A child with a deletion in the monocarboxylate transporter 8 gene: 7-year follow-up and effects of thyroid hormone treatment

Amnon Zung, Theo J Visser, André G Uitterlinden, Fernando Rivadeneira and Edith C H Friesema

Pediatric Endocrinology Unit, Kaplan Medical Center, Affiliated with the Hebrew University of Jerusalem, Rehovot 76100, Israel and Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands

(Correspondence should be addressed to A Zung; Email: amzung2@bezeqint.net)

Abstract

Objective: The monocarboxylate transporter 8 (MCT8; SLC16A2) has a pivotal role in neuronal triiodothyronine (T3) uptake. Mutations of this transporter determine a distinct X-linked psychomotor retardation syndrome (Allan–Herndon–Dudley syndrome (AHDS)) that is attributed to disturbed thyroid hormone levels, especially elevated T3 levels. We describe the genetic analysis of the MCT8 gene in a patient suspected for AHDS and the clinical and endocrine effects of L-thyroxine (LT4) or liothyronine (LT3) treatment intending to overcome the T3 uptake resistance through alternative transporters.

Methods: The six exons of the MCT8 gene were amplified individually by PCR. As multiple exons were missing, the length of the X-chromosomal deletion was determined by a dense SNP array, followed by PCR-based fine mapping to define the exact borders of the deleted segment. The clinical and endocrine data of the patient during 6.5 years of LT4 treatment and two periods (3 months each) of low- and high-dose LT3 were evaluated.

Results: A partial deletion of the MCT8 gene (comprising five of six exons) was detected, confirming the suspected AHDS. MCT8 dysfunction was associated with partial resistance to T3 at the hypothalamus and pituitary level, with normal responsiveness at the peripheral organs (liver and cardiovascular system). Thyroid hormone administration had no beneficial effect on the neurological status of the patient.

Conclusion: We identified a 70 kb deletion encompassing exons 2–6 of the MCT8 gene in our AHDS patient. Both LT4 and LT3 administration had no therapeutic effect. Alternatively, treatment of AHDS patients with thyroid hormone analogs should be considered.

Introduction

Over the last 7 years, we have been investigating a child with severe psychomotor retardation and thyroid dysfunction, characterized initially by high triiodothyronine (T3), borderline to low thyroxine (T4), and normal TSH levels. The clinical phenotype and the thyroid hormonal pattern were suspected of the Allan–Herndon–Dudley syndrome (AHDS; OMIM #300523) (1). AHDS is known to be caused by mutations in the SLC16A2 gene that encodes the monocarboxylate transporter 8 (MCT8) (2). This transporter is expressed in numerous human tissues including brain, heart, placenta, lung, kidney, skeletal muscle, and liver (3–5) and facilitates cellular T3 uptake (6, 7). Subsequently, MCT8 mutations result in a diminished intracellular T3 concentration (8, 9). Patients with MCT8 gene mutations present with severe psychomotor retardation, generalized dystonia combined with spasticity, lack of verbal communication, and poor head control and coordination (1, 8–17). These neurological characteristics were attributed to impaired MCT8 transporter activity into the neurons of the CNS (8, 18). All reported cases were males (except one female patient with unfavorable non-random X-activation (19)), since the SLC16A2 gene is located on chromosome Xq13.2. Currently, no therapy is available for these patients, although a few interventions have been suggested to cope with some aspects of the syndrome (17, 20).

We describe here the genetic study and a follow-up of a child with AHDS who was treated with LT4 for about 6.5 years and with low- and high-dose liothyronine (LT3) for a short period of time. The treatment with high-dose LT3 was intended to overcome the T3 uptake inhibition at the CNS through alternative transport routes. Such routes could involve MCT10 and the organic anion transporting polypeptide 1A2 (OATP1A2), which facilitate T3 uptake similar to MCT8 (3, 21).
Case study

The patient

The male patient was born by vaginal delivery to non-consanguineous parents, with a birth weight of 3950 g. At the age of 2 months, he was evaluated for poor head control, hypotonia of the shoulder girdle, and no visual tracking. An extensive genetic and metabolic work-up revealed normal karyotype, normal levels of urinary amino and organic acids, venous blood gases, and both serum and cerebrospinal fluid ammonia and lactate. Yet, liver enzymes were mildly elevated, with alanine aminotransferase (ALT) in the range of 35–38 U/l (normal levels 4–27 U/l) and aspartate aminotransferase (AST) in the range of 45–48 U/l (normal levels 8–29 U/l).

In addition, thyroid tests revealed an unusual pattern of elevated total T3 up to 7.0 nmol/l (normal range 1.1–3.0 nmol/l), low to normal free T4 (FT4) levels (8.0–8.2 pmol/l, normal range 7.4–21.0 pmol/l), and normal TSH levels (2.9–4.3 mU/l). A standard thyrotropin-releasing hormone (TRH) stimulation test was performed at the age of 5 months. In response to IV administration of 100 μg/m² TRH, TSH increased from a basal level of 4.3 to a peak of 8.4 mU/l after 30 min, concomitantly with prolactin elevation from 348 to 583 mU/l. Since TSH elevation was below the expected values (22, 23), other pituitary hormones were examined, with normal response of GH to glucagon stimulation (peak level of 17 ng/ml) and cortisol to ACTH (synacthen) stimulation (peak level of 1397 nmol/l). At 7 years of age, a comprehensive evaluation of thyroid function of the index case and his family (father, mother, and two brothers) revealed high T3 (4.06 nmol/l; normal range 1.4–2.5) and remarkably elevated T3/T4 (reverse T3) ratio (22.6; normal range 3.1–13.0) in the patient, with minute elevation of T3 in one sibling (2.81 nmol/l) and normal tests in all other family members.

Brain magnetic resonance imaging (MRI) was performed at 6 months of age, revealing hypoplastic corpus callosum and delayed myelination of the brain. Auditory evoked response (at 8 months of age) and nerve conduction and needle electromyography (EMG) studies (at 7 years of age) were within the normal range. An abdominal ultrasound was performed at 10 months of age and revealed atrophic left kidney with normal right kidney and collecting system. This imaging was performed as part of a work-up for hypertension that was documented in the patient and spontaneously resolved after a short period of time. Renal function, expressed by serum creatinine levels, was normal over the years.

Between 6 months and 7 years of age, the patient was treated with LT4 at daily doses that ranged from 25 to 64 μg (2.6–4.0 μg/kg body weight). In response, TSH levels decreased from initial levels of 2.9–4.3 mU/l to

![Figure 1](https://www.eje-online.org)

**Figure 1** (A) Analysis of serum of the patient shows a negative correlation between TSH and free thyroxine (FT4) levels (log TSH versus FT4, R = −0.52, P < 0.01). The regression analysis included all TSH and FT4 values that were measured before and after triiodothyronine (LT3) administration (filled and open circles respectively). The shaded area represents the normal range of serum TSH and FT4 concentrations. (B) Analysis of serum of the patient shows no correlation between TSH and total triiodothyronine (T3) levels (log TSH versus T3, R = −0.14, P = NS). The regression analysis included all TSH and T3 values that were measured before and after LT3 administration (filled and open circles respectively). The shaded area represents the normal range of serum TSH and T3 concentrations. (C) Analysis of serum of the patient shows a positive correlation between FT4 and T3 levels during LT4 treatment (filled circles; R = 0.859, P < 0.001). Thyroid hormone levels before treatment and during LT4 therapy are depicted by open and filled triangles respectively.
levels in the range of 0.01–1.99 mU/l, maintaining a negative correlation with serum FT4 (R = −0.52, P < 0.01; Fig. 1A) but not with T3 levels (R = −0.14, NS; Fig. 1B). During LT4 treatment, there was a positive correlation between serum FT4 and T3 levels (R = 0.859, P < 0.001; Fig. 1C). The T3 levels before LT4 treatment are well above this regression line with serum FT4, suggesting that in untreated MCT8 patients, the elevated serum T3 are derived at least in part from increased thyroidal T3 secretion. Over those years, the patient was consistently growing along the 50th percentile in height and slightly below the 3rd percentile in weight.

Neurological examination at 7.5 years of age showed severe global developmental retardation, mainly in the motor and verbal aspects. The prominent signs were severe hypotonia mainly in the trunk with poor head control and no verticalization. The patient presented with increased muscle tone in the hamstrings and gastrocnemius muscles and Achilles tendons, mild contracture of the knees, and augmented deep tendon reflexes without clonus. Fine motor skills were severely disturbed with inability to hold small objects to bring them to mouth or to pass them from side to side. The patient had involuntary, choreiform movements, repetitive dystonic smiles, and only rudimentary, non-verbal communication expressed by social smile and intentional look toward his family members and some medical persons.

After several years of LT4 treatment at a final dose of 64 μg/day (4.0 μg/kg body weight), we initiated a washout period of 5 months, followed by LT3 (cytomel) treatment in two doses of 12.5 μg twice daily and 25 μg twice daily, each dose administered over a period of 3 months. We initiated this LT3 treatment in an attempt to improve some neurological functions of the patient. The treatment was approved by the Ethics Committee of Kaplan Medical Center and we obtained written informed consent from both parents.

The clinical and endocrine data of this intervention are summarized in Table 1. Before LT3 initiation, a heart rate of 114 was recorded with normal blood pressure. During treatment, the patient developed mild hypertension during both low- and high-dose LT3 administration with only mild elevation in heart rate. There were no other signs compatible with hyperthyroidism, as body weight was increased during therapy, and no excessive sweating or hyperactivity was observed. Along with an increment in serum T3 levels, FT4, and TSH levels were progressively decreased, liver transaminases were mildly increased, and sex hormone-binding globulin (SHBG) was remarkably increased in response to the elevation in LT3 doses. Yet, this intervention failed to induce any change in the patient’s motor or cognitive functions. The cognitive skills of the patient were examined before and after LT3 administration by a pediatric neurologist who found no change in verbal abilities and intentional facial expression.

**Cytogenetic and SNP microarray studies**

The DNA of the patient, his parents, and two brothers was extracted from peripheral lymphocytes by routine methods. PCR analysis of the MCT8 gene (SLC16A2) showed the presence of exon 1, while exons 2–6 were missing in the patient but not in his parents and brothers. Using this approach, it is not possible to discriminate between having one or two copies of the gene, meaning that we could not exclude that the mother was a heterozygous carrier of this X-chromosome deletion (Fig. 2A).

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**Table 1** Clinical parameters, thyroid hormones, and biochemical tests before and during low- and high-dose LT3 administration. Normal ranges for age are in parenthesis. Values out of normal range are in bold.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>End of LT4</th>
<th>0 time (5 m off therapy)</th>
<th>+ 3 months</th>
<th>+ 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapy (μg/day)</td>
<td>LT4 64</td>
<td>LT4 12.5 twice daily</td>
<td>LT4 25 twice daily</td>
<td>LT4 End of therapy</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>16.4; −3.3a</td>
<td>16.6; −3.3</td>
<td>17.0; −3.2</td>
<td>17.8; −2.9</td>
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<td>Pulse (73–113)</td>
<td>100</td>
<td>114</td>
<td>115</td>
<td>120</td>
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<tr>
<td>Blood pressure (112/71)b</td>
<td>102/70</td>
<td>106/77</td>
<td>126/80</td>
<td>137/86</td>
</tr>
<tr>
<td>Free T4 (pmol/l; 11.0–22.0)</td>
<td>11.3</td>
<td>8.6</td>
<td>4.3</td>
<td>3.0</td>
</tr>
<tr>
<td>T3 (nmol/l; 0.9–2.8)</td>
<td>4.4</td>
<td>4.4</td>
<td>5.2</td>
<td>9.4</td>
</tr>
<tr>
<td>TSH (mU/l; 0.28–4.3)</td>
<td>1.43</td>
<td>2.68</td>
<td>1.64</td>
<td>0.22</td>
</tr>
<tr>
<td>SHBG (nmol/l; 13–71)</td>
<td>280</td>
<td>250</td>
<td>290</td>
<td>360</td>
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<tr>
<td>AST (U/l; 8–28)</td>
<td>33</td>
<td>29</td>
<td>29</td>
<td>49</td>
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<tr>
<td>ALT (U/l; 4–27)</td>
<td>32</td>
<td>29</td>
<td>29</td>
<td>67</td>
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<tr>
<td>LDH (U/l; 185–764)</td>
<td>443</td>
<td>398</td>
<td>502</td>
<td>72</td>
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<tr>
<td>GGT (U/l; 5–45)</td>
<td>152</td>
<td>174</td>
<td>237</td>
<td>20</td>
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<tr>
<td>Alk Phos (U/l; −410)</td>
<td>0.43</td>
<td>0.36</td>
<td>0.33</td>
<td>181</td>
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<tr>
<td>Creatinine (mg/dl; 0.2–0.7)</td>
<td>193</td>
<td>165</td>
<td>181</td>
<td></td>
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<tr>
<td>Cholesterol (mg/dl; 100–200)</td>
<td>41</td>
<td>49</td>
<td>72</td>
<td></td>
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</tbody>
</table>

SHBG, sex hormone binding globulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alk Phos, Alkaline Phosphatase; LDH, lactate dehydrogenase; GGT, gamma-glutamyl transpeptidase.

aWeight in Z-score.
b90th percentile for age.
In order to define the approximate size of the deletion, to see whether other genes were located within the deletion, and to determine whether the mother was a carrier, we used the Illumina Infinium II HumanHap 610K SNP Genotyping BeadChip (Illumina, Inc., San Diego, CA, USA). Genotyping procedures were followed according to Illumina manufacturer’s protocols. Using the genomic DNA of the patient and his mother together with two randomly selected female controls, we determined that the deletion was only present in the DNA of the patient and therefore concluded that the deletion was likely of a de novo origin since the mother was not a carrier. We observed the complete loss of nine SNPs between rs511721 and rs16991777 spanning maximal 113.7 kb (Fig. 2B). Finally, the exact borders of the deleted segment were defined by PCR-based fine mapping, yielding a deletion spanning 69 546 bp on chromosome Xq13.2 and comprising five of six exons of SLC16A2 (Fig. 2B) confirming that the deletion in this patient was limited to the SLC16A2 gene.

Discussion

We had the chance to follow a patient with AHDS and to evaluate the clinical and endocrine response to a long-term treatment with LT4 and to a 6-month period of LT3 therapy. His severe global development retardation derives from an almost complete deletion (five of six exons) of the SLC16A2 gene and thus likely involves complete inactivation of MCT8 function. Unlike our patient, several reported AHDS patients with mutations in the SLC16A2 gene showed residual activity of the MCT8 (1, 8) and some of them developed walking and speech capacity (1). It has been suggested that these patients with partial inactivation of MCT8 might benefit from thyroid hormone treatment (7).

Although our patient was not an ideal candidate for such intervention at his relatively advanced age and conceivably complete inactivation of the MCT8, we hypothesized that the complete cellular resistance to T3 uptake can be partially overcome through alternative T3 transporters, e.g. MCT10 and OATP1A2 (SLCO1A2). MCT10 is a thyroid hormone transporter that preferentially facilitates cellular T3 uptake more effectively than MCT8. MCT10 (SLC16A10) mRNA is expressed in various tissues, with relatively low levels of expression in the brain (21), although recent studies performed by Alkemade et al. (24) showed high expression of MCT10 in specific areas of the human hypothalamus. OATP1A2 facilitates the transmembrane transport of thyroid hormones including T3 and is expressed in multiple tissues including the brain. Although it has a low specificity for thyroid hormones and probably plays a minor role as a thyroid hormone transporter, it has been suggested that it may serve as a back-up system in case of other transporters malfunction (3). LT3 treatment was examined only once in an AHDS patient with a complete loss of function of the MCT8 transporter, yielding no clinical improvement (17). In spite of these discouraging results, we aimed to examine the effect of higher doses of LT3 for a longer period of time, since LT3 in the previous study was administered only for about a month and later for a week, periods that might be too short to conclusively dismiss possible effects of LT3 treatment, especially in the CNS. Similar to the gradual response of peripheral organs to increasing levels of serum T3 (17), we assumed that the CNS might respond to T3 concentrations that exceed a certain threshold. Notably, we have started LT3 administration at serum T3 levels that were significantly lower than levels recorded during infancy (4.4 vs 7.0 nmol/l respectively).

During administration of high-dose LT3, we observed a significant elevation of T3 levels that exceeded the highest levels recorded during infancy, but no improvement of motor or cognitive skills could be detected by the pediatric neurologist. There are several possible explanations for the unresponsiveness to the treatment. First, the harmful effect of neuronal T3 deprivation in utero, a critical period for brain development, may be irreversible and permanent after birth. While athyreotic infants or those with an inability to synthesize T4 maintain essential levels of thyroid hormones in utero by trans-placental transfer from the mother (25), those with MCT8 inactivation cannot compensate for T3 absence in the neurons, even by remarkably elevated T3 levels. This may also explain the remarkable severity of AHDS patients compared with the old reports of patients with untreated congenital hypothyroid, especially in motor skills (26).

The devastating irreversible effect of thyroid hormone transport resistance can also be reflected by the brain structural damage associated with MCT8 dysfunction. Our patient displayed hypoplastic corpus callosum and delayed myelination of the brain in MRI performed at 6 months of age. Similarly, others have shown subtle cortical and subcortical atrophy (17) or delayed myelination from infancy (15, 27). The thyroid hormone treatment failure may alternatively be attributed to the introduction of the hormones late in life, i.e. well beyond a window of opportunity that may theoretically exist during the first weeks of life. Finally,
it is possible that the alternative transporters, namely MCT10 and OATP1A2, cannot adequately compensate for the MCT8 dysfunction and its associated severe neurological consequence.

A different approach was suggested by Wemeau et al. (20), who demonstrated a significant weight gain in a malnourished adolescent with AHDS, in response to a combined administration of propylthiouracil and LT4. Yet, this treatment had no beneficial effect on the neurological condition of the adolescent patient.

Several years of LT4 treatment and a short exposure of our patient to LT3 shed some light on the effects of thyroid hormone at the hypothalamus/pituitary level as well as the peripheral organs such as heart and liver. The pituitary gland was resistant to the suppressing effect of initial high T3 levels on TSH secretion, as TSH levels were within the normal range in spite of T3 levels up to 7.0 nmol/l. This resistance to T3 was observed over the years, demonstrated by a lack of TSH suppression in response to elevated T3 serum levels (Fig. 1B). Nevertheless, it was only a partial resistance since TSH levels (and subsequently FT4 levels) were remarkably suppressed in response to the gradual elevation in T3 levels during low- and especially high-dose LT3 treatment (Table 1). Furthermore, basal T3 imposed some suppressing effect on TSH elevation during TRH stimulation test, an observation that was reported by others (17, 28). In the human pituitary gland, MCT8 is expressed in the folliculostellate cells rather than the TSH-producing cells (29). It is assumed that these sites are involved in the negative feedback control of TRH in the hypothalamus and TSH in the pituitary gland by thyroid hormones (7). In our patient, who conceivably had complete inactivation of the MCT8 protein due to the deletion of an extended segment of the SLC16A2 gene, it is possible that the observed T3 effects were mediated by alternative T3 transporters (e.g., MCT10 and OATP1A2) located in the pituitary gland and the hypothalamus.

Unlike partial resistance to T3, the patient was sensitive to even mild elevation in FT4 levels during LT4 administration, presented as a reverse correlation between FT4 and TSH serum levels (Fig. 1A). LT3 treatment was previously reported in three other AHDS patients with a single-nucleotide substitution or deletion (15–17), compared to an almost complete deletion of the MCT8 gene in our patient. Yet, in spite of the distinct genetic differences, all four patients presented with a similar suppression of TSH and no change in cardiovascular indices or neurological functions in response to LT4 administration. Based on these limited data, it is conceivable that the response to LT4 is unrelated to the type of the genetic defect. Similarly, we could not detect an association between thyroid hormones and TSH levels at baseline and the type of mutation or size of deletion. Hence, while our patient presented with very high T3 and normal TSH levels, another patient with a large deletion in MCT8 gene of about 25 000 bp (8) presented with high levels of both TSH (8.8 mU/l) and T3 (6.1 nmol/l). Notably, high levels of T3 (7.3 nmol/l) were documented in a patient with a missense mutation (10) whereas normal levels of TSH were reported in many patients with all kinds of small size mutations and deletions (7, 8, 17, 20).

At the periphery, the cardiovascular system was responding to high T3 levels by mild tachycardia and hypertension (both chronotropic and inotropic effects) and the liver responded by SHBG elevation (and mild elevation in transaminase enzymes) in parallel with LT3 dose and T3 levels. Our patient had unilateral atrophic kidney. Although MCT8 is expressed in the kidney of rats (2) and mice (30), structural defects of the kidney were not reported in AHDS; hence, this finding in our patient is probably incidental. Unlike the reported increased sweating (17) and impaired weight gain that was attributed to a deleterious effect of high T3 at the adipose tissue (17, 20), our patient did not display these signs of clinical hyperthyroidism but rather gained weight during LT3 administration. We suggest that the low weight gain, common in AHDS (1), is derived from feeding problems in severely retarded patients rather than from high T3-related hypermetabolic state. Indeed, Wemeau et al. (20) found no significant change in resting energy expenditure measured in their AHDS patient, in spite of T3 suppression to normal levels during propylthiouracil (PTU) treatment. Alternatively, different responses of peripheral organs to elevated serum levels of T3, due to variability in the residual function of MCT8 transporter, may account for differences in hyperthyroid symptoms, including weight gain and loss among these patients. It should be emphasized that although weight loss in hyperthyroidism is common despite an increase in appetite, the occasional patient gains weight if caloric intake exceeds the metabolic rate (31). The mother of our patient has reported some better compliance for feeding during the LT3 course but no significant change in food intake.

In conclusion, even in a severe case with extended deletion of the MCT8 gene and apparently complete inactivation of the protein, some central responsiveness to T3 was observed along with peripheral responsiveness at the heart and the liver. These observations may reflect variability in MCT8 expression at different tissues or a role of alternative T3 transport systems. Unfortunately, these transporters could not mediate any motor or cognitive improvement in our patient, even in response to high doses of LT3. An early administration of thyroid hormones during infancy, especially in AHDS patients with partial activity of the MCT8, should be examined (7). Another therapeutic option in the future might be the administration of a thyroid hormone analog with reduced dependence on the MCT8 for cellular uptake that showed promising results in MCT8 knockout mice (32).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements
We thank Hans van Toor and Mila Jhamai for excellent technical assistance.

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Received 22 April 2011
Revised version received 17 August 2011
Accepted 5 September 2011