## Title

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**BRIEF REPORT**

# Sorting Variants of Unknown Significance Identified by Whole Exome Sequencing: Genetic and Laboratory Investigations of Two Novel *MCT8* Variants

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Mutations in the cell membrane thyroid hormone (TH) transporter monocarboxylate transporter (MCT) 8 produce severe neuropsychomotor defects and characteristic thyroid function test (TFT) abnormalities. Two children with mild neurological phenotypes and normal TFTs were found to harbor *MCT8* gene variants of unknown significance (VUS), MCT8-R388Q that occurred *de novo,* and MCT8-Q212E. Normal TH transport and action in fibroblasts of MCT8-R388Q was demonstrated in a novel nonradioactive functional assay measuring the intracellular TH availability after L-T3 treatment. No genotype–phenotype correlation was found in additional family members carrying MCT8-Q212E. For the field of MCT8 deficiency, it is important to assess the significance of *MCT8* gene VUS.

**Keywords:** MCT8, Allan–Herndon–Dudley syndrome, thyroid hormone transporter, whole exome sequencing, variants of unknown significance

## Introduction

The monocarboxylate transporter (*MCT*) 8 gene (*SLC16A2*) encodes a specific thyroid hormone (TH) cell membrane transporter (1). Mutations in *MCT8* gene are responsible for the Allan–Herndon–Dudley syndrome (AHDS) (2–4). All reported patients have characteristic serum thyroid function tests (TFTs) abnormalities including elevated serum T3 and decreased serum T4 and reverse T3 that serve as biomarkers for AHDS, requiring confirmation by *MCT8* gene sequencing. Severe psychomotor defects including poor head control, truncal hypotonia, cognitive delay, and spastic quadriplegia have been observed in all reported with MCT8 deficiency. Most are unable to sit, stand, walk, or talk. However, patients harboring specific mutations have been reported to acquire the ability to walk with ataxic gait and/or develop some dysarthric speech (4).

### *Patients*

Proband-1 (Supplementary Data, Supplementary Fig. S1, II-2), a 12-year-old boy born to parents of northern European origin, was conceived by *in vitro* fertilization and born at 38 weeks by C-section after an uneventful pregnancy. A fraternal twin brother was reported to have autism. By two months of age, the proband was noted to have decreased muscle tone, excessive tongue thrusting, back arching, and difficulty holding his head. He started walking at 14 months and had dyspraxia. Extensive physical and occupational therapies begun at three years resulted in significant improvement. He learned to ride a bike, swim, and ski, although with some difficulties. He was left handed and able to write with some apraxia and talked with some speech difficulties. Physical examination was unremarkable, except for scoliosis with winged scapulae and decreased

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reflexes. No clinical thyroid phenotype was noted and his TFTs were normal (Supplementary Fig. S1).

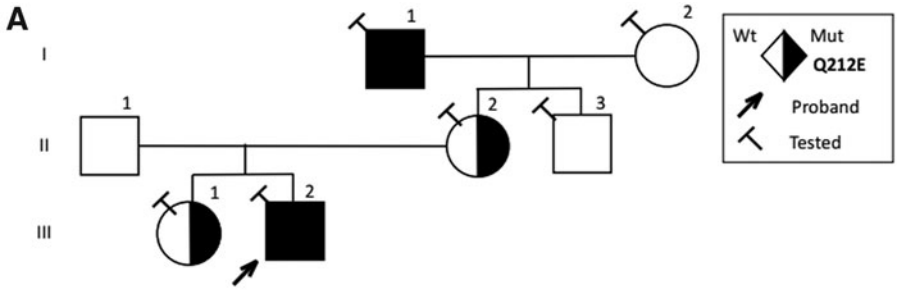
Proband-2 (Fig. 1A, III-2) is an 8-year-old boy born to a mother of German Mexican origin and father of Argentinian and Puerto-Rican origin. The proband had seizures since the age of 5.5 years, and was treated with lamotrigine. He was able to participate in sport activities for children with special needs, had problems with school performance ,and stuttering. He underwent weekly physical, occupational, and speech therapies. Physical examination revealed mildly decreased axial tone, hand weakness, and decreased deep tendon reflexes. No clinical thyroid phenotype was noted and his TFTs in him and all family members were normal.

## Results

Whole exome sequencing (WES) was performed to screen for a genetic cause for their condition. Hemizygous missense variants of unknown significance (VUS) were identified, MCT8-R388Q that occurred *de novo* in Proband-1 (Supplementary Fig. S1) and MCT8-Q212E (numbering according to the long MCT8 isoform with 613 amino acids) in Proband-2 (Fig. 1A), and their functional relevance was questioned.

Function predictions analyzing the MCT8-R388Q VUS with *in silico* algorithms provided conflicting results (Supplementary Table S1). To address the effect of the *de novo* MCT8-R388Q on TH transmembrane transport, we designed a new *in vitro* functional study in cultured primary human skin fibroblasts to compare the T3 uptake of fibroblasts from Proband-1 with that of fibroblasts from two MCT8-deficient patients with a severe phenotype (2), as well as a normal individual. The magnitude of the luciferase response to incremental T3 doses added to the medium of fibroblasts from Proband-1 was similar to that of fibroblasts from the normal individual, while it was greatly reduced in fibroblasts harboring MCT8-L512P and MCT8-A404Afs12* (Fig. 1B). Of note, these same negative and positive control fibroblasts were previously tested in a radioactive $^{125}$I-T3 uptake assay (5). These findings suggest that the R388Q mutation might have little or no effect on the MCT8-specific TH transport at physiological hormone concentrations.

For the MCT8-Q212E VUS, functional *in silico* predictions suggested a benign substitution. Fibroblasts could not




| Age (y) | 10 | 8 | 70 | 42 | 37 | 67 | Normal range |
|---|---|---|---|---|---|---|---|
| TT4 (nmol/L) | 99 | 102 | 97 | 94 | 86 | 109 | 64 - 154 |
| TT3 (nmol/L) | 2.2 | 2.4 | 2.0 | 1.6 | 2.2 | 1.6 | 1.2 - 2.9 |
| TrT3 (pmol/L) | 508 | 505 | 508 | 526 | 361 | **636** | 246 - 553 |
| FT4I | 9.0 | 8.5 | 8.1 | 7.8 | 8.2 | 8.9 | 6 - 10.5 |
| TSH (mU/L) | 3.0 | 3.0 | 1.6 | 1.8 | 1.7 | 2.2 | 0.4 - 3.6 |
| TG (µg/L) | 10 | 7 | 19 | 16 | 13 | 10 | 2 - 38 |
| TGab/TPOab | <0.4/<0.4 | <0.4/<0.4 | <0.4/<0.4 | <0.4/<0.4 | <0.4/<0.4 | <0.4/<0.4 | <0.4 / <0.4 |

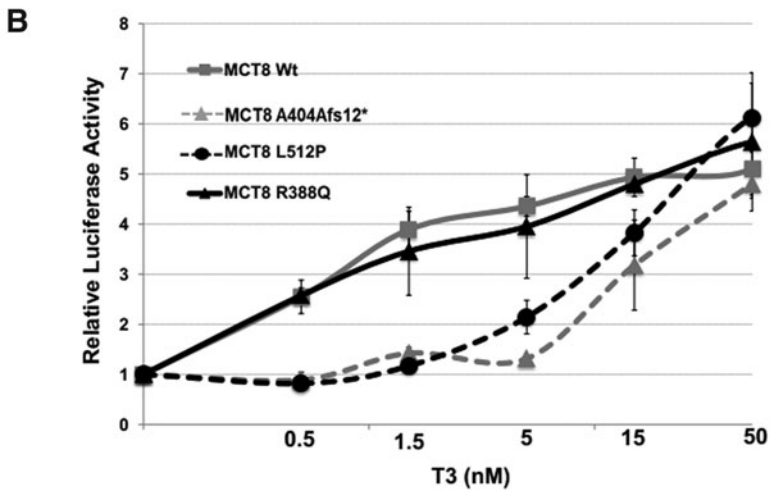



**FIG. 1.** (**A**) Pedigree of family 2 and thyroid function tests. Individuals are identified by generation (roman numerals on the left) and by a number on the right of each symbol. Results are aligned with each symbol representing a family member. Values outside the normal range are indicated in bold numbers. (**B**) Relative luciferase activity of fibroblasts treated with different amounts of T3. The results are presented as relative fold increase of the luciferase activity. T3 concentrations in the medium are shown in nM. The genotype of fibroblast is indicated in the legend on the top left of the figure. rT3, reverse T3; T3, triiodothyronine; T4, thyroxine; TG, thyroglobulin; TGab, antibodies against thyroglobulin; TPOab, antibodies against thyroperoxidase; TrT3, total rT3; TSH, thyrotropin; TT3, total T3; TT4, total T4.

be obtained from Proband-2. Thus, we next evaluated genotype–phenotype correlations in additional family members on the maternal side. The MCT8-Q212E VUS was also found in the proband's mother, sister, and maternal grandfather (Fig. 1A, II-2, III-1, and I-1), but not in the maternal uncle and grandmother (Fig. 1A, II-3, and I-2). The maternal grandfather had normal TFTs and did not have neurological abnormalities, thus confirming the benign functional behavior of this VUS.

## Discussion

Characteristic TFT abnormalities are a distinct biochemical signature in MCT8 deficiency that, together with the neurological manifestations, prompt investigation by candidate gene sequencing (2–4). When *MCT8* VUS are identified through WES in individuals with less typical neurodevelopmental manifestations, their clinical relevance must be questioned and TFTs should also be determined. If TFTs are not characteristic of MCT8 deficiency, the diagnostic significance of the detected VUS requires further functional investigations, as there is interest in the MCT8 field for genotype–phenotype correlation.

We report two such *MCT8* VUS and a new nonradioactive functional assay that assesses intracellular TH transfer in cultured human skin fibroblasts. This assay did not demonstrate a TH transport defect in the MCT8-R388Q VUS, but confirmed loss of function in previously characterized mutations associated with the AHDS. These findings are in agreement with absence of TFT abnormalities that are pathognomonic of MCT8 deficiency, and thus clarify the ambiguous *in silico* analysis results. For the MCT8-Q212E VUS, exclusion of a pathogenic effect could be established by genotype–phenotype studies of maternal relatives. This study emphasizes the importance of functional assays and/or establishing genotype–phenotype correlations to characterize the impact of novel VUS.

## Author Disclosure Statement

No competing financial interests exist.

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## Supplementary Material

Supplementary Data
Supplementary Table S1
Supplementary Figure S1

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