

## REPORT

# *DHTKD1* Mutations Cause 2-Amino adipic and 2-Oxo adipic Aciduria

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Abnormalities in metabolite profiles are valuable indicators of underlying pathologic conditions at the molecular level. However, their interpretation relies on detailed knowledge of the pathways, enzymes, and genes involved. Identification and characterization of their physiological function are therefore crucial for our understanding of human disease: they can provide guidance for therapeutic intervention and help us to identify suitable biomarkers for monitoring associated disorders. We studied two individuals with 2-amino adipic and 2-oxo adipic aciduria, a metabolic condition that is still unresolved at the molecular level. This disorder has been associated with varying neurological symptoms. Exome sequencing of a single affected individual revealed compound heterozygosity for an initiating methionine mutation (c.1A>G) and a missense mutation (c.2185G>A [p.Gly729Arg]) in *DHTKD1*. This gene codes for dehydrogenase E1 and transketolase domain-containing protein 1, which is part of a 2-oxoglutarate-dehydrogenase-complex-like protein. Sequence analysis of a second individual identified the same missense mutation together with a nonsense mutation (c.1228C>T [p.Arg410\*]) in *DHTKD1*. Increased levels of 2-oxo adipate in individual-derived fibroblasts normalized upon lentiviral expression of the wild-type *DHTKD1* mRNA. Moreover, investigation of L-lysine metabolism showed an accumulation of deuterium-labeled 2-oxo adipate only in noncomplemented cells, demonstrating that *DHTKD1* codes for the enzyme mediating the last unresolved step in the L-lysine-degradation pathway. All together, our results establish mutations in *DHTKD1* as a cause of human 2-amino adipic and 2-oxo adipic aciduria via impaired turnover of decarboxylation 2-oxo adipate to glutaryl-CoA.

L-lysine degradation is a complex multistep mechanism involving mitochondrial, cytosolic, and peroxisomal enzymes. It converges with the degradative pathways of L-hydroxylysine and L-tryptophan at the levels of 2-amino adipic-6-semialdehyde and 2-oxo adipate, respectively (Figure 1). Although the molecular causes of most inherited deficiencies of lysine degradation have been elucidated, those of 2-amino adipic and 2-oxo adipic aciduria (MIM 204750) remain to be explained. Over 20 individuals with this metabolic disease are known. More than half of them are asymptomatic, whereas others have developed mild to severe intellectual disability, muscular hypotonia, developmental delay, ataxia, and epilepsy.<sup>1,2</sup> A defect in 2-oxo adipate dehydrogenase was first suggested as an underlying cause, but there was no experimental evidence. Furthermore, it was shown that the 2-oxoglutarate-dehydrogenase complex (OGDHc) also has substrate specificity for 2-oxo adipate.<sup>3</sup> Individuals with inherited 2-oxoglutarate-dehydrogenase deficiency usually present in the newborn period or infancy with severe neurological symptoms, as well as elevated 2-oxoglutarate and lactate.<sup>4</sup> Fiermonte and coworkers suggested a deficiency of the human 2-oxo adipate mitochondrial carrier as the underlying cause.<sup>5</sup>

To unravel the metabolic origin of 2-amino adipic and 2-oxo adipic aciduria, we studied two individuals who pre-

sented with the characteristic metabolic profile of this disease. Both were born to nonconsanguineous parents after an uneventful pregnancy and delivery and had an unremarkable family history.

Individual 1, a girl, presented with moderately delayed psychomotor development (sitting alone at age 10 months and walking alone at age 25 months) in infancy, and this improved by physiotherapy and occupational therapy. Metabolic analysis at the age of 2 years showed elevated 2-oxo adipate (range = 10–120 mmol/mol creatinine) and 2-hydroxy adipate (range = 5–40 mmol/mol creatinine) in urine; these are usually not detected. The plasma concentration of 2-amino adipate was also elevated (120  $\mu$ mol/l). Moderate protein restriction was introduced but did not influence the disease course and was thus discontinued. Serial cranial magnetic-resonance-imaging studies did not reveal any morphological abnormalities, and neuropsychological testing at the age of 8 years revealed an intelligence quotient (IQ) of 117. At 11 years of age, she was diagnosed with attention deficit hyperactivity disorder. Symptoms improved after implementation of methylphenidate. At the age of 14 years, her health is in excellent condition, and she is attending regular school and is following a normal diet. Neurological examination was normal.

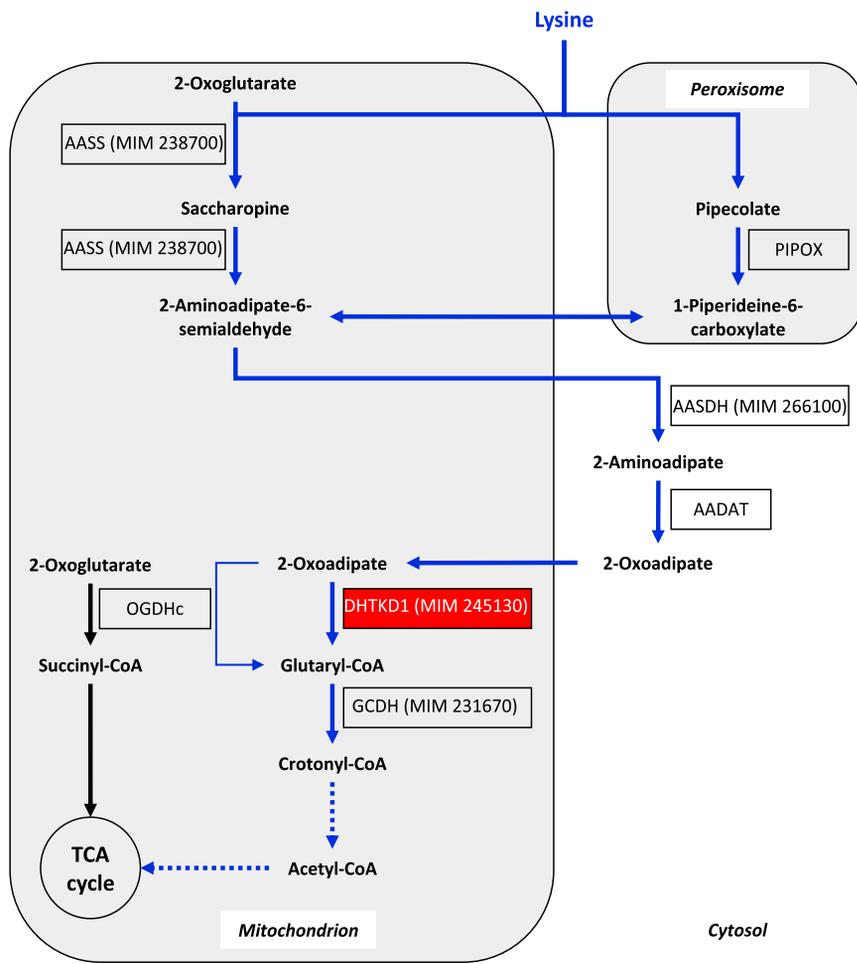
Individual 2, a girl, presented with microcephaly, mild motor developmental delay (sitting alone at the age of

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**Figure 1. Proposed Multicompartimental Degradative Pathway of L-lysine**

L-Lysine degradation is initiated by either peroxisomal  $\alpha$ -deamination (pipecolate pathway) or mitochondrial  $\epsilon$ -deamination (saccharopine pathway). Both pathways converge in 2-amino adipate semialdehyde, which is subsequently metabolized to 2-amino adipate and 2-oxoadipate. Our data strongly indicate that DHTKD1 is part of a 2-oxoglutarate-dehydrogenase-complex (OGDHc)-like enzyme and is involved in the decarboxylation of 2-oxoadipate to glutaryl-CoA. A minor route for this reaction via the tricarboxylic-acid (TCA)-cycle enzyme OGDHc might exist but does not compensate for the lack of DHTKD1. Succinyl-CoA and glutaryl-CoA are known to inhibit OGDHc (not shown). DHTKD1 might be an interesting drug target for glutaric aciduria type I, which is caused by inherited deficiency of glutaryl-CoA dehydrogenase (GCDH). MIM numbers of inherited diseases of the lysine degradative pathway are provided (e.g., MIM 245130 for 2-amino adipic and 2-oxoadipic aciduria). Dotted lines indicate multiple enzymatic steps. The following abbreviations are used: AADAT, 2-amino adipate transaminase; AASDH, 2-amino adipate-6-semialdehyde dehydrogenase; AASS, 2-amino adipate-6-semialdehyde synthetase; DHTKD1, proposed E1 subunit of an OGDHc-like complex; and PIPOX, pipecolate oxidase. Mitochondrial transporters are not shown in this figure.

9 months and walking alone at the age of 22 months), and predominant speech delay of unknown origin in early childhood. Sensorineural hearing loss was excluded. When she was 2 years old, metabolic analyses of urine and plasma revealed the characteristic biochemical profile of 2-amino adipic and 2-oxoadipic aciduria (2-oxoadipate, 520–970 mmol/mol creatinine; 2-hydroxyadipate, 100–150 mmol/mol creatinine; 2-amino adipate, elevated). Moderate protein restriction was transiently introduced but did not influence the disease course. At the age of 12 years, she still has significant problems with expressive and receptive speech (limited active and passive vocabulary, dyslalia, and dysgrammatism). Her IQ is 87. Except for mild muscular hypotonia, neurological examination was normal.

Written informed consent was obtained from all participants or their guardians at the recruiting center, and the study was approved by the ethical committee at the Technical University of Munich.

We performed exome sequencing as described previously<sup>6</sup> to identify mutations responsible for 2-amino adipic and 2-oxoadipic aciduria in individual 1. In brief, exonic sequences were enriched with the use of the SureSelect Human All Exon 50 Mb kit from Agilent and were subsequently sequenced as 100 bp paired-end runs on a

Hiseq2000 system (Illumina). Data analysis included read alignment with the Burrows-Wheeler Aligner (version 0.5.8) and variant calling with SAMtools (version 0.1.7). We produced 8.98 Gb of mappable sequences with an average exome coverage of 102.8 $\times$ , and >91% of the target was covered more than 20 $\times$ .

We next applied additional filters under the assumption of autosomal-recessive inheritance and a low allele frequency of causal mutations in controls. Accordingly, we excluded variants with a minor allele frequency (MAF) > 0.4% in SNP databases (HapMap and 1000 Genomes) and in 1,458 exomes from individuals with unrelated phenotypes.

This analysis identified eight candidate genes carrying predicted compound heterozygous or homozygous variants (Table 1). No mutation was detected in *ODC*, mainly excluding a dysfunctional 2-oxoadipate mitochondrial carrier as the underlying cause.<sup>5</sup> Of the eight candidates, three (*ADAMTS10*, *AIRE*, and *C8orf38*) had been previously linked to unrelated phenotypes and were therefore considered to be not involved. Among the remaining five genes (*DHTKD1*, *OVOS2*, *CAMTA2*, *LPPR3*, and *RADIL*), only one, *DHTKD1* (RefSeq accession number NM\_018706.5), carried two probably pathogenic variants as predicted by in-silico-analysis software (PolyPhen-2

**Table 1. Variants Identified in an Individual with 2-Amino adipic and 2-Oxo adipic Aciduria**

	Number of Variants
Synonymous	11,229
Nonsynonymous	10,252
Rare nonsynonymous	313
Genes with $\geq$ two nonsynonymous variants	8

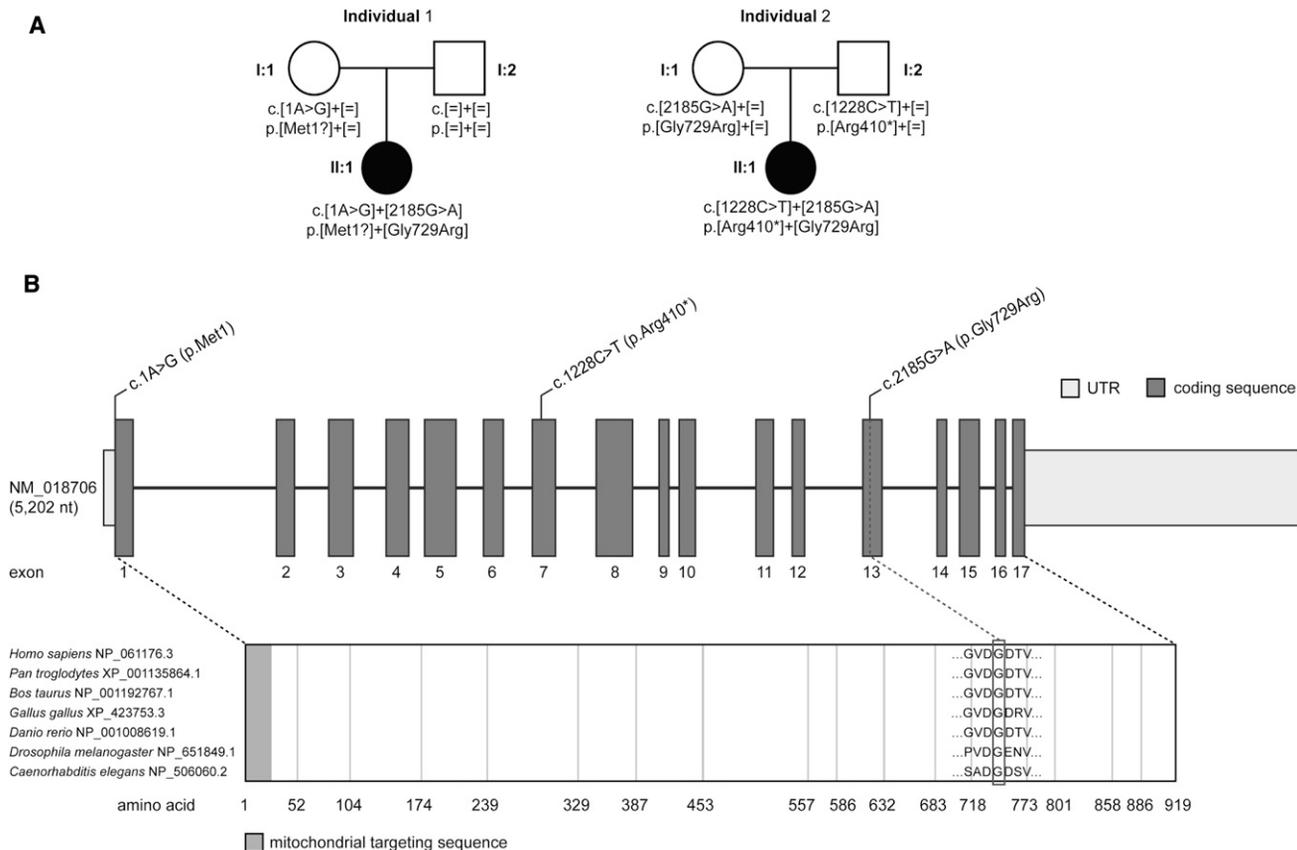
"Rare" indicates a frequency  $< 0.4\%$  in 1,458 control exomes, HapMap, and the 1000 Genomes Project. Nonsynonymous variants include missense, nonsense, stoploss, and splice-site mutations, as well as insertions and deletions.

and MutationTaster<sup>7</sup>). The first mutation, c.1A>G (p.Met1?), affects the start codon and might cause out-of-frame usage of the next AUG triplet 160 nucleotides downstream; it is therefore expected to produce a nonfunctional protein. The second mutation, c.2185G>A, is predicted to cause an amino acid exchange, p.Gly729Arg, affecting an amino acid residue evolutionarily conserved from *Homo sapiens* to *Caenorhabditis elegans* and *Drosophila melanogaster*. This mutation is listed in dbSNP (rs117225135) and was found in the National Heart, Lung, and Blood Institute (NHLBI) Exome Variant Server

with a MAF of 0.17%, corresponding to 19 heterozygous but no homozygous carriers among 5,379 individuals. Investigation of the carrier status of the parents showed that the c.1A>G mutation is present on the maternal allele but that the c.2185G>A mutation is absent from both the maternal and the paternal allele (Figure 2).

Sanger sequencing of the *DHTKD1* coding regions from individual 2 with the use of intronic primers (sequences are available upon request) identified compound heterozygosity for an exon 7 nucleotide transition, c.1228C>T, which is predicted to generate a stop codon (p.Arg410\*), as well as heterozygous mutation c.2185G>A, also observed in individual 1. Both parents were found to carry one mutation each in the heterozygous state. Identification of *DHTKD1* mutations in a second individual with the same metabolic profile supports a causal role for these mutations in the pathogenesis of 2-amino adipic and 2-oxo adipic aciduria. A summary of the genetic, biochemical, and clinical phenotype of individuals 1 and 2 is provided in Table 2.

Although both individuals carried one predicted loss-of-function allele, the effects of the shared missense mutation on *DHTKD1* activity were unclear. In order to confirm the detrimental functional consequences of the c.2185G>A (p.Gly729Arg) mutation and to verify the causal role of



**Figure 2. Pedigrees of Investigated Families and *DHTKD1* Structure and Conservation of Identified Mutations**

(A) Mutation status of affected (closed symbols) and unaffected (open symbols) family members.

(B) Structure of *DHTKD1* with localization and conservation of affected amino acid residues of identified mutations.

**Table 2. Genetic, Biochemical, and Clinical Phenotype of Individuals with *DHTKD1* Mutations**

ID	Sex	<i>DHTKD1</i> Mutations Identified		Biochemical Investigations			Clinical Features	
		cDNA (RefSeq NM_018706.5)	Protein (RefSeq NP_061176)	2-OA <sup>a</sup>	2-OHA <sup>a</sup>	2-AA <sup>a</sup>	Age of Diagnosis	Clinical Presentation
1	female	c.1A>G c.2185G>A	p.Met1? p.Gly729Arg	10–120 mmol/mol creatinine	5–40 mmol/mol creatinine	120 μmol/l	2 years	moderate psychomotor developmental delay and attention deficit hyperactivity syndrome
2	female	c.1228C>T c.2185G>A	p.Arg410* p.Gly729Arg	520–970 mmol/mol creatinine	100–150 mmol/mol creatinine	above normal	2 years	slight psychomotor developmental delay, predominant speech delay, and mild muscular hypotonia

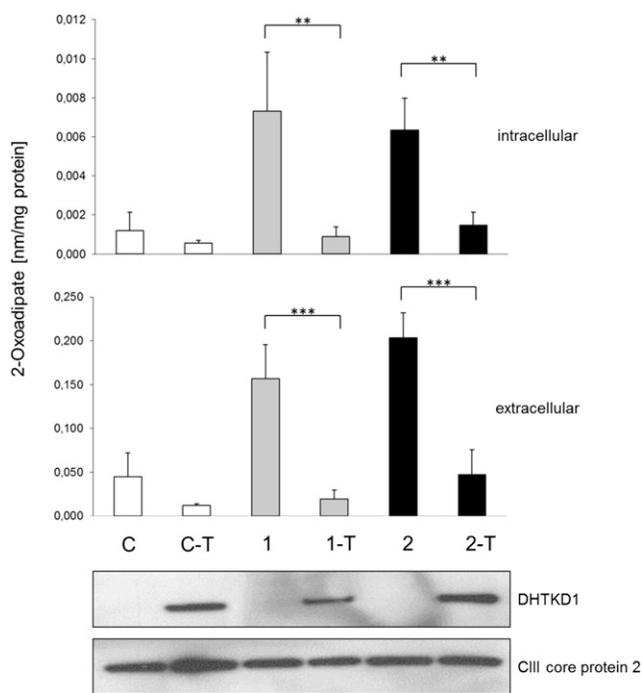
The following abbreviations are used: 2-OA, 2-oxoadipate; 2-OHA, 2-hydroxyadipate; and 2-AA, 2-aminoadipate.

<sup>a</sup>The reference range is <5.

the identified variants in *DHTKD1*, we performed complementation experiments in fibroblast cell lines established from both individuals 1 and 2. By using a feline-immunodeficiency-virus-based lentiviral system, we transduced an expression vector (Genecopoeia) containing the cDNA of *DHTKD1* and a neomycin resistance gene as described previously.<sup>8</sup> Immunoblotting with an antibody against *DHTKD1* (Sigma-Aldrich) on mitochondria-enriched samples confirmed higher protein levels after transduction (Figure 3). Next, we cultivated primary fibroblasts, as well as fibroblasts expressing wild-type *DHTKD1*, from individuals 1 and 2 and from a healthy control in MEM medium (PAA) containing 10% fetal calf serum (GIBCO, Life Technologies) and penicillin and streptomycin (PAA) for 4 days. Afterward, 2-oxoadipate levels were detected in cells and media with the use of gas chromatography/mass spectrometry (GC/MS) according to a previously described method<sup>9</sup> (fragment ion *m/z* 302). Compared to the control cell line (NHDF-neo, Lonza), both cell lines of the affected individuals showed increased levels of 2-oxoadipate in cells and media. Expressions of the wild-type *DHTKD1* in fibroblasts of individuals 1 and 2 led to a strong decrease in intracellular and extracellular 2-oxoadipate concentrations to the levels of those in metabolically competent control fibroblasts (Figure 3). To pinpoint the role of *DHTKD1* in lysine metabolism, we cultivated primary fibroblasts, as well as fibroblasts expressing the wild-type *DHTKD1*, in MEM medium as described above, but we supplemented them with 4 mmol/l 4,4,5,5-*d*<sub>4</sub>-lysine (CDN Isotope). The production of deuterium-labeled 2-oxoadipate was detected by GC/MS<sup>9</sup> (fragment ion *m/z* 306). Indeed, 4,4,5,5-*d*<sub>4</sub>-labeled 2-oxoadipate could be shown in medium and cells of individuals 1 and 2, whereas its levels were strongly reduced when the wild-type *DHTKD1* was expressed in these cells (Figure 4). In control fibroblast cells, 4,4,5,5-*d*<sub>4</sub>-labeled 2-oxoadipate was not detected. All together, our data provide strong evidence that reduced *DHTKD1* activity is responsible for 2-aminoadipic and 2-oxoadipic aciduria in the investigated individuals.

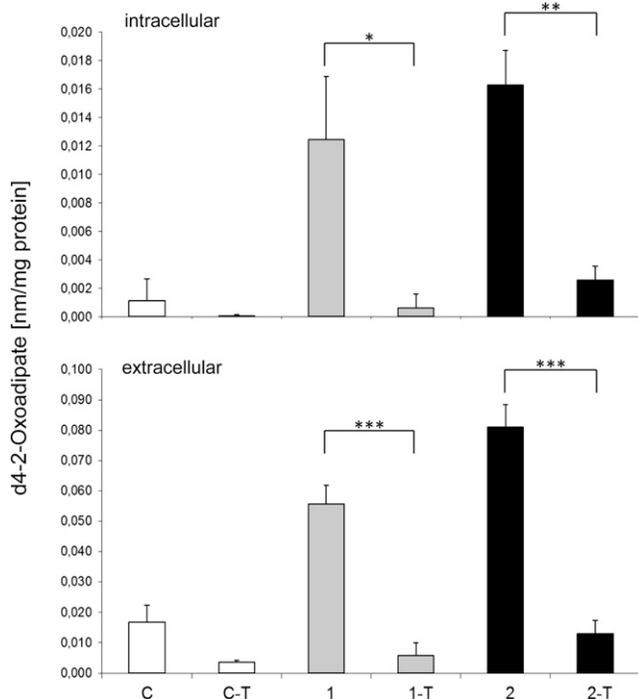
We applied an unbiased exome-sequencing-based approach to identify *DHTKD1* mutations as an underlying

molecular-genetic cause of a rare metabolic disorder, 2-aminoadipic and 2-oxoadipic aciduria. Although this metabolic profile is extremely rare, elucidation of the



### Figure 3. Metabolic Function of *DHTKD1*

Primary fibroblasts of several controls (C) and of individuals 1 and 2, as well as fibroblasts expressing wild-type *DHTKD1* from individuals 1 and 2 (1-T and 2-T, respectively) and a healthy control (C-T) were cultivated in MEM medium for 4 days. Fibroblasts from the affected individuals showed increased intracellular and extracellular levels of 2-oxoadipate. Expressions of wild-type *DHTKD1* in these cells reduced intracellular and extracellular 2-oxoadipate concentrations to the levels seen in metabolically competent control fibroblasts. Data are presented as the mean of five independent experiments  $\pm$  the standard deviation (\*\**p* < 0.01, \*\*\**p* < 0.001). Immunoblotting of the same cell lines was performed on mitochondria-enriched samples. The antibody against *DHTKD1* (Sigma-Aldrich, SAB 1400619, dilution 1:1,000) demonstrated increased levels of *DHTKD1*. An antibody against the complex III core protein 2 (Abcam, ab14745, dilution 1:1,000) was used as a loading control.



**Figure 4. d<sub>4</sub>-Oxoapipate Production by Fibroblasts**  
 Several control (C) fibroblasts and fibroblasts from individuals 1 and 2 with compound heterozygous *DHTKD1* mutations were cultivated in MEM medium supplemented with 4 mM 4,4,5,5-d<sub>4</sub>-lysine. Detection of d<sub>4</sub>-oxoapipate in cells and media showed an overproduction of d<sub>4</sub>-oxoapipate in fibroblasts of individuals 1 and 2; this overproduction was reduced by the expression of wild-type *DHTKD1* (C-T, 1-T, and 2-T). Data are presented as the mean of four independent measurements  $\pm$  the standard deviation. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

genetic defect and subsequent characterization of enzymatic activity of the gene product have provided new insights into a basic cellular pathway of amino acid degradation.

DHTKD1 is one of two known isoforms of the E1 subunit of the OGDHc. It has been speculated that the 2-oxoglutarate-dehydrogenase-like proteins have different substrate affinities.<sup>10</sup> The OGDHc catalyzes the decarboxylation of 2-oxoglutarate to succinyl-CoA within the citrate cycle. Our data suggest that DHTKD1 is part of an OGDHc-like supercomplex responsible for the decarboxylation of 2-oxoapipate to glutaryl-CoA within the common degradative pathways of L-lysine, L-hydroxylysine, and L-tryptophan.

In line with these findings, defective DHTKD1 activities resulted in increased levels of 2-oxoapipate and 2-oxoaminoapipate. In contrast to individuals who have other inherited disorders of amino acid degradation and who present with accumulating toxic metabolites and a severe clinical phenotype, such as phenylketonuria (MIM 261600), maple syrup urine disease (MIM 248600), tyrosinemia type I (MIM 276700), nonketotic hyperglycinemia (MIM 605899), and glutaric aciduria type I (MIM 231670), the individuals investigated in this study dis-

played rather mild and unspecific phenotypes. Of note, both individuals carried the same missense mutation, which might allow residual protein function. Therefore, it can be speculated that either complete loss of function leads to a more severe clinical phenotype or the mild clinical phenotype is explained by the low toxicity of accumulating metabolites. DHTKD1 is located one enzymatic step above glutaryl-CoA dehydrogenase in the lysine degradative pathway. Deficiency of glutaryl-CoA dehydrogenase causes glutaric aciduria type I, a cerebral organic acidemia that causes a severe neurological phenotype if untreated. Pharmacologic inhibition of DHTKD1 might be an interesting new drug target for glutaric aciduria type I in that it converts this disease into 2-aminoapipate and 2-oxoapipate aciduria, which has a less severe neurological phenotype. Interestingly, it has been reported that an apparent 2-aminoapipic aciduria also occurred during an antiepileptic treatment with vigabatrin and was probably caused by the inhibition of the metabolizing transaminase.<sup>11</sup>

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## Web Resources

The URLs for data presented herein are as follows:

MutationTaster, <http://www.mutationtaster.org>

NHLBI Exome Sequencing Project Exome Variant Server, <http://evs.gs.washington.edu/EVS>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>

PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2>

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