Hemolytic anemia and severe rhabdomyolysis due to compound heterozygous mutations of the gene for erythrocyte/muscle isozyme of aldolase: ALDOA\(^{(Arg303X/Cys338Tyr)}\)

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Abstract

Aldolase [E.C. 4.1.2.13], a homotetrameric protein, encoded by the ALDOA gene, converts fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Three isozymes are encoded by distinct genes. The sole aldolase present in red cells and skeletal muscle is the A isozyme. We report here the case of a female patient of Sicilian descent with aldolase A deficiency. Clinical manifestations included transfusion-dependent anemia until splenectomy at age three and increasing muscle weakness, with death at age four associated with rhabdomyolysis and hyperkalemia. Sequence analysis of the ALDOA coding regions revealed two novel heterozygous ALDOA mutations in conserved regions of the protein. The paternal allele encoded a nonsense mutation, Arg303X, in the enzyme active site. The maternal allele encoded a missense mutation, Cys338Tyr, predicted to cause enzyme instability. This is the most severely affected patient reported to date, and only the second with both rhabdomyolysis and hemolysis.
Introduction

Aldolase A is necessary for production of ATP in erythrocytes and muscle fibers, which depend on glycolysis for energy. The protein is a homotetramer, encoded by the ALDOA gene on chromosome 16q22-24. Aldolase A deficiency has been reported as a rare, autosomal recessive disorder. Clinical manifestations in four previously reported patients have been variable (refs 1-4, summarized in Table 1). Hemolysis has been associated with this disorder in each case while myopathy in one and mental retardation in another. Two prior reported cases were analyzed for mutations (Table 1).

Study Design

Patient and case report

A girl of Sicilian ancestry, born to non-consanguineous parents, presented as a newborn with jaundice, pyropoikilocytosis and anemia requiring transfusion. The patient’s father had normal blood counts and peripheral smear. Her mother had a history of newborn jaundice and elliptocytes on peripheral blood smear consistent with dominant (benign) hereditary elliptocytosis. The patient had no sibs. Initially, hereditary pyropoikilocytosis was considered. A low-expression spectrin mutation, alpha-LELY (5), was sought in the father, but not found. From age one to three, the patient had recurrent episodes of pneumonia, croup and one episode of disseminated Pseudomonas, without neutropenia. Neurological examination at age two, prompted by a seizure, revealed Gower’s sign without other evidence of weakness. Cognitive function was normal. The patient required blood transfusions every 6-8 weeks until splenectomy at age 3, which relieved the transfusion requirement. After splenectomy (off transfusions), the peripheral smear became more abnormal, with many elliptocytes...
and Howell-Jolly bodies. The patient complained of leg pain while climbing stairs. Elevated creatine phosphokinase (CPK) levels in the plasma were noted with febrile illnesses. On a “healthy” day at 48 months, RBC and plasma enzyme levels were obtained (Table 2). Elevated serum CPK was noted at 13,800 U/L, yet serum aldolase was normal at 6.2 U/L, suggesting relative muscle aldolase deficiency.

Subsequent studies revealed that the patient’s red cell aldolase level was low at 0.3 U/g Hb while other glycolytic enzymes, phosphofructokinase, glucose phosphate isomerase, phosphoglycerate kinase, hexokinase, lactate dehydrogenase and pyruvate kinase, were normal or elevated (Table 2). Episodes of rhabdomyolysis were more prominent as the patient grew older. She succumbed to severe hyperkalemia and acute rhabdomyolysis during a febrile illness with GI hemorrhage at age 54 m. Post-mortem muscle biopsy revealed myopathic changes with small atrophic fibers and large hypertrophic fibers with increased internal nuclei, suggesting a long-standing myopathic process. Immunohistochemical stains for ß-sarcoglycan and spectrin were reduced while other sarcolemmal proteins were normal. Ragged red fibers were absent. Enzyme assays on the necropsy muscle tissue were uninformative due to problems with tissue preservation.

**Erythrocyte aldolase and LDH analysis**

Aldolase and lactate dehydrogenase (LDH) activities were determined from red cell lysates as described by the International Committee for Standardization in Haematology (6). Briefly, erythrocytes were isolated with Ficoll, washed and lysed in isotonic buffer. Enzyme activities were measured by a loss in absorbance at 340nm. Aldolase assay
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used a coupled assay with fructose 1,6-bisphosphate as the substrate (7) and LDH assay substrate was pyruvate.

**ALDOA gene sequencing**

Genomic DNA from patient’s fibroblasts and parental whole blood samples were prepared via Puregene (Gentra Systems, Research Triangle Park, NC). Nine exons including intron/exon junctions were amplified with primers designed based on human aldolase A gene sequence (Genbank genomic sequence X12447). Primer sequences are available upon request. Amplified PCR fragments were purified by QIAquick Extraction (Qiagen, Valencia, CA) and both strands were sequenced using the standard automated DNA sequence methodology. The sequence was confirmed for the patient and both parents. We designed an Amplification Refractory Mutation System (ARMS) assay (8) to confirm the mutations in genomic DNA (i.e. to rule out PCR contaminations) and to facilitate rapid screening for the mutation without sequencing.

**Results and Discussion**

Sequence analysis of patient’s ALDOA gene (all eight coding exons and the 5’ untranslated exon IB) revealed that the patient was heterozygous for two distinct novel mutations in highly conserved regions of the protein, each carried by one parent (Figure 1A). The paternal point mutation, 931C>T, introduces a premature stop codon Arg303X. This nonsense mutation truncates the protein, producing a “null” allele. It is interesting that this nonsense allele is at Arg303, which is crucial for enzymatic activity. The guanidino group interacts with both the C1- and C6-phosphate at different points during catalysis (9, 10, 11) and acts as a “trigger” residue in conformational changes in the C-terminal region associated with catalysis (11). The maternal point mutation, 1037G>A,
encodes missense mutation, Cys338Tyr. Although Cys338 is not crucial for catalysis (12), it is near a critical “hinge” region for the conformational change in the C-terminus and may be important for maintaining the structure (13). Substitution of Cys338 with tyrosine may disrupt structure in a temperature sensitive fashion. Both Arg303 and Cys338 are conserved in vertebrates (Figure 1B).

Compared to previously reported aldolase A deficiency cases, the clinical consequences for our patient were more severe and ultimately lethal. Myopathic symptoms in the case report of Kreuder et al. were attributed to the aldolase tetramer instability (4). Based on the present sequence analysis, we postulate that the severity of our patient’s anemia and myopathy can be attributed to one null allele and the thermolabile nature of the remaining aldolase protein. This would explain her decompensation with fever; the instability of the protein would render it insufficient to compensate for the needs of erythrocyte and muscle energy consumption when ill. Aldolase A is reported to be the predominant or sole aldolase in leukocytes (14). It is possible that the recurrent infections in the patient, in particular disseminated *Pseudomonas*, resulted from defective bacterial killing. This possibility was not tested directly. Despite the severity of the enzymatic defect in this patient, her cognitive function was entirely normal. It is possible that the reported case of mental retardation was coincidental (1).

Were the elliptocytosis and enzymopathy in our patient related? We believe they were coincidental. Non-spherocytic hemolytic anemia is a hallmark of glycolytic enzyme disorders of erythrocytes. In general, these disorders are not directly related to red cell
membrane phenotypes. A single case report in 1986 described an infant with a partial deficiency in enolase with spherocytosis (15). In addition to hemolytic anemia and severe, progressive myopathy, our patient appears to have had a combination of dominant (mild) hereditary elliptocytosis inherited from her mother and recessive compound heterozygosity aldolase A deficiency. We cannot rule out an interaction between these genetic disorders. The glycolytic defect may have made the membrane defect more severe - for example by intracellular ATP depletion.

Aldolase is required for life. Isozymes A, B and C have distinctive tissue distributions. Because of overlap in many tissues, deficiency of the A isozyme is predicted to be most severe in erythrocytes and muscle, where it is the sole isozyme. We conclude that the combination of hemolysis and evidence of weakness, muscle pain or myopathy should prompt specific evaluation of erythrocyte aldolase, in addition to evaluation of other glycolytic enzymes known to cause the combination of hemolytic anemia and myopathy (e.g. phosphofructokinase (OMIM 232800), triose phosphate isomerase (OMIM 190450), glucose phosphate isomerase (OMIM 172400) and phosphoglycerate kinase (OMIM 311800)).
Acknowledgements

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References


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FIGURE LEGENDS

Figure 1A. **Sequence analysis of ALDOA Exon VIII and IX and predicted amino acid change.** A heterozygous mutation in Exon VIII was found in the patient and her father. Maternal Exon VIII (not shown) was wild type. In Exon IX, the patient and her mother share a heterozygous mutation, which was absent in the father.

Figure 1B. **ALDOA sequence alignment.** Partial human aldolase A polypeptide sequences from exons VIII and IX, aligned to highly conserved orthologs from other species (Genbank cDNA sequence: Xenopus, AAH46673; human, AAH16800; mouse, NP_031464; rat, NP_036627; rabbit, P00883 and zebrafish, AAN04476). The patient’s sequence, divergent at two otherwise invariant residues, is shown below.
Table 1

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Consanguinity</th>
<th>Mutation DNA</th>
<th>Amino Acid</th>
<th>Clinical Description</th>
<th>REFERENCE</th>
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<tr>
<td>“Canadian Jewish”</td>
<td>Yes</td>
<td>Not Reported</td>
<td>Not Reported</td>
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<td>Japanese</td>
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<td>Not Reported</td>
<td>Yes, No, No</td>
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<tr>
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<td>Probable</td>
<td>386A&gt;G</td>
<td>Asp128Gly</td>
<td>Yes, No, No</td>
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<td>German</td>
<td>No</td>
<td>619G&gt;A</td>
<td>Glu206Lys</td>
<td>Yes, Yes, No</td>
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<tr>
<td>Sicilian</td>
<td>No</td>
<td>931C&gt;T, 1037G&gt;A</td>
<td>Arg303X, Cys338Tyr</td>
<td>Yes, Yes, No</td>
<td>This report</td>
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Table 1. Reported cases of Aldolase A deficiency. DNA sequences numbered from A of the ATG start codon.
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Table 2

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<th>Enzyme</th>
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<th>Patient</th>
<th>Normal Range*</th>
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<tr>
<td>Erythrocyte Aldolase</td>
<td>1.5 ± 0.2 U/g Hb</td>
<td><strong>0.3 U/g Hb</strong></td>
<td>1.3-2.8 U/g Hb</td>
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<tr>
<td>Erythrocyte LDH</td>
<td>260 U/L</td>
<td>360 U/L</td>
<td>90-180 U/L</td>
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<th>RBC Enzyme</th>
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<th>Normal Range (U/g Hb)</th>
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<td>Glucose phosphate isomerase</td>
<td>69</td>
<td>48-90</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>3.8 (↑)</td>
<td>1.0-2.5</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>196 (↑)</td>
<td>141-179</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>40.5 (↑)</td>
<td>9.0-22.0</td>
</tr>
<tr>
<td>Phosphofructokinase (PFK)</td>
<td>5.7</td>
<td>3.0-6.0</td>
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<table>
<thead>
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<th>Patient (U/L)</th>
<th>Normal Range (U/L)</th>
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</thead>
<tbody>
<tr>
<td>Aldolase</td>
<td>6.2↑, 1 (↓)</td>
<td>(3-12)</td>
</tr>
<tr>
<td>Creatine Phosphokinase</td>
<td>13,800 (↑↑)</td>
<td>(4-150)</td>
</tr>
</tbody>
</table>

* normal ranges are represented as ± 2 SD from the mean.

¶ unexpected ‘normal’ value.

Table 2. **Erythrocyte enzyme study.** Enzyme activities of red cell aldolase A and LDH were measured in EDTA-anticoagulated blood from patient and mother at 30°C. Patient’s RBC aldolase is markedly decreased. Enzyme normal ranges are derived from Internal Committee for Standardization in Hematology (7). **Additional Enzymatic studies.** RBC and plasma enzyme levels were obtained at age 48 m. Markedly elevated CPK levels are noted. The glycolytic enzymes aside from aldolase A are either normal or slightly elevated, as expected in hemolytic anemia. Plasma aldolase is derived primarily from muscle. The low level in the face of marked CPK elevation supports the diagnosis of muscle aldolase deficiency.
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Figures

Exon VIII

931C>T → Arg303X

\[
\begin{array}{c}
\text{wt} \\
\text{mutant}
\end{array}
\begin{array}{cccccccc}
tgg & gcc & ctg & acc & ttc & tcc & tac & ggc \\
295 & Trp & Ala & Leu & Thr & Phe & Ser & Tyr
\end{array}
\begin{array}{cccccccc}
cag & gtc & tgt & gtc & cag & gtc & tgt & gtc \\
Gly & Arg & Ala & Leu & Gin & Ala & Ser & Ala
\end{array}
\begin{array}{c}
310 \\
\end{array}
\]

Exon IX

1037G>A → Cys338Tyr

\[
\begin{array}{c}
\text{wt} \\
\text{mutant}
\end{array}
\begin{array}{cccccccc}
cga & gcc & ctg & ggc & aac & agc & ctt & gct \\
330 & Arg & Ala & Leu & Ala & Asn & Ser & Ala
\end{array}
\begin{array}{cccccccc}
cau & gga & aag & lac & act & ccg & agc \\
Cys & Gin & Gly & Lys & Tyr & Thr & Pro
\end{array}
\begin{array}{c}
345 \\
\end{array}
\]

Figure 1A
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FIGURE 1B
Hemolytic anemia and severe rhabdomyolysis due to compound heterozygous mutations of the gene for erythrocyte/muscle isozyme of aldolase: ALDOA(Arg303X/Cys338Tyr)

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