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


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Abstract

Context: Thyroid hormones (TH) are essential for brain development and function. The TH transporters monocarboxylate transporter 8 (MCT8) and organic anion transporter 1 C1 (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 is 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 aspartic acid 252 to asparagine (D252N). In vitro, the mutated OATP1C1 displays 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.
Funding Division of Pediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway; Netherlands Organisation for Health Research and Development (project number 113303005, 2014).
Research in context (no word restrictions).
Evidence before this study
This section should include a description of all the evidence that the authors considered before undertaking this study. Authors should briefly state: the sources (databases, journal or book reference lists, etc) searched; the criteria used to include or exclude studies (including the exact start and end dates of the search), which should not be limited to English language publications; the search terms used; the quality (risk of bias) of that evidence; and the pooled estimate derived from meta-analysis of the evidence, if appropriate.

We searched PubMed for relevant studies using the following search terms “OATP1C1” “SLCO1C1”, “Tria”, “Thyroid hormone and brain”, “thyroid hormone and positron electron transmission (PET) scanning”. We searched for previously described single nucleotide variants (SNVs) in SLCO1C1 in the following databases: http://exac.broadinstitute.org/, http://gnomad.broadinstitute.org/, https://www.ncbi.nlm.nih.gov/SNP/. We registered the identification of a putative disease causing variant in SLCO1C1 in GeneMatcher, (http://www.genematcher.org), a web-based tool with the goal of identifying additional individuals with rare phenotypes who had variants in the same candidate disease gene. We searched https://clinicaltrials.gov/, for clinical trials, with the following search terms “MCT8”, “Allan-Herndon-Dudley syndrome”, “Tria”.

Added value of this study
Authors should describe here how their findings add value to the existing evidence.

We describe for the first time isolated brain hypothyroidism due to mutation of OATPIC1. The disease was followed by neurodegeneration demonstrated in a series of MRI examination, accompanied by loss of cognitive and motor functioning, and lowered body temperature. At cellular level, we demonstrated reduced levels of the mutant OATP1C1 in the plasma membrane, resulting in reduced uptake of thyroid hormone. The progression of this clinical entity was halted by the T3 analog Triac. The condition of the patient stabilized and even improved and body temperature reversed to normal, suggesting that Triac treatment should be provided to future OATPIC1 deficient patients.
Implications of all the available evidence

Authors should state the implications for practice or policy and future research of their study combined with existing evidence.

Since the OATP1C1 homozygous mutation in the patient was identified by exome sequencing in a cohort of patients with unexplained progressive encephalopathy, it is expected that other diagnostic centers soon will make a similar discovery.

OAT1PC1 deficiency is a differential diagnosis in neurodegenerative diseases with childhood onset. Hypothermia in presence of normal plasma concentration of thyroid hormone is a clue to this condition. A vital message is that treatment with the T3 analog Triac is available and should be attempted as early as possible, since the disease progresses relentlessly.

Research in context panels should not contain references; key studies mentioned here should be referenced in the main text.

Introduction

Thyroid hormone, thyroxine (T4) and its active metabolite triiodothyronine (T3), is critical for brain development and functioning, underscored by the devastating consequences of congenital hypothyroidism.\(^1\) Important steps leading to thyroid hormone 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 receptors in these cells, T3 initiates powerful genetic control on myelination\(^5\) and neuronal differentiation in various brain regions\(^6\) (Figure 1A). In the human brain, T4 transport across the blood-brain barrier is 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 describe such a patient showing features of brain hypothyroidism. Progressive neurological deterioration and low body temperature were halted by treatment with the T3 analogue triiodothyroacetic acid (Triac).

**Case report**

The patient was a 15.5-year-old girl, born to healthy parents in a rural area in Norway. Her two younger sisters are 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”, for example that in the toddler age movements were clumsy and that vocal sounds and language seemed unexpectedly slow to develop. Between 2 and 3 years she appeared aggressive with stereotypic behaviour and was considered to be autistic. Intellectual disability was suspected at 4 years by the local health service. 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 jump 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. The latter could partly be evaluated with the Posture and Postural Ability Scale, PPAS10 (Table 1 and Figure 1B). 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 and spasticity of the lower limbs (Movie S1 in the Supplementary Appendix). At
14 years, she lost the ability to use her hands. Electroencephalographic examination showed intermittent slow frequency of 6 Hz without epileptic discharges. There was no clinical suspicion of epilepsy. Startle response episodes were easily provoked accompanied by apnoea of 30 seconds duration and cyanosis. She had myoclonic-like movements in the hands. Electromyography and nerve conduction studies did not indicate peripheral nerve pathology. Cerebral magnetic resonance imaging (MRI) examinations showed atrophy starting in the cerebral cortex, continuing into subcortical white matter and cerebellum (Figure 1C). 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. PET-CT with $^{18}$F-flutemetamol showed no amyloid-β aggregation (Figure 1D and Figure S1), consistent with normal concentrations of amyloid-β and tau in the cerebrospinal fluid. All serum and cerebrospinal fluid measurements are presented in Table S1 in the Supplementary Appendix. 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 (Table S1 in the Supplementary Appendix). She suffered inexplicable intolerance to cold and had cold hands and feet and frequent shivering and preferred to dress warmly even on hot days. A lowered body temperature was documented with a rectal thermometer (Figure 1E
left). Once, during general anaesthesia for a hospital procedure, she needed full-body warming blankets for hypothermia. Hypothyroidism was considered, however serum thyroid function tests were normal (Table S1 in the Supplementary Appendix). Growth charts of the patient are displayed in Figure S2 in the Supplementary Appendix).

Methods
The parents provided written informed consent to genetic studies and to publish results, photos and video. They consented to treatment with Triac. The Ethics Committee approved the genetic studies and the treatment. The methods used for cerebrospinal fluid and PET-CT examinations, evaluation of postural ability, molecular studies, and treatment with Triac, are described in Methods in the Supplementary Appendix.

Results
Molecular studies
Exome sequencing of the family trio identified in the patient a homozygous variant in \textit{SLCO1C1} (NM_001145946.1)12:g.20870143G>A, changing a highly conserved aspartic acid at position 252 to asparagine (Asp252Asn) in the OATP1C1 protein (Figure S3A and B in the Supplementary Appendix). In the patient the variant was part of a region of autozygosity of estimated minimum size of 4.38 Mb, compatible with the parents being second cousins (Table S2 in the Supplementary Appendix). Parents and one sister were heterozygous for the variant (Figure S3C in the Supplementary Appendix). It was not reported in public databases of sequence variants and not identified in 378 controls Sanger sequenced. 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,\textsuperscript{11} 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,\textsuperscript{12} and at ~50 kDa, most likely representing its immature form (Figure 2B), as has been described for OATP2B1.\textsuperscript{13} 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 (Figure S4 in the Supplementary Appendix). 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 \textit{E. coli} multidrug transporter MdfA (PDB#4ZP0) (Figure 2E and Figure S5 in the Supplementary Appendix). 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 extra-cellular end of transmembrane domain 8 (Figure 2F). Such an inter-helical interaction is most likely important for proper protein folding and stability.
Treatment with Triac

Treatment with Triac was started at 14.5 years. 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. Rectal temperature increased to normal values (P<0.001) (Figure 1E right). After the treatment with Triac was started, there was no further decrease in postural ability and in two postural positions the level of ability increased (Figure 1B). As Triac suppresses TSH, and consequently also thyroid hormone secretion, serum T4 was maintained at low-normal levels by administration of levothyroxine (Table S1 in the Supplementary Appendix). A slight increase in the heart rate during Triac treatment was considered tolerable (median=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. The compromised brain glucose metabolism was compatible with a hypothyroid state of the brain.14 The progressive course appeared to be halted by treatment with the T3 analogue Triac. 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),\textsuperscript{15} where single amino acid changes cause cytoplasmic protein retention.\textsuperscript{17} Both in vitro and in silico analyses demonstrated a damaging effect of the patient mutation. In the brain, impaired trafficking of the mutated OATP1C1 to the plasma membrane likely caused low T4 uptake in astrocytes and reduced conversion to T3. Thus, 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. The single nucleotide polymorphism, rs73069071, located downstream SLC01C1 and affecting its expression, was associated with increased risk for hippocampal sclerosis in elderly patients,\textsuperscript{16} further supporting causality between OATP1C1 defect and neurodegeneration. 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. Myelination is an age-dependent process involving several proteins, including myelin basic protein, regulated by T3 in the brain.\textsuperscript{5} T3 also exerts a regulatory effect on cortical interneurons,\textsuperscript{17} found reduced in numbers in children with autism.\textsuperscript{18} Cerebellar atrophy and ataxia, known consequences of hypothyroidism,\textsuperscript{19} were also present in the patient. Despite the severe phenotype in the patient, neither Oatp1c1\textsuperscript{20} nor Mct8\textsuperscript{21} 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.\textsuperscript{22} The therapeutic effect of Triac, a physiological T3 metabolite was evident in the double knockout model.\textsuperscript{23} Triac reaches target cells in the brain independent of transport by
Mct8 and Oatp1c1. Triac restores impaired neural differentiation caused by T3 deprivation in Mct8/Oatp1c1 double knockout mice. In mct8+/− zebrafish larvae, administration of Triac after the formation of the blood-brain barrier fully restores myelination and axonal outgrowth deficiencies. Currently, a Triac treatment trial is ongoing for patients with the Allan-Herndon-Dudley syndrome caused by MCT8 mutations (NCT02060474). Progression in the clinical course appeared to be halted or even improved by treatment with Triac. Notably, 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. Body temperature normalization with Triac may have been mediated by hypothalamic regulatory mechanisms although a direct thermogenic effect of Triac on brown adipose tissue is not excluded. 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. In conclusion, we describe a novel neurodegenerative disease associated with mutation of the T4 brain transporter OATP1C1. Treatment with Triac, even at a late stage, is advisable.

**Contributors**

PS recruited the patient, performed clinical evaluation. PS, DM, EF, HH, WEV, TJV designed the study. LHJ evaluated the gross motor functioning. AB performed PET-CT scan. 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, TJV wrote the first draft. All authors contributed to the analysis and the interpretation of the data.
Declaration of interest

The authors have no competing interests.

Acknowledgments

We are grateful to the family for taking part in this study. The study was supported by grants from Division of Pediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway and by a grant from the Netherlands Organisation for Health Research and Development (project number 113303005, 2014) (to WEV). The sequencing service was provided by the Norwegian Sequencing Centre, a national technology platform supported by the ‘Functional Genomics' and ‘Infrastructure' programs of the Research Council of Norway and the Southeastern Regional Health Authorities.
References


17 Westerholz S, de Lima AD, Voigt T. Regulation of early spontaneous network activity and GABAergic neurons development by thyroid hormone. *Neuroscience* 2010; **168**: 573-589.


Figure 1: Thyroid hormone transport and distribution, and pathology of the patient
Legend to Figure 1:

(A) Thyroid hormone physiology in the brain. The thyroid gland produces the pro-hormone T4 and to a lesser extent the active hormone T3. 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 DIO2 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.

(B) Postural ability, a part of gross motor functioning, evaluated with PPAS at different ages. A score of 7 indicates 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 trunk forwards-backwards).
(C) Series of T2 and T1-weighted cerebral MRI examinations at 7 and 13 years (top and bottom panels, respectively). 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.

(D) PET brain images at 14 years using $^{18}$F-FDG and $^{18}$F-flutemetamol. The uptake intensity scale for each tracer is shown to the right. The axial basal ganglia (BG) view showed severely decreased $^{18}$F-FDG uptakes in the frontal, temporal and parietal lobes and in the thalami (arrow head), and normal uptake in the occipital cortex and the basal ganglia (arrow). The coronal (Cor) view also demonstrated decreased $^{18}$F-FDG uptakes in the hypothalamus (arrow head) and hippocampus (arrow). $^{18}$F-Flutemetamol uptake was not noticed in grey matter implying absence of amyloid-β deposition in this part of the cortex. The overall $^{18}$F-Flutemetamol uptake seen on axial and midline sagittal views in the patient was less than in the control, corresponding with loss of white matter shown in MRI. The examinations were performed as PET-CT, but only PET images are shown in the figure.

(E) 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** T4 Uptake capacity

**B** Protein expression levels

**C** Cell surface expression

**D** Subcellular localization

**E** OATP1C1 homology model

**F** Location of Asp252 and role in protein stabilization

Legend to Figure 2:
(A) OATP1C1-mediated T4 transport in transiently transfected JEG-3 cells expressed as percentage of added T4. The Asp252Asn (D252N) patient mutation and the Asp252Ala (D252A) artificial mutation both 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. 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 posttest (***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 posttest 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 posttest 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) shows molecular dynamic simulations (Methods in the Supplementary 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.
<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>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 TV</td>
<td>Interested in surroundings; laughs at TV</td>
<td></td>
</tr>
<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; Inarticulate; Some understanding</td>
<td>Inarticulate; Some understanding</td>
<td></td>
<td></td>
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<tr>
<td><strong>Expressive; Impressive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></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</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 36°C (rectal)§</td>
<td>Always cold; usually 36°C (rectal)</td>
<td>Feeling less cold</td>
<td>Feeling less cold; usually 37°C (rectal)§</td>
<td></td>
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</table>
Legend to Table 1: Neurological functioning before and during treatment with Triac

* 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 always increased in a cyclic fashion with a build-up of sleep deprivation, ending with exhaustion and prolonged sleep. Melatonin was introduced at 13 years and improved sleep. Sleep also improved on Triac, lasting usually from approximately 5 hours before to 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 1B, 1E.