"Mutations in the D-2-hydroxyglutarate dehydrogenase gene cause D-2-hydroxyglutaric aciduria."

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**ABSTRACT**

d-2-hydroxyglutaric aciduria is a neurometabolic disorder with both a mild and a severe phenotype and with unknown etiology. Recently, a novel enzyme, d-2-hydroxyglutarate dehydrogenase, which converts d-2-hydroxyglutarate into 2-ketoglutarate, and its gene were identified. In the genes of two unrelated patients affected with d-2-hydroxyglutaric aciduria, we identified disease-causing mutations. One patient was homozygous for a missense mutation (c.1331T-->C; p.Val444Ala). The other patient was compound heterozygous for a missense mutation (c.440T-->G; p.Ile147Ser) and a splice-site mutation (IVS1-23A-->G) that resulted in a null allele. Overexpression studies in HEK-293 cells of proteins containing the missense mutations showed a marked reduction of d-2-hydroxyglutarate dehydrogenase activity, proving that mutations in the d-2-hydroxyglutarate dehydrogenase gene cause d-2-hydroxyglutaric aciduria.

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Mutations in the D-2-Hydroxyglutarate Dehydrogenase Gene Cause D-2-Hydroxyglutaric Aciduria

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D-2-hydroxyglutaric aciduria is a neurometabolic disorder with both a mild and a severe phenotype and with unknown etiology. Recently, a novel enzyme, D-2-hydroxyglutarate dehydrogenase, which converts D-2-hydroxyglutarate into 2-ketoglutarate, and its gene were identified. In the genes of two unrelated patients affected with D-2-hydroxyglutaric aciduria, we identified disease-causing mutations. One patient was homozygous for a missense mutation (c.1331T>C; p.Val444Ala). The other patient was compound heterozygous for a missense mutation (c.440T>G; p.Ile147Ser) and a splice-site mutation (IVS1-23A>G) that resulted in a null allele. Overexpression studies in HEK-293 cells of proteins containing the missense mutations showed a marked reduction of D-2-hydroxyglutarate dehydrogenase activity, proving that mutations in the D-2-hydroxyglutarate dehydrogenase gene cause D-2-hydroxyglutaric aciduria.
Table 1

D-2-Hydroxyglutarate Dehydrogenase Activity in HEK-293 Cells Overexpressing the Wild-Type or Mutated Human Enzyme

<table>
<thead>
<tr>
<th>Transfected</th>
<th>Activitya (pM/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-2-Hydroxyglutarate Dehydrogenase</td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>2.00 ± 0.3</td>
</tr>
<tr>
<td>Val444Ala mutant</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Ile147Ser mutant</td>
<td>0</td>
</tr>
</tbody>
</table>

NOTE.—Transient transfections were performed as described elsewhere (Achouri et al. 2004). The nucleic acid sequences of the mutated plasmids were confirmed by sequence analysis. The enzymatic activity was assayed by use of the radiochemical assay in the presence of 2 μM D-2-hydroxyglutarate without added Co2+. Activities are expressed as the activity observed after subtraction of the value in nontransfected HEK cells (0.16 ± 0.02 pM/min/mg protein). Data are mean ± SEM of three or four transfections.

bitemporal diameter, a prominent forehead, and micrognathia. The patient exhibited psychomotor retardation, experienced episodes of vomiting, and suffered from tonic, tonic-clonic, and myoclonic seizures that were not responsive to antiepileptic treatment. He died at 2 years of age. Quantitative measurements of D-2-HG showed urine levels at 502 mM/M creatinine (normal, <18 mM/M creatinine), plasma levels at 26 μM/l (normal, <1 μM/l), and cerebrospinal fluid levels at 6 μM/l (normal, <0.4 μM/l). L-2-HG was within the reference range, in all samples. Two siblings died with symptoms similar to those observed in the patient (no data available), and one sibling is unaffected.

In the D-2-hydroxyglutarate dehydrogenase gene of patient 1, a homozygous T→C transition (c.1331T→C) that results in the substitution of alanine for valine at position 444 (p.Val444Ala) was found in exon 9 (see fig. 1). The parents were heterozygous for this mutation, proving homozygosity in their affected child. Valine and alanine belong to the same group of amino acids with the substitution of serine for isoleucine (p.Ile147Ser). Isoleucine is conserved in the mouse D-2-hydroxyglutarate dehydrogenase protein and in the actin-interacting protein 2 of S. cerevisiae (Achouri et al. 2004). The conservation of isoleucine, the differences in the chemical properties of serine versus isoleucine, and the absence of this mutation in 210 control chromosomes suggest that this substitution is pathogenic. Indeed, overexpression in HEK-293 cells of the mutant protein containing p.Ile147Ser proved that the amino acid change results in marked reduction of D-2-hydroxyglutarate dehydrogenase activity (table 1).

The heterozygous mutation in intron 1, IVS1-23A→G, was not found in 210 control chromosomes and thus could represent a pathogenic mutation. RT-PCR was performed on RNA isolated from cultured fibroblasts of the patient and showed that only the allele containing the missense mutation was present, which indicates that the second allele is a null allele. mRNA was isolated from fibroblasts that were incubated for 5 h with cycloheximide (0.25 mg/ml), an inhibitor of protein synthesis, which avoids nonsense-mediated decay. RT-PCR analysis using this mRNA identified the structure of the second mutant allele. It arises as a result of the creation of an alternative spliceacceptor site located 19 nt upstream of the wild-type splice-acceptor site, leading to the insertion of 19 nt into the mature mRNA (r.295_296ins19), and it involves the IVS1-23A→G site. This insertion results in a frameshift that causes a premature stop (p.Cys100fsX9). The combination of a null allele and a pathogenic missense mutation indicates that, in this patient, D-2-HGA is caused by a deficiency of D-2-hydroxyglutarate dehydrogenase.

Our data show, for the first time, that mutations in the D-2-hydroxyglutarate dehydrogenase gene can cause D-2-HGA. This result proves that D-2-HGA is an inborn error of metabolism with autosomal recessive inheritance and is consistent with our previous studies in which we found increased levels of D-2-HG in culture media of fibroblasts from patients with D-2-HGA (Struys et al. 2003). The low activities of D-2-hydroxyglutarate dehydrogenase in fibroblasts, leukocytes, and lympho-
Figure 1   Schematic presentation of the D-2-hydroxyglutarate dehydrogenase gene, showing exons (numbered blackened boxes) and introns (see Genbank for genomic DNA [accession number 27465811] and cDNA [accession number 22477763]). The three pathogenic mutations that were found in two patients affected with D-2-HGA are shown. The IVS1-23A→G mutation creates an alternative splice-acceptor site located 19 nt upstream of the wild-type splice-acceptor site (r.295_296ins19). Figure is drawn to scale.

cytes, however, do not allow accurate measurement by radiochemical assay (Achouri et al. 2004), which is the only available method for this enzyme to date. In this procedure, racemic [2-3H]D/L-2-HG is used as the substrate, requiring the separation of D-2-hydroxyglutarate dehydrogenase and L-2-hydroxyglutarate dehydrogenase (Rzem et al. 2004) prior to the enzyme assay. This is obligatory, since both D-2-hydroxyglutarate dehydrogenase and L-2-hydroxyglutarate dehydrogenase act on [2-3H]D/L-2-HG, yielding titrated water, which makes the assay nonspecific. Alternatively, if the racemic substrate [2-3H]D/L-2-HG could be separated into enantiomeric pure substrates by chromatographic techniques, then the assay would become specific for the corresponding hydroxyglutarate dehydrogenase. Unfortunately, both the separation of the enzymes from fibroblasts, leukocytes, and lymphocytes and the separation of the racemic substrate are not feasible at this time.

Although the pathophysiologic mechanism by which D-2-HGA causes neurologic dysfunction remains unknown, the elucidation of disease-causing mutations in the D-2-hydroxyglutarate dehydrogenase gene extends prenatal diagnoses from the established measurement of D-2-HG in amniotic fluid to DNA analysis during the first trimester for families with proven pathogenic mutations.

Acknowledgments

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Electronic-Database Information

The accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for genomic DNA [accession number 27465811] and cDNA [accession number 22477763])

References