Supplement to

Clinical and functional consequences of C-terminal variants in MCT8: a case series

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Supplemental results

Clinical description of individuals with clinical signs atypical for MCT8 deficiency

P6 was born to non-consanguineous parents at term by caesarean section with a birth weight of 2780 grams (-1.7 SD). He presented at the age of 7 years with moderate intellectual disability, microcephaly and a low body weight for age. Motor development was delayed. Independent sitting was achieved by the age of 11 months and he was able to walk independently by the age of 4 years. He was able to speak his first words by the age of 5 years. By the age of 8 years, he developed tonic-clonic seizures with epileptic abnormalities detectable on EEG. At time of last evaluation at the age of 9 years and 4 months, he still exhibited mild clumsiness with difficulties in climbing the stairs and he could only speak 3-4 words. Social interaction was normal. Body weight was low for age (-3.23 SD) and height was within the low-normal range (-1.9 SD). He did not present either marked hypotonia, or spasticity and dystonic features. Dysmorphic features included an elongated face, bulbous nose, large ears and convergent exotropia. Auditory brain stem responses and ophthalmology evaluation were normal. Cardiac and abdominal ultrasound examinations did not detect any anomalies. Brain MRI performed at 12 months and 3 years of age were normal. A metabolic diagnostic work-up, including plasma amino acids and plasma acylcarnitine profile, urinary organic acids and glycosaminoglycans, urinary oligosaccharides, blood and urine creatine-derived metabolites, was unremarkable. Isoelectric focusing of serum transferrin showed an increase of di- and tri-sialotransferrins. Thyroid function tests at the age of 7 years exhibited serum T3 concentrations within normal range and (free) T4 concentrations at the lower end of the normal range (**Table 1**).

P7 is a 5-year old boy born to non-consanguineous parents. He first presented at the age of 4 years with mild global developmental delay without additional health problems other than recurrent upper respiratory tract infections. Achievement of motor milestones and speech was delayed. He was 5 years at time of referral when he was able to walk and jump and speak in simple sentences. He reportedly had reduced exercise tolerance and was unable to walk for long distances. Mild swallowing difficulties

were reported by his parents. Physical examination did not reveal apparent abnormalities. He did not present hypotonia and dystonic and spastic features were absent, although he reportedly experienced increased muscle tone during emotional distress. Seizures were absent. Body weight and height were normal for age and resting tachycardia and excessive perspiration were absent during physical examination. Serum T3 concentrations were within normal range, whereas serum rT3 and (free) T4 concentrations were at the lower end of the normal range (**Table 1**).

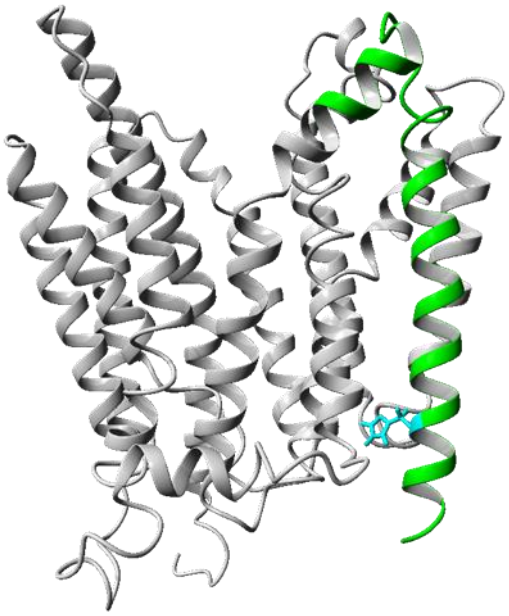
Supplemental Tables

Supplemental Table S1 Antibody Table

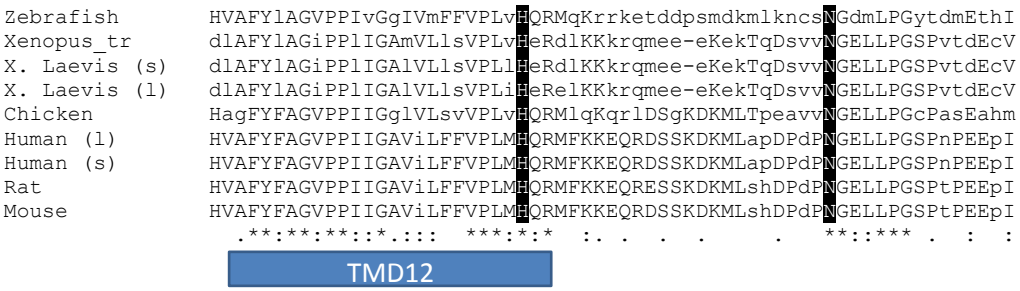
Target protein/ antigen	Antigen sequence (if known)	Name of AB	Species raised (P or M)	Manufacturer (and catalog number)	Dilution used for WB	Dilution used for ICH	RRID	Ref
hsMCT8	AA 52-155	MCT8	Rabbit (P)	ATLAS (HPA003353)	1:2000	1:1000	AB_1079343	(1)
GAPDH		GAPDH	Mouse (M)	Millipore (Mab 374)	1:20000		AB_2107445	(2)
ZO1		ZO1	Mouse (M)	Thermo Fisher (33-9100)		1:1000	AB_2533147	(3)
Rabbit IgG		IRDye800	Goat	LI-COR (926-32211)	1:20000		AB_621843	(4)
Mouse IgG		IRDye680	Goat	LI-COR (926-68020)	1:20000		AB_10706161	(5)
Rabbit IgG		Alexa 488	Goat	Thermo Fisher (A11008)		1:1000	AB_143165	(6)
Mouse IgG		Alexa 633	Goat	Thermo Fisher (A21050)		1:1000	AB_2535718	(7)

AB: antibody; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; ZO-1: zona occludens 1;P: polyclonal antibody; M: monoclonal antibody; WB: Western Blot ; ICH: immunohistochemistry

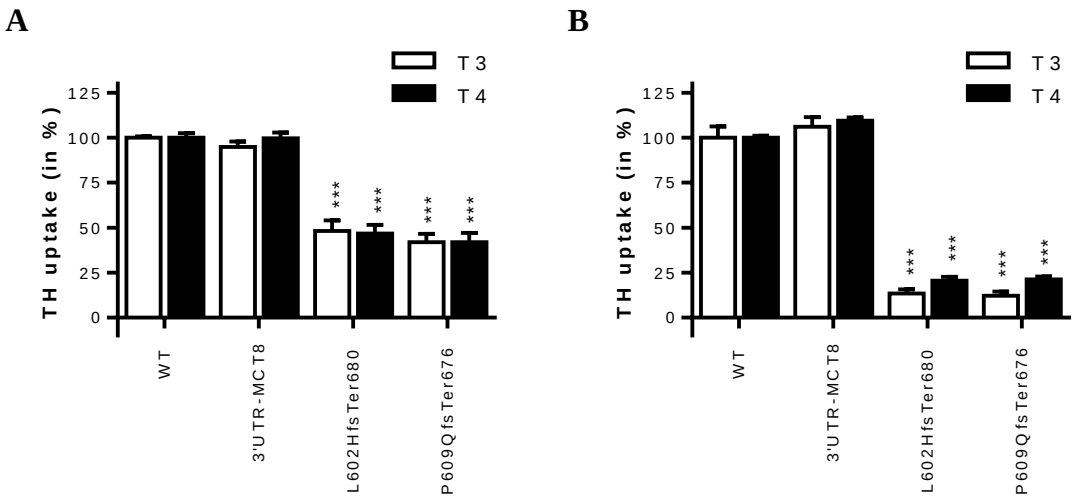
Supplemental Figures



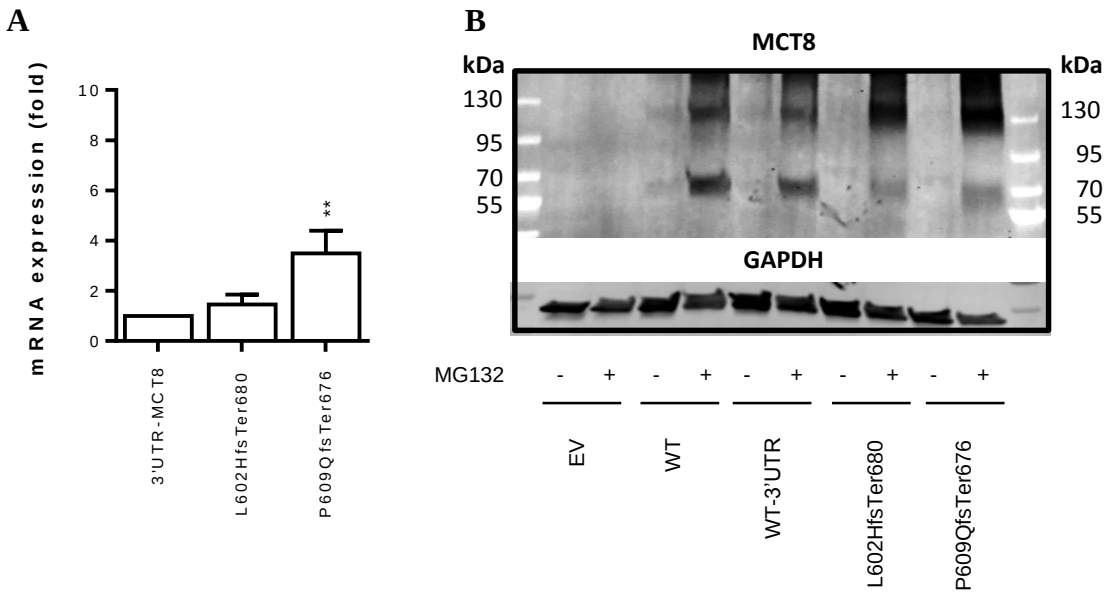
Supplemental Figure S1 MCT8 homology model (8) in which the residues encoded by exon 6 are highlighted in green. The His575 residue is highlighted in blue. The Asn599 residue is not included in the currently available MCT8 homology models (8,9) due to poor sequence alignment between the intracellular C-terminal tail of MCT8 and the available template structures.



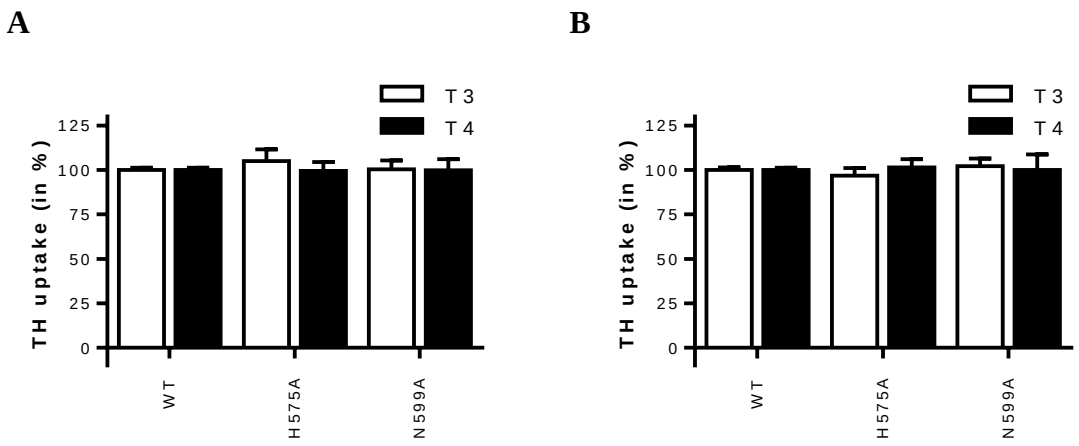
Supplemental Figure S2 Multiple sequence alignment of the C-terminal end of the MCT8 protein. This region includes transmembrane domain (TMD)12 and the intracellular C-terminal tail (see **Figure 1A** and **B**). Amino acid sequences of human, mouse, zebrafish, *Xenopus Tropicalis* (Xenopus tr), the short (s) and long (l) isoforms of *Xenopus laevis* (X. Laevis), chicken, and rat were included. Identical amino acids in all species are indicated with an *. Conservation between amino acids with strongly similar properties (equivalent to scoring > 0.5 in the Gonnet PAM 250 matrix) is indicated with an :, whereas conservation between amino acids with weakly similar properties (equivalent to scoring 0< score <0.5 in the Gonnet PAM 250 matrix) are indicated with a . . Multiple sequence alignments were generated using Clustal Omega. The His575 and Asn599 residues in human MCT8 and their corresponding (His and Asn) residues in other species are highlighted in black.



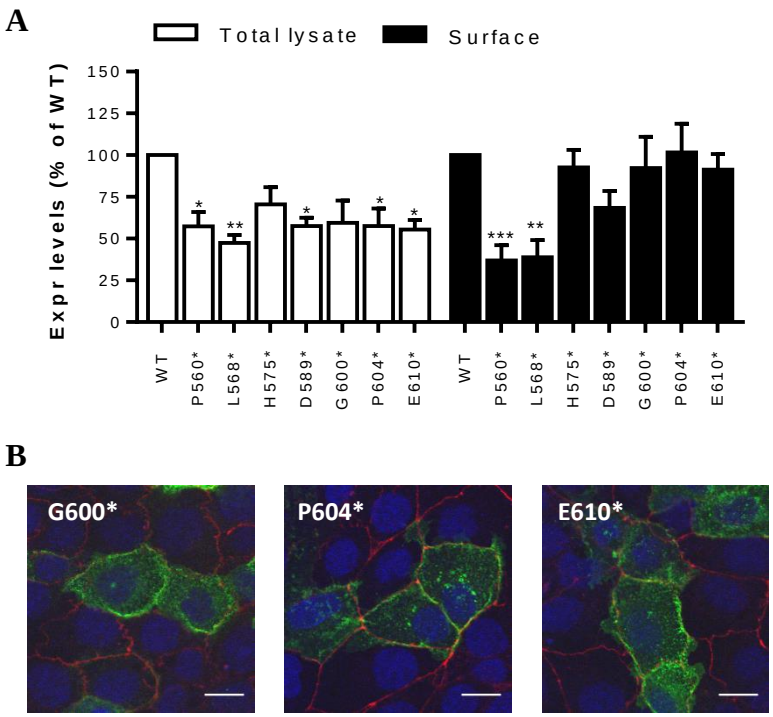
Supplemental Figure S3 T3 and T4 uptake in transiently transfected COS-1 **(A)** or JEG-3 **(B)** cells in presence of CRYM, after 30 min incubation at 37 °C. Uptake levels are corrected for those observed in pcDNA3 empty vector (EV) transfected control cells and expressed relative to standard wild-type (WT) MCT8. Data are presented as means ± SEM based on at least 2-3 independent experiments in duplicate. Two-way ANOVA with Bonferroni post-tests were performed to assess for statistically significant differences between WT MCT8, 3'UTR-MCT8 (WT MCT8 construct containing an extension of 227-nucleotide of the 3'UTR) and indicated MCT8 frameshift variants (generated using the 3'UTR MCT8 construct as a template; $p<0.005$ ***).



Supplemental Figure S4 (A) mRNA expression levels of 3'UTR-MCT8 and indicated frameshift variants in transiently transfected human JEG-3 choriocarcinoma cells. MCT8 expression levels are corrected for the housekeeping gene cyclophilin A, and expressed as fold difference over 3'UTR-MCT8. Data are presented as means ± SEM of a single experiment performed in triplicate. Statistical significance was tested using One-way analysis of variance analyses followed by Dunnett multiple-comparison test ($p<0.01$, **). **(B)** Representative immunoblot on total lysates of JEG-3 cells transfected with pcDNA3 EV, WT or 3'UTR-MCT8, or indicated frameshift variants. JEG-3 cells were cultured in the absence (-) or presence (+) of the proteasomal inhibitor MG132 (1 µM) for 24 hours prior to lysate preparation. Bands at ~60 kDa and ~120 kDa represent MCT8 monomer and homodimer, respectively (n=2).



Supplemental Figure S5 T3 and T4 uptake in transiently transfected COS-1 **(A)** or JEG-3 **(B)** cells in presence of CRYM, after 30 min incubation at 37 °C. Uptake levels are corrected for those observed in pcDNA3 empty vector (EV) transfected control cells and expressed relative to wild-type (WT) MCT8. Data are presented as means ± SEM based on at least 3 independent experiments in duplicate. Two-way ANOVA with Bonferroni post-tests were performed to assess for statistically significant differences between WT and indicated MCT8 variants.



Supplemental Figure S6 (A) Quantification of WT and indicated mutant MCT8 protein in total lysates and the biotinylated cell surface fraction in transiently transfected COS-1 cells (representative blots are shown in **Figure 4E** and **4F**). Expression levels were quantified using ImageJ software and expressed relative to WT MCT8. The means ± SEM from N=3 independent experiments are displayed and compared to WT MCT8 expression levels using Two-way ANOVA with Bonferroni post-tests. Statistically significant differences are indicated as follows: p<0.05,*; p<0.01, **; p<0.001, ***. **(B)** Immunocytochemistry in JEG-3 cells transiently transfected with indicated MCT8 variants using antibodies against MCT8 (green) and the membrane marker ZO-1 (red). Cell nuclei were stained with DAPI (blue). Images are presented as an overlay image. The scale bar represents 20µm.

References

1. RRID:AB_1079343, https://scicrunch.org/resolver/AB_1079343.
2. RRID:AB_2107445, https://scicrunch.org/resolver/AB_2107445.
3. RRID:AB_2533147, https://scicrunch.org/resolver/AB_2533147.
4. RRID:AB_621843, https://scicrunch.org/resolver/AB_621843.
5. RRID:AB_10706161, https://scicrunch.org/resolver/AB_10706161.
6. RRID:AB_143165, https://scicrunch.org/resolver/AB_143165.
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