deficiencies and therapies

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In all vertebrates, thyroid hormone (THs, T4 and T3) activation of thyroid nuclear receptors induce the transcription of hundreds of genes, and regulate fundamental physiological processes including embryonic development, neurogenesis, and metabolism. In order to function, THs require specific transporter proteins that facilitate their uptake and efflux across the cell membrane. Insufficient TH uptake by the target cells in the brain causes hypothyroidism and mental retardation. We developed a zebrafish model for Allan-Herndon-Dudley syndrome (AHDS), a psychomotor retardation associated with mutations in the TH transporter Mct8 (monocarboxylate transporter 8). Pharmacological treatment with TH analogs and blood-brain-barrier (BBB) targeted gene therapy rescued the neurological deficiencies in *mct8* mutant (*mct8*-/-) larvae. However, Mct8 is not the sole transmembrane TH transporter, and other TH transporters have been functionally described in mammals. The organic anion-transporting polypeptide 1C1 (Oatp1c1) is expressed primarily in the BBB and facilitate the transport of T4 into the cell, where it is converted into the active ligand T3. Transgenesis and co-localization experiments showed that, similar to mammals, *oatp1c1* is primarily expressed in BBB endothelial cells, neurons and astrocytes, but not in oligodendrocyte. CRISPR-mediated *oatp1c1* mutant (*oatp1c1*-/-) zebrafish model was generated. In this work, transcriptome profiling, neuronal and behavioral deficiencies, as well as the restorative effect of TH analogs in TH transporter mutant zebrafish, will be presented.

Using induced Pluripotent Stem Cells derived oligodendrocyte lineages as model for the neurological phenotype in MCT8 deficiency

Nilhan Gunhanlar, Stefan Groeneweg, Robin Peeters, Steven Kushner and W. Edward Visser

Thyroid hormone (TH) transporter protein MCT8 is crucial for TH signaling in brain. MCT8 mutations result in a devastating disorder called MCT8 deficiency (also known as the Allan-Herndon-Dudley syndrome) comprising severe neurological and metabolic features. Importantly, brain imaging consistently shows impaired myelination in MCT8 deficiency. Given the expression of MCT8 in oligodendrocytes and the importance of T3 for development of these cells, MCT8 deficiency likely disrupts the myelination process, ultimately resulting in severe neurological features. We aim to unravel the direct effect of MCT8 deficiency on abnormal oligodendrocyte development as another pathogenic mechanism, beyond abnormal transport across the blood-brain barrier, underlying MCT8 deficiency.

Induced pluripotent stem cells (iPSCs) technology has largely advanced the understanding of human diseases, by allowing for the first time to study living cells from patients. A recent unprecedented protocol demonstrated a highly reliable method for generating functional neural networks derived from iPSCs which allows simultaneous generation of neurons, astrocytes and oligodendrocytes in one culture (Gunhanlar & Kushner unpublished data). This protocol allows cells to differentiate in matrigel that creates a 3D environment and, thus, mimics the *in vivo* environment such as formation of organized structures and generation of cortical layers. Until now, no spontaneous myelination in such cultures has been reported. However, importantly, long-term 3D cultures have shown that this new protocol results in spontaneous myelination of neurons (Gunhanlar & Kushner, unpublished data). Consistent with the white matter abnormalities in MCT8 deficient patients and the key role of T3 for oligodendrocyte maturation, MCT8-deficient oligodendrocytes derived from iPSCs and the effect of oligodendrocytes on other neural lineages would help to understand the disease mechanism of MCT8 deficiency. Therefore, the knowledge gap can be accommodated using iPSCs from MCT8-deficient patients to model the “brain-thyroid hormone” interaction and to understand the effects of T3 in oligodendrocyte lineage cell and myelination.

Drug rediscovery strategies for the treatment of AHDS

Jerran Santos and Bruce Milthorpe

Developing a functional treatment for current AHDS patients is paramount for reducing the impact of the disease and improving their quality of life. Current chemical treatment strategies of triiodothyronine (T_3) analogues, DITPA, TRIAC and TETRAC have had minimal and impactful results. These molecules utilise the identical transmembrane route via MCT8 in the BBB and neural membrane to act upon the T_3 nuclear receptor. Due to the misfolding in the MCT8, the traversal rates of these molecules across the BBB would also be low. Thus alternate molecules are required, that have an equivalent action to T_3, and however can be translocated via other mechanisms or transporters.

The compounds that are commercially available and FDA/TGA approved are not limited to the above mentioned analogues. There are approximately 100,000 FDA approved compounds with millions of combinations and permutations, constituting New Molecular Entities (NMEs). This wealth of commercially available libraries of drug compounds has been utilised extensively in recent years to identify and repurpose a number of these NMEs for the treatment of varying diseases. Herein lays a perfect opportunity to test and trial the effectiveness of a panel of available drugs for the purpose of finding a treatment strategy for AHDS/MCT8 patients using a model for the disease “in a dish” from induced pluripotent cells.

Modeling AHDS associated delayed myelination using MCT8-deficient iPSCs

Gad Vatine and Clive Svendsen

Delayed myelination is the prominent pathophysiological symptom associated with AHDS. To study the role of MCT8 in myelination, we differentiated iPSCs from MCT8-deficient patients oligodendrocyte progenitor cells (OPCs) and followed their maturation into mature oligodendrocytes both, *in vitro* and *in vivo*. Surprisingly, the *in vitro* maturation of (OPCs) into mature O4+ oligodendrocytes was not influenced by T3. In order to test the ability of MCT8-deficient OPCs to differentiate and mature under physiological conditions, we transplanted MCT8-deficient OPCs into neonates of an immunodeficient congenital hypomyelinated mouse model. Our results demonstrated that MCT8-deficient OPCs survived, integrated and migrated through the mouse brain. Furthermore, OPCs were able to differentiate into mature MBP+ oligodendrocytes and populate both, the corpus callosum the cerebellum. Moreover, MCT8-deficient oligodendrocytes were able to create myelin sheath and myelinate axons *in vivo*. Interestingly, MCT8-deficient OPCs produced less myelination per transplanted OPC when compared to healthy control OPCs. These results suggest that MCT8 plays a role in oligodendrocyte maturation in a cell autonomous manner *in vivo*. In order to test the potential of OPC transplantation in a hypothyroid brain we have introduced a *rag2* mutation mutation in the background of the MCT8/OATP1C1 double KO (dKO) mouse, thereby generating an immunodeficient and CNS-hypothyroid triple KO (tKO) mouse model. Interestingly, healthy control OPCs transplanted into the triple KO mouse brain failed to mature into oligodendrocytes, and remained in an undifferentiated state. These results indicate that the hypothyroid brain of the tKO mouse does not support OPC maturation, and confirms that MCT8 plays a crucial role in the entry of T3 into the brain.

On the pathogenesis of MCT8 defect

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It is believed that the MCT8 defect is caused by profound brain hypothyroidism from fetal stages due to lack of adequate transport of thyroid hormones through the blood-brain barrier. In rodents, the Mct8 defect impairs the transport of T3 but not T4, due to the presence of the T4 transporter Oatp1c1, allowing T4 to enter the brain and generate local T3 by type 2 deiodinase activity. Similar compensation does not take place in humans due to low expression of OATP1C1. In mice, inactivation of Mct8 and either Oatp1c1 or Dio2 would, therefore, be more adequate as models of the disease. We have investigated the extent of cerebral hypothyroidism achieved with these models by analyzing the expression of 100 transcriptional T3 targets in the cortex and striatum. The results show that gene expression is less affected in the mouse genetic models than in mice made hypothyroid with antithyroid drugs. This indicates that transport of T4 and T3 is not completely suppressed in the double KO, possibly through the activity of alternative transporters. The implications of these results for the pathogenesis of MCT8 defect will be discussed

Desynchronized activity of neurons and glial cells in *mct8* mutant zebrafish

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The Allan-Herndon-Dudley syndrome (AHDS) is a severe X-linked intellectual disability disorder accompanied by abnormal thyroid hormone (TH) levels. AHDS is caused by inactivating mutations in the monocarboxylate transporter 8 (MCT8), a specific TH transporter widely expressed in the CNS. Here, we study the effect of Mct8 deficiency on the activity of neurons and radial glia cells (RGCs) in the brain of *mct8* mutant (*mct8*-/-) zebrafish. Radial glia cells (RGCs) function as stem and progenitor cells and play fundamental roles in the maintenance and development of the brain. In embryos and adults, RGCs can differentiate into neurons, astrocytes and oligodendrocytes. RGC activity along their fibers regulate neurogenesis and cell migration during early development. We monitored simultaneous activity of RGCs and tail motion using two-photon live imaging of the genetically encoded calcium indicator GCaMP and high-speed camera, respectively. We found that extensive tail movement is followed by a burst of synchronized activity of RGCs. In *mct8*-/- larvae, which exhibits hypomyelination, reduced axon branching and reduced synaptic density, these synchronized events are altered. In addition, we found alteration in neuronal activity in the midbrain and hindbrain. These findings suggest new role for RGCs in regulating behavior and highlight their importance in psychomotor retardation. Furthermore, the results offer an in vivo model that can be used to test the effect of pharmacological and gene therapies on brain activity.

Correlation of brain MRI images with childhood MCT8 phenotypes at different ages

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Characterization of the neuropsychomotor phenotype of patients with MCT8 transporter deficiency has been redefined in the past few years. A better understanding of the disease and how it affects the neuropsychomotor phenotype has been progressively improving due to dedicated evaluations of distinct parts of the disease spectrum. Our part of the spectrum is to assess the genotype, the motor phenotype, and the developing brain of patients with MCT8 transporter deficiency using dedicated genetic assessments, neurological evaluations, and advanced brain MR images. Eleven pediatric patients with genetically proven MCT8 transporter deficiency (age range from 2-14 years) and 20-age matched control boys were recruited for brain MR images with dedicated sequences to quantify the volume of the cerebral structures, the microstructural integrity of the gray and white matter, and the integrity of the brain connections. The eleven MCT8 transporter deficiency patients also had a general examination, a neurological examination, and a movement disorder examination.

The MRI images revealed statistically significant differences in volume and microstructural integrity of the gray and white matter cerebral structures. The genotype in our small MCT8 group of patients showed more than the expected numbers of *de novo* mutations. All the patients had their motor disability classified and quantified by a movement disorder neurologist allowing us to assess potential clinical-genotypic-image correlations.