Supplemental Data

Detailed Case reports

Family #8 (subjects A1 and A2)

The affected monozygotic twin boys, hereafter referred to as subject A1 and A2, were born in Israel, to Ashkenazi, non consanguineous parents living in Australia. The mother, carrier of Gaucher disease, had gestational diabetes mellitus which was managed with diet. Amniocentesis revealed normal male karyotype. Born at 36-37 weeks gestation, weights of twins 1 and 2 respectively were 2490 and 2733 gm (10^{th} to 25^{th} centile). Apgar scores were 9 and 9 for both twins. Delay in ability to make eye contact until 3 months and to smile until 4 months brought the twins to medical attention. At age 5 months both were admitted for bronchiolitis. The twins were referred to a Child Developmental Center and found to have gross and fine motor delay. By the age of 10 months they could roll over but not sit unsupported. More specifically, they had a prominent axial hypotonia with poor head control and lag. They also had dystonic posture with hands fisted and held in extreme supine position, and hyperextension of the legs. Deep tendon reflexes were hyperactive with extensor plantar response, but no clonus. The twins had broad foreheads, wide nasal bridge, high arched palate, dolichocephaly (circumferences on the 10^{th} centile), and some bitemporal hollowing.

Chemistry was normal except for high lactic acid, ammonia and creatine kinase. Blood and urine amino acids, uric acid, pyruvic acid acyl carnitine, acyl glycine and urine guanidinoacetate were normal. TSH was 4.7 and 4.1 mU/L (normal range 0.35 – 5.5). Examination of twin 2 cerebrospinal fluid for neurotransmitter disorders gave a negative result. Electroencephalograms (EEG), awake and asleep, were normal. At 9 months both twins showed delayed myelination on magnetic resonance imaging (MRI) and a large choline peak on magnetic resonance spectroscopy (MRS) (1).

At 18 months of age, we found that in twin 1 and 2, respectively, serum T_3 was 54 and 60 % above the upper limit of normal (ULN), T_4 was 26 and 20 % below the lower limit of

normal (LLN) and rT_3 was 44 and 37 % below the LLN with normal TSH of 4.0 and 3.7 mU/L. We also identified a mutation in the *MCT8* gene; a single nucleotide substitution (c.962 C>T) producing a missense mutation (P321L) located in the 5th transmembrane domain of the molecule. The mother is heterozygous for the mutation and has a normal brother.

Their postnatal growth was similar to other subjects with MCT8 deficiency. While length progressed between the 10th to 25th centile, by the age of 6 months their weight dropped below the 1st centile.

Treatment with DITPA was started at 25 months of age in both twins.

Family #10 (subject B)

The affected boy, hereafter referred to as subject B, was born at term to non-consanguineous white European (Swiss) parents. His birth weight and length were 2840 g and 47.5 cm and Apgar score 9/9/10. Neonatal screen TSH was <15 mU/L (the cut off value for the program). Hypotonia was noted at 1 month of age and thyroid tests at 4 months showed a FT_4 15% below the LLN for age and total T_3 63% above the ULN. The *MCT8* gene, sequenced in Dr. Theo Visser's laboratory, showed a single nucleotide substitution (c.733 C>T) producing a stop codon (R245X). The mother was heterozygous for the same mutation; a maternal uncle, now aged 22 years, does not walk or talk and has seizures. MRI obtained at the ages of 3, 8 and 13 months showed various degrees of retarded myelination of the white matter, especially bifrontally. There was an increased myoinositol peak on MRS. Currently, at age 45 months, he cannot talk or walk but has no dyskinesia or seizures.

Treatment with DITPA was started at 8.5 months of age.

Family #11 (subject C)

The affected boy, hereafter referred to as subject C, was born in Canada to non consanguineous

Iranian parents. Born at 40 week gestation, his birth weight and length were 3875 g and 50 cm. Neonatal screen TSH was <17 mU/L (the cut off value for the program). At 3 months of age, long crying spells and poor sleep were attributed to colic and the infant was treated with Ranitidine. By 4 months of age his grand mother noted poor head control and by 5 months hypotonia was obvious. This initiated a number of analyses, including, blood and urinary amino acids and blood quantitative acylcarnitines which showed no gross abnormalities. Karyotype was normal. At 5 months MRI of the brain showed delayed myelination. This was still present, though at a lesser degree at 17 mo of age. Muscle biopsy showed a decrease in cytochrome oxidase and an increase in citrate synthase. He was 18 months old when thyroid tests were obtained showing a FT_4 40% below the LLN and a FT_3 60% above the ULL. It is at this point that genetic diagnosis was sought. We identified a single nucleotide substitution (c.1238 C>T) in the *MCT8* gene, producing a stop codon (Q380X). This is a de-novo mutation as it was not found in the mother. The child was growing normally between the 50^{th} and 75^{th} centile for length and between the 10^{th} and 25^{th} centile for weight. Dyskinetic episodes were noted only during febrile illnesses. At 21 months basal metabolic rate was +79%.

Treatment with DITPA was started at 25 months of age.

Source of DITPA and Permission for its use

DITPA produced by Sigma was purchased by the parents from either Titan Pharmaceuticals, Inc (South San Francisco, CA) or Syngene (Bangalore, India). In Australia the capsules were initially prepared by NxGen, a local pharmaceutical company and subsequently by Stenlakes Compounding Pharmacy, both in Sydney. In Switzerland, they were prepared by the hospital pharmacy and in Canada by Pharmachoice, Waterloo, ON. The concentration of DITPA in the capsules was verified by the Chicago laboratory as well as by other laboratories (Stenlake Compounding Chemist, Bondi Junction, Australia and Syngen International Inc). For details see supplemental Table 1.

Permissions were granted for the compassionate use of DITPA as follows: For subjects A1 and A2 in Australia, by the Therapeutic Goods Administration, of the Federal Government of Australia and also approved by the Medical Administration of Sydney Children's Hospital with written informed consent for experimental use of the drug signed by both parents. For subject B in Switzerland, approval was obtained from the national responsible body, Swiss Medic, and the Regional Ethics Committee. For subject C in Canada, permission was granted by the Hospital of Sick Children Executive Offices. Genetic testing was approved by the IRB of The University of Chicago and informed written consents were obtained.

Changes in length before and after of DITPA treatment expressed as SDS range

Subject A1: -1.6 to -0.6 before; -2.2 to -1.0 after

Subject A2: -1.8 to -0.9 before; -2.3 to 0.8 after

Subject B: -1.3 to -0.6 before; -1.9 to -0.6 after

Subject C: -0.9 to +1.8 before; -0.6 to +1.6 after

Note that variation in measurements is partly due to their spasticity.

1. **Gika AD, Siddiqui A, Hulse AJ, Edwards S, Fallon P, McEntagart ME, Jan W, Josifova D, Lerman-Sagie T, Drummond J, Thompson E, Refetoff S, Bönnemann CG, Jungbluth H** 2010 White matter abnormalities and dystonic motor disorder associated with mutations in the SLC16A2 gene. Dev Med & Child Neurol 52:475-482

Supplemental Table 1. DITPA content in capsule preparation

Subject	Date	Stated content	Measured Content	Laboratory
A1, A2	23 Feb, 2010	3.2	3.3	Chicago
A1, A2	6 March, 2010	3.6 10	3.7 9.1	Stenlake
C	30 Aug. 2010	2 4	1.9, 1.9 3.5, 3.6	Chicago
B	15 Dec, 2010	2 4	1.2 3.3	Chicago
C	31 Dec, 2010	2, 4	(97.4%)*	Syngene
A1, A2	17 July, 2012	2 10	1.9 9.3, 9.2	Chicago

Recovery from capsules is on the average 90%.
* Only purity given