Mutated thyroid hormone transporter OATP1C1 associates with severe brain hypometabolism and juvenile neurodegeneration


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**Keywords** Thyroid hormone transporter, SLCO1C1, OATP1C1, brain hypothyroidism, neurodegeneration, Triac
Abstract

Context Thyroid hormones (TH) are essential for brain development and function. The TH transporters monocarboxylate transporter 8 (MCT8) and organic anion transporter 1C1 (OATP1C1) facilitate the transport of TH across the blood-brain-barrier and into glia and neuronal cells in the brain. Loss of MCT8 function causes Allan-Herndon-Dudley syndrome (AHDS, OMIM 300523) characterized by severe intellectual and motor disability due to cerebral hypothyroidism. We describe the first patient with loss of OATP1C1 function. The patient was a 15.5-year-old girl with normal development in the first year of life, who gradually developed dementia with spasticity and intolerance to cold. Brain imaging demonstrated grey and white matter degeneration and severe glucose hypometabolism.

Methods We performed exome sequencing of the patient and parents to identify the disease-causing mutation and studied the effect of the mutation through a panel of in vitro experiments, including T4 uptake studies, immunoblotting, and immunocytochemistry. Furthermore, we describe the clinical effects of treatment with the T3 analogue triiodothyroacetic acid (Triac).

Results Exome sequencing identified a homozygous missense mutation in OATP1C1 changing the highly conserved Asp252 to an Asn. In vitro, the mutated OATP1C1 showed impaired plasma membrane localization and decreased cellular T4 uptake. After treatment with Triac the clinical condition improved in several domains.

Conclusions This is the first report of human OATP1C1 deficiency, compatible with brain-specific hypothyroidism and neurodegeneration.

Précis We describe a novel disease associated with mutated OATP1C1, a brain-specific thyroxine transporter expressed in astrocytes, characterized by brain hypometabolism and early-onset neurodegeneration.
**Introduction**

Thyroid hormone, common name for the pro-hormone thyroxine (T4) and its active form triiodothyronine (T3), is critical for brain development and functioning, underscored by the devastating consequences of congenital hypothyroidism (1).

Important steps leading to TH action in the brain include transport of T4 across the blood-brain barrier, uptake of T4 by astrocytes, conversion to T3 by type 2 deiodinase (DIO2), and supply of T3 to target cells such as oligodendrocytes and neurons (2-4).

By binding to nuclear T3 receptors in these cells, T3 initiates powerful genetic control on myelination (5) and neuronal differentiation in various brain regions (6). In the human brain, T4 transport across the blood-brain barrier is predominantly mediated by monocarboxylate transporter 8 (MCT8, encoded by SLC16A2; OMIM 300095), while T4 uptake by astrocytes is facilitated by organic anion transporting polypeptide 1C1 (OATP1C1 encoded by SLCO1C1; OMIM 613389) (7). MCT8 deficiency in humans causes intellectual disability, dystonia, spasticity and hypomyelination due to a hypothyroid state in the brain (Allan-Herndon-Dudley syndrome, OMIM 300523), and is associated with low serum T4 and high T3 levels resulting in a hyperthyroid state in peripheral tissues (8,9). Until now, patients with OATP1C1 defects have not been reported. We here describe such a patient showing features of brain hypothyroidism.

**Materials and Methods**

**Patient**

The patient was a 15.5-year-old girl, born to healthy parents in a rural area in Norway. Her two younger sisters were healthy. Birth measurements were normal. Eye contact was achieved at an early age and development was unremarkable in the first months. Although she walked independently at 10 months, the mother had an impression that
“something was wrong”. In the toddler age, movements were clumsy and vocal sounds and language seemed to develop unexpectedly slow. Between 2 and 3 years, she appeared aggressive with stereotypic behaviour and was considered to be autistic. Intellectual disability was suspected by the local health service at 4 years. Psychomotor functioning was best between 5 and 6 years, when she spoke in two- or three-word sentences, ate independently, was continent, could walk, and jumped on a trampoline. Due to suspected loss of skills she was referred to our hospital at 9.5 years. Despite extensive work up, no diagnosis was made. She started to lose expressive verbal language and gradually lost cognitive and motor functioning (Table 1 and Figure 1A). In addition, body weight and height development stunted and declined to the 2.5th percentile, prompting the placement of a percutaneous gastrointestinal feeding tube at the age of 11 years. At 13 years, she was demented and incontinent for bowel and bladder functioning. Later, urinary retention required daily bladder catheterization. She had no expressive verbal language, and walking was impaired due to gait apraxia, cerebellar ataxia, scoliosis, and spasticity of the lower limbs. At 14 years, she lost the ability to use her hands. There was no clinical suspicion of epilepsy. Startle response episodes became easily provoked accompanied by apnoea of 30 seconds duration and cyanosis. She had myoclonic-like movements in the hands. Electroencephalographic examination showed intermittent slow frequency of 6 Hz without epileptic discharges. Electromyography and nerve conduction studies did not indicate peripheral nerve pathology. Cerebral magnetic resonance imaging (MRI) examinations at 7 and 13 years showed progressive atrophy starting in the cerebral cortex, continuing into subcortical white matter and cerebellum (Figure 1B). Cerebral magnetic resonance spectroscopy with the voxels in the left basal ganglia and subcortical area at 9.5 and 11 years did not show abnormal metabolic peaks. Positron emission tomography–computed
tomography (PET-CT) examination using $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) demonstrated severely decreased glucose metabolism in nearly all areas of the brain at the age of 14 (Figure 1C). PET-CT with $^{18}$F-flutemetamol showed no amyloid-β aggregation (Figure 1C), consistent with normal concentrations of amyloid-β (712 ng/L, normal range >550 ng/L) and tau (155 ng/L, normal range <250 ng/L) in the cerebrospinal fluid. Neurofilament light protein concentration in the cerebrospinal fluid was markedly elevated, 2600 ng/L at 11 years and 1400 ng/L at 14 years (reference value below 380 ng/L), indicating rapid degeneration of the cytoskeleton of axonal fibers in the cerebrum, consistent with degeneration of central white matter observed on MRI. Glial fibrillary acidic protein was mildly elevated, 280 ng/L at 11 years and 180 ng/L at 14 years (reference value below 175 ng/L), indicating that astrocytes were also involved in the degenerative process. She suffered inexplicable intolerance to cold and had cold hands and feet and frequent shivering and preferred to dress warmly even on hot days. In accordance, body temperature was generally low (Figure 1D left), and she once needed full-body warming blankets for hypothermia during general anaesthesia. Hypothyroidism was considered, however serum thyroid function tests were normal (TSH 1.96 [0.5-4.9] mU/L, free T4 15.3 [10.0-18.0] pmol/L, and free T3 6.2 [3.6-8.3] pmol/L at the age of 11 years and also at the age of 14 years). At the age of almost 12 years, whole exome sequencing of child and healthy parents was carried out to unravel the underlying cause of the phenotype.

**Ethical considerations**

The current study was formally approved by the Regional Committees for Medical Research Ethics – South-East Norway, REK 2010/1152a and REK 2016/1227. Written informed consent was obtained from the parents to treat the patient with Triac and use
the medical information, video’s and pictures of the patient for scientific and educational purposes.

**Brain PET-CT scan and biochemical analyses**

Brain PET-CT (Siemens Biograph, Siemens Healthcare, Oslo, Norway) with 18F-FDG and 18F-flutemetamol was performed with time of flight system and 46-slice computed tomography and carried out following a well-defined protocol in pediatric patients (11,12) All measurements in cerebrospinal fluid and serum have been carried out using standard laboratory methods. Technical details are described in the Supplemental Appendix.

**Exome sequencing, data analysis and confirmation by sanger sequencing**

Exome libraries from DNA of the patient and healthy parents were prepared using standard methods and subjected to routine quality checks and sequenced on an Illumina HiSeq2000 with 100 bp paired-end reads. Reads that did not pass Illumina’s chastity filter were removed prior to alignment to the reference human genome (hg19). Variant calling was performed using GATK HaplotypeCaller and analysed using FILTUS program (13). All variants with allelic frequency >0.01 or with a predicted benign impact on protein function (Combined Annotation Dependent Depletion, CADD, score <15 (14)) were excluded and we focussed on missense, nonsense, frameshift, and small insertion/deletion variants. Sanger sequencing on DNA from the patient, parents and two healthy younger sisters was used to confirm the presence of a homozygous missense mutation in *SLCO1C1* in the patient. Full technical details are provided in the Supplemental Appendix.
Expression constructs, cell culture and transfection

The cloning of human OATP1C1 cDNA into pcDNA3.1 and human CRYM cDNA into pSG5 has been previously described (15,16). OATP1C1 cDNA was subcloned into pSG5 with addition of a C-terminal V5 tag (further referred to as wild-type OATP1C1-V5) which did not affect T4 transport function (data available upon request). The Asp252Asn and Asp252Ala variants were introduced into wild-type OATP1C1-V5 using QuickChange site-directed mutagenesis according to manufacturer’s protocol (Stratagene, Amsterdam, NL). All primers are available upon request. Correctness of the constructs was confirmed by complete sequencing of the inserts. JEG-3 human choriocarcinoma (CVCL_0363) and COS-1 African green monkey kidney cells (CVCL_0223) were obtained from ECACC (Sigma-Aldrich, Zwijndrecht, NL) and cultured and transfected under standard conditions (17,18). Technical details are provided in the Supplemental Appendix.

Functional studies

T4 uptake, cell surface biotinylation, immunoblotting and immunocytochemistry studies were essentially performed as recently described (17,18). Full technical details are available in the Supplemental Appendix.

OATP1C1 homology modelling

An OATP1C1 homology model was constructed similarly as we have recently done for MCT8 using YASARA Structure Software (www.yasara.org) (18-20). The E.coli multidrug transporter MdfA (PDB#4ZP0) in inward-open conformation was selected as the most suitable template. The KAZAL-like sequence motif present in extracellular loop (ECL) 5, spanning Arg470 to Cys523, was modelled separately based on the
KAZAL-type inhibitor infestin 4 (PDB#2ERW) and integrated into the OATP1C1 model. **Full details are available in the Supplemental Appendix and the final alignment between target and template in Figure S1.**

**Statistical analysis for functional studies**

All statistical analyses were performed as indicated in the Figure legends using GraphPad Prism Version 5 software (GraphPad Software Inc., San Diego, USA). Statistically significant differences are indicated as described in the legends of the corresponding Figures.

**Results**

A homozygous variant was identified in the TH transporter *SLCO1C1* (OATP1C1)

Exome sequencing of the family trio identified in the patient a homozygous variant in *SLCO1C1* Chr12(GRCh37):g.20870143G>A; NM_001145946.1:c.754G>A; p.(Asp252Asn) (Figure S2A and S2B), changing a highly conserved aspartic acid at position 252 to asparagine (Asp252Asn, D252N) in the OATP1C1 protein (Figure S2C) and **predicted** to be damaging to the protein function (CADD 34). Parents and one sister were heterozygous for the variant (Figure S2A and S2B). In the patient, the variant was part of a region of autozygosity of estimated minimum size of 4.38 Mb, and the total fraction of autozygosity in the patient was compatible with the parents being second cousins (data not shown). The variant was not reported in the public database of sequence variants Genome Aggregation database (gnomad.broadinstitute.org).

**Functional studies confirmed loss of T4 transport function in mutated OATP1C1**
In order to study if the identified mutation impairs OATP1C1 function, we performed complementary *in vitro* studies. To allow immunochemical detection, expression constructs were made of wild-type OATP1C1 and the Asp252Asn mutant equipped with a C-terminal V5 epitope. Since Asn residues may undergo hydrolysis to Asp (21), the native residue in OATP1C1, we also introduced the Asp252Ala mutation. The effect of both mutations on T4 transport by OATP1C1 was evaluated in transiently transfected JEG-3 cells. Compared to wild-type OATP1C1, T4 uptake was diminished by 70% by the patient mutation and nearly completely inhibited by the artificial Ala mutation (Figure 2A). We evaluated the impact of both mutations on OATP1C1 protein expression levels by immunoblotting on total lysates of JEG-3 cells transfected with wild-type or mutant OATP1C1. For wild-type OATP1C1-V5, bands were detected at ~75 kDa, representing the mature, glycosylated protein (22), and at ~50 kDa, most likely representing its immature form (Figure 2B), as has been described for OATP2B1 (23). The Asp252Asn mutation predominantly reduced the abundance of the mature protein, whereas the Asp252Ala mutation resulted in a marked reduction of both proteins (Figure 2B). Cell surface protein expression determined by surface biotinylation analyses showed that both OATP1C1 mutants were markedly reduced in the cell membrane fraction compared to mature wild-type OATP1C1 (Figure 2C). This corresponded with the predominant peri-nuclear localization of both mutants as revealed by immunocytochemistry (Figure 2D). Similar results were obtained in COS-1 cells (data not shown). These findings suggested that both Asp252 mutations affect OATP1C1 protein maturation, stability and intracellular trafficking. To substantiate this hypothesis, we generated an OATP1C1 structure homology model based on the crystal structure of *E. coli* multidrug transporter MdfA (PDB#4ZP0) (Figure 2E). Molecular dynamic simulations suggested that Asp252 forms hydrogen bonds with
Lys248, both predicted to be located at the extracellular end of transmembrane domain 5, and Ser389 at the extracellular end of transmembrane domain 8 (Figure 2F). Such an inter-helical interaction is likely important for proper protein folding and stability.

The condition of the patient stabilized with Triac treatment

Treatment with Triac was started at 14.5 years, with co-administration of low-dose levothyroxine to maintain serum T4 and T3 concentrations in the low-normal range. After 6 weeks she resumed eye contact and became more alert (Table 1). Her general condition and quality of life improved. Importantly, the startle response episodes were markedly reduced in number and severity. Painful muscle spasms almost disappeared, she could swallow her own tablets and much of her own food, and urinary retention causing the need for bladder catheterization became rare. After the treatment with Triac was started, there was no further decrease in postural ability, and in fact in two postural positions the level of ability increased (Figure 1A). However, scoliosis continued to progress. Rectal temperature increased to normal values (Figure 1D, right). A slight increase in the heart rate during Triac treatment was considered tolerable (median heart rate during treatment=87 beats per minute, number of 40 measurements; data not shown).

Discussion

We report for the first time a human disease associated with mutation of the brain specific T4 transporter protein OATP1C1. It presented in childhood, at first with developmental impairments, later evolving as a neurodegenerative disease with a distinct intolerance to cold and severely reduced brain glucose metabolism. The progressive course appeared to be halted by treatment with the T3 analogue Triac. In
in vitro evaluation of the Asp252Asn mutation identified in the patient demonstrated a marked decrease in OATP1C1-mediated T4 transport, caused by intracellular retention of the transporter. Further, in silico modelling suggested that the highly conserved Asp252 residue stabilizes the transporter by hydrogen bond formation with Lys248 and Ser389. Also, the Asp252 residue is located close to the most evolutionary conserved domain in OATPs ("signature sequence" 267-279) (24), where single amino acid changes have been found to cause cytoplasmic protein retention (25). Thus, both in vitro and in silico analyses demonstrated a damaging effect of the patient mutation.

In the brain, impaired OATP1C1 function likely reduces T4 uptake in astrocytes and its subsequent conversion to T3. Thus, the reduced availability of T3 to target cells within the central nervous system appears to be the critical consequence of the OATP1C1 mutation in this disorder (Figure 3) to which the observed developmental delay, abnormal energy metabolism and subsequent neurodegeneration can be attributed. The causality between defective OATP1C1 and the observed phenotype is strengthened by the following aspects. First, the compromised brain glucose metabolism is compatible with a hypothyroid state of the brain (26). Importantly, a high glycolytic rate is typically observed in astrocytes, the main site of OATP1C1 expression. Second, cerebellar ataxia, present in the patient, is a known consequence of hypothyroidism in the brain (e.g. (27)). The presence of transient autism may also be attributed to the hypothyroid state since T3 also exerts a regulatory effect on cortical interneurons (28), found reduced in numbers in children with autism (29). Third, the single nucleotide polymorphism rs73069071, located downstream SLC01C1 and affecting its expression, has been associated with increased risk for hippocampal sclerosis in elderly patients (30), which supports the link between defective OATP1C1 and neurodegeneration. Fourth, the most notable cerebrospinal fluid abnormality was a
vast increase in neurofilament light protein concentration, indicating cytoskeletal decay of myelinated axonal fibers compatible with subcortical white matter atrophy and shrinkage of the corpus callosum. Key-factors in the formation and maintenance of myelin in the brain include kruepel-like factor 9 and myelin basic protein, which are T3-dependent genes (6,31). Finally, progression of the clinical course in the patient appeared to be halted or even improved by treatment with the T3 analogue Triac.

Despite the severe phenotypes associated with human MCT8 and OATP1C1 deficiency, neither Oatp1c1 (32) nor Mct8 (33) deficient mice exhibit a neurological phenotype due to functional overlap of the two transporters in the mouse brain. However, deletion of both Mct8 and Oatp1c1 leads to brain hypothyroidism and neurological deficits (34). In these animals, the therapeutic effect of the T3-analogue Triac, which enters the cell independent of Mct8 and Oatp1c1, was evident by restoring impaired neural differentiation caused by T3 deprivation (35). In our patient, there was a rapid reduction in the number of exaggerated startle response episodes, which are pathological and involve glycine inhibitory circuits in the brain stem and are potentially lethal (36). Body temperature normalization with Triac may have been mediated by hypothalamic regulatory mechanisms (37) although a direct thermogenic effect of Triac on brown adipose tissue is not excluded (38). At present, we cannot exclude that deficiency of additional substrates for OATP1C1 contributed to the neurological phenotype, but the positive effects of Triac on key features suggested that brain hypothyroidism was an important hallmark of this disease. Moreover, a direct impact of OATP1C1 deficiency on the TH status in tissues other than brain cannot be excluded, although its expression levels are generally low in the peripheral tissues. The normal serum TH concentrations observed in the patient suggest that inactivation of OATP1C1 does not have a major impact on the hypothalamus-pituitary-thyroid axis, which is in
line with the normat serum TH concentration in the Oatp1c1 knockout mouse model (32). Future studies and identification of new patients will help to advance the mechanisms of disease underlying OATP1C1 deficiency.

The field of TH transport has largely expanded in the last 15 years. After decades of belief that cellular entry of TH occurs via passive diffusion, the identification of MCT8 as a specific transporter for TH and the subsequent identification of mutations therein in patients revealed the physiological relevance of TH transporters. In recent years, it has become clear that OATP1C1 is a specific T4-transporter, which is importantly expressed in human astrocytes. We hope that our report, describing a novel neurodegenerative disease associated with a mutation in the T4 transporter OATP1C1, will fuel further research in the field of TH transporters.

Contributors

PS recruited the patient and performed clinical evaluation. PS, DM, EF, HH, WEV, TJV designed the study. LHJ evaluated the gross motor functioning. AB performed and described PET-CT scans. HZ performed cerebrospinal fluid measurements. AT, AH, DM, EF performed the genetic studies. SG, ECL, CZ, EG, MM, RPP TJV performed functional studies at cellular and in silico level. PS, DM, EF, SG, TJV wrote the first draft. All authors contributed to the analysis and the interpretation of the data.

Acknowledgments

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**Figure legends and table.**

**Figure 1. Clinical and neuroimaging data.**

(a) Postural ability, a part of gross motor functioning, evaluated with the Posture and Postural Ability Scale (PPAS) at different ages (10). A score of 7 indicate performance at the highest and 1 at the lowest level of postural ability. Levels 1-2 indicate no ability to maintain a postural position, and levels 3-7 indicate varying degrees of postural control, to either maintain or change position without assistance. The scores decreased markedly in all four postural positions (supine, prone, sitting, standing) between 10 and 13.5 years. During treatment with Triac, started at 14.5 years, the scores either remained at the same level (supine and standing positions) or increased (prone and sitting positions). The most noticeable improvement was in the sitting position, which went from level 2 (placed in an aligned sitting posture but needs support) to between 3 and 4 (able to maintain sitting position when placed, and able to move the trunk forwards-backwards). *Scores were obtained in retrospect from relevant data in the patient’s chart by the local physiotherapist or paediatrician in our hospital.*

(b) Series of T2 and T1-weighted cerebral MRI examinations at 7 (top panel) and 13 (bottom panel) years. Axial views through the centrum semiovale (T2 Ax CSO) showed increased widening of the subarachnoid spaces, indicating progressive cortical atrophy. Axial views at the level of the basal ganglia (T2 Ax BG) showed progression from mild to marked cortical atrophy, accompanied by widening of the lateral ventricles due to loss of central white matter.

(c) PET-CT scan images of the brain of the patient at 14 years, compared to a healthy control and to a patient with Alzheimer disease using 18F-Fludeoxyglucose (FDG) (left) or 18F-Flutemetamol (Flut) as tracer.
In the patient, the axial basal ganglia (Ax BG) view showed severely decreased $^{18}$F-FDG uptake, in the frontal, temporal and parietal lobes and in the thalami (thick arrow), and normal uptake in the occipital cortex and the basal ganglia (thin arrow). The coronal (Cor) view also demonstrated decreased $^{18}$F-FDG uptake in the hypothalamus (arrow head) and hippocampus. As expected, the Alzheimer patient showed reduced $^{18}$F-FDG uptake compared to the control. However, glucose metabolism in the patient, as indicated by these examinations, was markedly diminished, also compared to the Alzheimer patient.

In the patient, $^{18}$F-Flutemetamol uptake was almost undetectable in grey matter implying absence of amyloid-β deposition in this part of the cortex. The overall $^{18}$F-Flutemetamol uptake seen on axial basal ganglia (Ax BG) and sagittal midline (Sag Mid) views in the patient was less than in the control, corresponding with loss of white matter shown in MRI. To illustrate the contrast, extensive $^{18}$F-Flutemetamol uptake was documented in cerebral cortex in the patient with Alzheimer disease, reflecting typical aggregation of amyloid-β in this disease.

The examinations were performed as PET-CT, but only PET images are shown. The uptake intensity scale for each tracer is shown at the bottom of the panel.

(d) Home rectal temperature measurements of the patient before and during treatment with Triac (1050 mg/day). Before treatment the mean temperature was 36.65°C (number of measurements=18), during treatment the mean temperature increased to 36.95°C (number of measurements=52) (### P<0.001).
Figure 2. Functional in vitro analyses of the patient’s mutation.

(a) Organic anion transporter1 C1 (OATP1C1)-mediated thyroxine (T4) transport in transiently transfected JEG-3 cells. The D252N mutation identified in the patient and the D252A artificial mutation both display markedly reduced T4 transport by OATP1C1. Some residual activity of the Asp252Asn mutant may be due to partial hydrolysis of Asn252 to the native Asp residue.11 The results are presented as means ± SEM of 3 experiments, each performed in triplicate. Statistical significance of the differences was determined by one-way ANOVA with Bonferroni post-test (**P<0.001 versus wild-type).

(b) Representative immunoblot on total lysates of JEG-3 cells transfected with wild-type or mutant OATP1C1-V5 construct. The band at 75 kDa represents mature, glycosylated OATP1C1, and the band at 50 kDa supposedly represented an immature, non-glycosylated form. The quantity of the 75 kDa band was significantly reduced by the Asp252Asn and Asp252Ala mutations. Quantification of the 75 kDa band was performed using imaging software and levels were shown relatively to wild-type (100%) after normalization for GAPDH signal (presented as means ± SEM of 3 experiments). One-way ANOVA with Dunnett’s post-test was used to test for statistically significant differences between wild-type and mutant protein expression levels (*P<0.05, **P<0.01).

(c) Representative surface biotinylation assay in transfected JEG-3 cells, indicating the reduced abundance of the Asp252Asn and Asp252Ala mutants at the plasma membrane. Cell surface OATP1C1 protein expression levels were expressed as OATP1C1 (surface)/GADPH (total lysate) ratio relative to wild-type OATP1C1 (100%) and presented as mean ± SEM of 2 independent experiments. One-way ANOVA with Dunnett’s post-test was used to test for statistically significant
differences between wild-type and mutant surface expression levels (*P<0.05, **P<0.01).

(d) Subcellular distribution of wild-type and mutant OATP1C1-V5 protein (in green) in transfected JEG-3 cells. Plasma membrane localization is indicated by co-localization with tight junction protein ZO-1 (in red). Nuclear DNA is stained with DAPI (in blue). Wild-type OATP1C1 co-localized with ZO-1 at the plasma membrane, while both mutants showed a predominant peri-nuclear staining, suggesting abnormal protein trafficking.

(e) OATP1C1 homology model in inward-open conformation, based on the crystal structures of the *E. coli* multidrug transporter MdfA (PDB# 4ZP0) and KAZAL-type inhibitor infestin 4 (PDB#2ERW). A T4 molecule (blue) is docked in the substrate channel. The black box indicates the region magnified in Panel F.

(f) Molecular dynamic simulations (Methods in the Supplemental Appendix) indicating hydrogen bonds formation (purple dots) of Asp252 with Lys248 and Ser389, which may be important for proper protein folding and exposure of glycosylation sites. The lipid bilayer is depicted in grey.
**Figure 3. Thyroid hormone physiology in the brain.**

The thyroid gland produces the pro-hormone T4 and to a lesser extent its active hormone T3. In the body, the majority of T3 is produced locally by deiodination of T4. The main access of thyroid hormone to the brain is across the blood-brain-barrier, where MCT8 transports both T4 and T3. T4 uptake in astrocytes is mediated mainly by OATP1C1. T4 in astrocytes is converted by the DIO2 deiodinase to T3, which is released into the brain parenchyma by an unknown transporter. By this mechanism, astrocytes supply neurons and oligodendrocytes with sufficient amounts of T3 for proper neural differentiation and functioning. A defective OATP1C1 results in impaired uptake of T4 in astrocytes and therefore insufficient availability of T3 in neural cells. Triac, a T3 analogue with T3-like biological effect, normally excreted at very low concentration, passes the blood-brain-barrier and enters brain cells through an unknown transporter.
Table 1. Neurological functioning before and during treatment with Triac.

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>Timeline</th>
<th>9.5–10.5 years</th>
<th>11–12 years</th>
<th>13–14 years</th>
<th>During Triac treatment</th>
<th>6-14 weeks</th>
<th>26 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General condition</strong></td>
<td>Neurological regression</td>
<td>Further regression</td>
<td>Further regression</td>
<td>Further regression</td>
<td>Neurological improvement</td>
<td>Plateau*</td>
<td></td>
</tr>
<tr>
<td><strong>Mental status</strong></td>
<td>Autistic rituals†; socially active</td>
<td>Staring gaze, described as “being in her own world”</td>
<td>In her “own world” for longer periods</td>
<td>Almost no contact; stopped laughing</td>
<td>Gives social response; laughs at television</td>
<td>Interested in surroundings; laughs at television</td>
<td></td>
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<tr>
<td><strong>Language</strong></td>
<td>Unclear, diminished; understands commands</td>
<td>Increasingly absent; understands less</td>
<td>Absent; almost absent</td>
<td>Absent; almost absent</td>
<td>Inarticulate; some understanding</td>
<td>Inarticulate; some understanding</td>
<td></td>
</tr>
<tr>
<td><strong>Expressive; Impressive</strong></td>
<td></td>
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<tr>
<td><strong>Paroxysmal events</strong></td>
<td>Not recorded</td>
<td>Not recorded</td>
<td>Startle response easily provoked</td>
<td>Startle response, 10/day, accompanied by apnea</td>
<td>Startle response less frequent</td>
<td>Startle response, 1/day</td>
<td></td>
</tr>
<tr>
<td><strong>Muscle tone‡</strong></td>
<td>Increased with discrete limb contractures</td>
<td>Spastic in lower limbs, slightly spastic in arms</td>
<td>Increased spasticity; adducted thumbs; painful muscle spasms</td>
<td>Muscle spasms increasingly painful</td>
<td>Diminished muscle spasms</td>
<td>Muscle spasms almost disappeared</td>
<td></td>
</tr>
<tr>
<td><strong>Gross motor skills</strong></td>
<td>Broad-based gait; walks independently; frequent falls</td>
<td>Walks with support; falls from sitting position</td>
<td>Mostly wheelchair bound</td>
<td>Wheelchair bound</td>
<td>Rises from wheelchair; takes a few steps with support</td>
<td>Walks 10-15 steps with support; performance restricted by scoliosis</td>
<td></td>
</tr>
<tr>
<td><strong>Fine motor skills</strong></td>
<td>Slightly clumsy</td>
<td>Impaired ability to initiate movements</td>
<td>Few spontaneous movements; intention tremor</td>
<td>No spontaneous movements; intention tremor and myoclonic hand jerking</td>
<td>Uses switches and handles; hand myoclonus almost absent</td>
<td>Reaches out as before; hand myoclonus provoked by cold or fatigue</td>
<td></td>
</tr>
<tr>
<td><strong>Swallowing</strong></td>
<td>Impaired swallowing; open mouth-drooling§</td>
<td>Swallowing increasingly difficult</td>
<td>Most meals via a gastric tube</td>
<td>All meals via a gastric tube</td>
<td>Swallows her own tablets</td>
<td>Swallows 2 meals/day</td>
<td></td>
</tr>
<tr>
<td><strong>Bladder control</strong></td>
<td>Occasionally incontinent; uses diapers</td>
<td>Incontinent</td>
<td>Incontinent</td>
<td>Urinary retention; bladder catheterization 1-2/day</td>
<td>Almost no need for catheterization</td>
<td>Almost no need for catheterization</td>
<td></td>
</tr>
<tr>
<td><strong>Body temperature</strong></td>
<td>Feels often cold; cold hands and feet</td>
<td>Increasingly cold, even on warm days</td>
<td>Always cold; usually &lt;37 °C (rectal)‡</td>
<td>Always cold; usually &lt;37°C (rectal)</td>
<td>Feeling less cold</td>
<td>Feeling less cold; usually 37°C (rectal)</td>
<td></td>
</tr>
</tbody>
</table>
**Legend.** * Interestingly, the feet had stopped growing at 7 years with shoe size remaining the same (European shoes size 32, foot length 19 cm) for 8 years. Twenty-six weeks after onset of Triac treatment, the shoe size had increased to shoes size 35.

† The autistic rituals worsened in a cyclic fashion along with a build-up of sleep deprivation, ending with exhaustion and prolonged sleep. Melatonin was introduced at 13 years and improved sleep. Triac also seemed to improve sleep also, lasting usually approximately 5 hours before and 9 hours per night during treatment.

‡ Persisting plantar inversion and hyperreflexia were noted from age 10.5 years.

§ Open mouth with drooling persisted unchanged throughout the observation time.

¶ See Figure 1D.

$ The patient was treated with oral Triac 0.35 mg daily during the first two weeks, 0.35 mg twice daily during the following two weeks, continuing with 0.35 mg three times daily.
References