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CASE REPORT

New Cases of *DHTKD1* Mutations in Patients with 2-Ketoadipic Aciduria

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Abstract 2-Ketoadipic aciduria (OMIM 204750), a defect in the catabolic pathway of tryptophan, lysine, and hydroxylysine, is characterized by elevations in 2-ketoadipic, 2-aminoadipic, and 2-hydroxyadipic acids. Patients with the aforementioned biochemical profile have been described with a wide range of clinical presentations, from early-onset developmental delay, epilepsy, ataxia, and microcephaly to completely normal. This broad range of phenotypes has led some to question whether 2-ketoadipic aciduria represents a true disease state or if the biochemical abnormalities found in these patients merely reflect an ascertainment bias. We present four additional individuals from two families, with 2-ketoadipic aciduria with compound heterozygous or homozygous mutations in *DHTKD1*, three of which remain asymptomatic.

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Introduction

Organic acid analysis has identified elevated 2-ketoadipic acid in patients with a wide variety of symptoms ranging from psychomotor retardation, hypotonia, epilepsy, ataxia, and failure to thrive to no clinical phenotype at all. To date over 20 individuals have been reported, about half of whom were asymptomatic (Przyrembel et al. 1975; Fischer and Brown 1980; Duran et al. 1984; Danhauser et al. 2012). With no known genetic etiology, 2-ketoadipic aciduria was thought to represent ascertainment bias with clinical symptoms being coincidental findings (Duran et al. 1984; Danhauser et al. 2012; Saudubray et al. 2012).

In 2012, whole-exome sequencing (WES) of a patient with a biochemical diagnosis of 2-ketoadipic aciduria identified compound heterozygous variants in dehydrogenase E_1 and transketolase domain-containing protein 1 (*DHTKD1*). Sanger sequencing of *DHTKD1* in a second unrelated patient identified a missense mutation on one allele and a nonsense variant on the other (Danhauser et al. 2012). These patients presented with hypotonia and variable degrees of psychomotor delay, speech delay, and attention deficit hyperactivity disorder with an otherwise unremarkable neurological examination. Due to the phenotypic variability associated with 2-ketoadipic aciduria, functional studies in primary fibroblasts from these patients were employed in order to elucidate the genetic etiology which revealed increased levels of 2-ketoadipic acid in cells and medium that was corrected with expression of wild-type *DHTKD1* (Danhauser et al. 2012).

We report mutations in two additional families and highlight the fact that while genetic abrogation of *DHTKD1* can lead to the accumulation of 2-ketoadipic, 2-aminoadipic, and 2-hydroxyadipic acids, this disruption does not always result in an observed clinical phenotype.

Table 1 Summary of the clinical findings of patients with DHTKD1 deficiency

	Patient 1	Patients 2–4	Patient 3 (Danhauser et al. 2012)	Patient 4 (Danhauser et al. 2012)
cDNA (NM_018706.5)	c.2143C>T	c.915G>C	c.1A>G	c.1228C>T
	c.2185G>A	c.915G>C	c.2185G>A	c.2185G>A
Protein (NP_061176)	p.Arg715Cys	p.Gln305His	p.Met1?	p.Arg410*
	p.Gly729Arg	p.Gln305His	p.Gly729Arg	p.Gly729Arg
Elevated urine/plasma metabolites	+	+	+	+
Motor delay	+	–	+	+
Speech delay	+	–	+	+
Microcephaly	+	–	–	+
Hypotonia	–	–	–	+
ADHD	+	–	+	–
Brain MRI abnormalities	–	N/A	–	–
Improvement over time	+	N/A	+	+

DHTKD1 dehydrogenase E1 and transketolase domain-containing protein 1, *ADHD* attention deficit hyperactivity disorder, *MRI* magnetic resonance imaging, *N/A* not applicable

Methods

Amino acid analysis in urine and plasma and urine organic acid analysis was performed in established biochemical genetics laboratories using proprietary methods.

Exome sequencing in both patients was performed prior to the discovery that mutations in *DHTKD1* cause 2-ketoadipic aciduria. WES for patient 1 was performed as a trio with both parents at UCLA Molecular Diagnostics Laboratories, Los Angeles, CA. WES for the second family was performed on the proband and both parents by BGI Americas and analyzed at the University of Colorado Denver. Identified *DHTKD1* mutations were evaluated for possible effects on protein structure and function by MutationTaster, SIFT, PROVEAN prediction, MutationAssessor, and PolyPhen-2. Variant frequencies were derived from the 1,000 genomes database.

Neuropsychological evaluations were performed at CHOC Children's Division of Psychology using NEPSY-II, Preschool Language Scale, 4th Edition; Adaptive Behavior Assessment System, 2nd Ed.; Behavior Assessment System for Children, 2nd Ed.; Behavior Rating Inventory of Executive Function, Preschool Version; Child Development Inventory, Sensory Profile, Wechsler Preschool and Primary Scale of Intelligence, 3rd Ed.; and Woodcock Johnson Tests of Achievement, 3rd Edition.

Case Reports

Our first patient, a girl, was born at full term after a pregnancy that was complicated by maternal hypertension with no postnatal complications to non-consanguineous parents of Filipino and Northern European ancestry. At 15 months of age, she presented with a history of failure to thrive (weight <3rd percentile), seizure-like episodes, and biochemical abnormalities consistent with 2-ketoadipic aciduria. Her height remained between the 10th and 25th percentile (length/weight ratio <3rd percentile) and head circumference was below the 3rd percentile. Of note, maternal head circumference was also below the 3rd percentile. A metabolic assessment revealed elevated levels of plasma 2-aminoadipate (37 μmol/L, nl < 4) with elevated 2-ketoadipate (434 mmol/mol creatinine, nl < 2) and 2-hydroxyadipate (28 mmol/mol creatinine, nl < 2) in urine (Table 1). Over time, she has suffered chronic episodes of headaches/migraines with persistent head tilting, nausea, and emesis. Neurological evaluation was performed and electroencephalogram, magnetic resonance imaging, and magnetic resonance angiogram of the brain were unremarkable. At 24 months of age, she began to show mild developmental delay. Initial neuropsychological evaluations measured less than the 10th percentile in verbal fluency and processing speed. Over the course of a 4-year follow-up, trends of improvement were noted in these areas; however, following the most recent evaluation, she is now below the 1st percentile for auditory attention and was given a diagnosis of a reading disorder.

WES revealed two heterozygous variants, c.2143C>T; p.Arg715Cys and c.2185G>A; p.Gly729Arg, in *DHTKD1*. While the latter variant, c.2185G>A; p.Gly729Arg, was previously reported in two patients with autosomal recessive 2-ketoadipic aciduria (Danhauser et al. 2012), the former variant, c.2143C>T; p.Arg715Cys, is novel. Both

variants have been observed in the general population with minor allele frequencies of 0.05% and 0.18%, respectively.

Our second patient, a girl, was born to consanguineous parents of Maltese origin in 1978. Routine urine screening performed at 6 weeks of age detected increased glutamate. Subsequent amino acid and organic acid testing showed elevated urinary 2-aminoadipic, 2-ketoadipic, and glutaric acids in the proband as well as in two elder brothers, ages 5 and 7. All three affected siblings were described as clinically normal when identified and have been followed over time and remain asymptomatic in adulthood (Wilcken et al. 1980; Wilcken 2014, personal communication).

Data analysis of the proband's exome identified a novel homozygous variant in *DHTKD1*, c.915G>C; p.Gln305His. Sanger sequencing showed that the proband's siblings are also homozygous for this variant and that each parent is a carrier. This variant has been observed in the general population with a minor allele frequency of 0.05%.

No other candidate genes were found during the WES analysis for either patient sequenced. All variants identified are predicted by several algorithms to be disease causing (MutationTaster), damaging to protein function (SIFT), deleterious to protein structure (PROVEAN), and probably damaging to protein structure and function (PolyPhen-2) and are predicted to have a high functional impact for c.2143C>T; p.Arg715Cys and c.915G>C; p.Gln305His and a medium functional impact for c.2185G>A; p.Gly729Arg (MutationAssessor).

Discussion and Conclusions

2-Ketoadipic acid is formed through three routes: from 2-aminomuconate in the oxidation of tryptophan, from 2-aminoadipic acid formed in the mitochondrial oxidation of lysine via the saccharopine pathway, and from 2-aminoadipic acid formed in the brain-specific, peroxisomal oxidation of lysine via the pipecolic acid pathway (Posset et al. 2015). The subsequent conversion of 2-ketoadipic acid to glutaryl-CoA, which is common to all three pathways and has long been assumed to involve a multienzyme complex similar to those that act on pyruvate, branched-chain keto acids and 2-ketoglutarate; indeed, the activities of the 2-ketoglutarate and 2-oxoadipic dehydrogenases in porcine heart could not be separated (Hirashima et al. 1967).

Several subjects have been described with 2-ketoadipic aciduria, including a 14-month-old girl with hypotonia, intermittent metabolic acidosis, and developmental delay (Przyrembel et al. 1975), a 14-year-old retarded boy and his intellectually normal sister (Wilson et al. 1975), a 9-year-old boy with a mild learning disability and his normal brother (Fischer et al. 1974; Fischer and Brown 1980), a 10-year-old retarded girl (Casey et al. 1978), a 9-year-old retarded boy with a history of seizures (Duran et al. 1984), a 7-year-old girl with cerebellar ataxia (Vianey-Liaud et al. 1985), two unrelated children with developmental delay (Danhauser et al. 2012), and three apparently normal siblings detected by a newborn screening program in Australia (Wilcken et al. 1980; Wilcken 2014, personal communication), whose genetic findings and outcome are presented here. Evidence for defects in the metabolism of 2-ketoadipic acid in these patients have included increased excretion of 2-ketoadipic and/or 2-hydroxyadipic acid in urine, often together with 2-aminoadipic acid, and delayed clearance of 2-aminoadipic and 2-hydroxyadipic after an oral lysine load. These studies are not strictly comparable because of the variability in biochemical testing among them. Recent studies on two unrelated patients with 2-ketoadipic aciduria revealed mutations in *DHTKD1*, a nuclear gene that encodes a protein similar to the E_1 component of a 2-ketoglutarate dehydrogenase complex (Danhauser et al. 2012). Our data extends these observations, showing mutations in the same gene in several additional patients with 2-ketoadipic aciduria. One of these patients has symptoms, but the other three are asymptomatic adult siblings.

The finding that all three patients with the same mutation, i.e., c.2158G>A; p.Gly729Arg (Danhauser et al. 2012; this paper), are symptomatic may suggest a relation to the clinical phenotype. The population frequency of this variant may approach one in 650 (188 of 120,740 chromosomes in the Exome Aggregation Consortium (ExAC) cohort as of April 2015). While this frequency seems high, it could be consistent as an underlying cause of mild developmental delay as observed in these patients. The other known sequence variants appear to be much less frequent; c.915G>C; p.Gln305His was present only once out of 121,316 chromosomes examined, and c.2143C>T; p.Arg715Cys was present in two of 121,298.

While 2-ketoadipic aciduria can occur without apparent clinical manifestations in childhood, and the relation to clinical manifestations may be due only to sampling bias, it is difficult to exclude the possibility that asymptomatic individuals will develop a clinical phenotype later in life or that particular mutations cause clinical disease and others do not. Indeed, knockdown of *DHTKD1* expression in a variety of cell lines suggests that this protein plays a role in mitochondrial function and energy production (Xu et al. 2013); and a proteomic/metabolomic study in mice implicated *DHTKD1* in glucose homeostasis through its connection to 2-aminoadipic acid (Wu et al. 2014). These studies suggest the possibility that further phenotypes, perhaps with a later onset, may be associated with genetic abrogation of *DHTKD1*. Of particular note is the implication of a c.1455T>G; p.Tyr485* mutation in *DHTKD1* as the cause

of dominantly inherited Charcot-Marie-Tooth disease in one family in China (Xu et al. 2012). The c.1228C>T; p. Arg410* mutation found in a 2-ketoadipic aciduria patient (Danhauser et al. 2012) might affect the E_1 protein in a similar manner as p.Tyr485*, and it will be of interest to determine if a similar neurological phenotype emerges in this patient or in the parent with the same mutation.

In summary, we present four additional patients with 2-ketoadipic aciduria with variants in *DHTKD1*. Three of these individuals have remained phenotypically normal in to adulthood, while the other shows clinical characteristics similar to the previously reported patients with 2-ketoadipic aciduria (Danhauser et al. 2012). These findings support the genetic etiology of 2-ketoadipic aciduria and continue to highlight the phenotypic variability historically seen in the reported patients.

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Synopsis

Our paper highlights that while genetic abrogation of *DHTKD1* leads to the accumulation of 2-ketoadipic, 2-aminoadipic, and 2-hydroxyadipic acids resulting in a biochemical diagnosis of 2-ketoadipic aciduria, this disruption does not always result in an observed clinical phenotype.

Compliance with Ethics Guidelines

Conflict of Interest

Ashlee Stiles, Leah Venturoni, Grace Mucci, Michael Woontner, Stephen Goodman, and Jose Abdenur declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Animal Rights

This article does not contain any studies with animal subjects performed by any of the authors.

Contributions of Individual Authors

Ashlee Stiles and Leah Venturoni contributed equally to the manuscript. Each helped to draft the case report presented.

Grace Mucci performed all neuropsychological testing and provided detailed summary of the data for the case report.

Naser Elbalalesy provided direct patient care, performed neurological examinations, and interpreted neurology test results.

Michael Woontner helped to perform data analysis of WES and edited the manuscript to provide critical feedback.

Steve Goodman and Jose Abdenur contributed equally to the manuscript. Each helped to interpret the biochemical test results and draft and review the case report providing critical feedback.

Competing Interests

The authors have no competing interests.

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