

BRIEF REPORT: INHERITED METABOLIC MYOPATHY AND HEMOLYSIS DUE TO A MUTATION IN ALDOLASE A

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GLYCOLYSIS is the most important source of energy in red cells and working muscles. Inherited defects of glycolysis can cause hemolytic anemia, neurologic abnormalities, and myopathy with exercise intolerance. The severity of each of these cardinal manifestations may vary depending on the particular step in the glycolytic pathway that is involved and on residual enzyme activity, the expression of tissue-specific isozymes, and physicochemical properties of the mutant enzyme.^{1,2} Aldolase A is one of the three isozymes of aldolase (the other two are B and C) responsible for the conversion of fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate in the glycolytic pathway.³ Deficiency of aldolase A has been reported as a rare cause of nonspherocytic hemolytic anemia.^{4,5} In this report we describe a boy with predominantly myopathic symptoms of aldolase A deficiency due to the substitution of a single amino acid within the subunit interface most essential for the tetrameric structure of this enzyme.

CASE REPORT

A 4½-year-old boy was admitted to our hospital because of muscle weakness and premature muscle fatigue: he was unable to walk for more than 10 minutes or climb more than 20 steps at a time. Two days previously, fever and symptoms of an upper respiratory tract infection had developed.

The boy weighed 2.75 kg at birth and was 48 cm long. He was born after an uneventful pregnancy and delivery. Phototherapy was required for postnatal unconjugated hyperbilirubinemia. Several unexplained episodes of jaundice and anemia requiring blood transfusions occurred during the first year of life. Examination of the family history revealed no cases of chronic hemolytic anemia or myopathy. The boy's parents were healthy and nonconsanguineous.

On physical examination, the patient had slight jaundice, diminished muscle mass, reduced muscle tone, and proximal muscle weakness. The liver was 3 cm below the right costal margin, and the spleen was palpable 2 cm below the left costal margin. He weighed 12.8 kg (3.4 SD below the normal mean for age), was 100.2 cm tall (1.8 SD below the normal mean for age), and had a head circumference of

49.1 cm (2.2 SD below the normal mean for age). Motor development and language acquisition were slightly delayed.

Laboratory examinations revealed the following values: hemoglobin, 9.7 g per deciliter; hematocrit, 26.8 percent; mean corpuscular volume, 86.7 μm^3 ; mean corpuscular hemoglobin, 31.4 pg; reticulocyte count, 6.5 percent; white-cell count, 19,800 per cubic millimeter; and platelet count, 242,000 per cubic millimeter. The creatine kinase concentration was markedly elevated (2620 U per liter; normal, <60). The levels of the following were also abnormal: aspartate aminotransferase, 71 U per liter (normal, <30); alanine aminotransferase, 39 U per liter (normal, <35); lactate dehydrogenase, 535 U per liter (normal, <300); total bilirubin, 2.9 mg per deciliter (49.6 μmol per liter; normal, <1.0 mg per deciliter [18 μmol per liter]); and haptoglobin, 0.3 g per liter (normal, 0.4 to 2.2). Coombs' test and a test for anti-erythrocytic antibodies were negative; osmotic resistance was normal. Urinalysis revealed mild hemoglobinuria and myoglobinuria. The results of electromyography, studies of nerve-conduction velocities, and electroencephalography were normal.

The boy's creatine kinase concentration dropped to 186 U per liter two weeks after he recovered from the febrile illness, and the hemoglobin level increased to 14.6 g per deciliter six weeks after his recovery. The size of the liver and spleen became normal. On follow-up, several episodes of rhabdomyolysis with creatine kinase elevations of up to 6480 U per liter were observed during febrile illnesses. Less severe elevations of creatine kinase were also seen after exercise and general anesthesia for tonsillectomy. The hemoglobin level and red-cell count were normal on repeated measurements during illness-free periods.

METHODS

The research proposal for this study was approved by the ethics committee of the Justus-Liebig University, Giessen, Germany. Informed consent was obtained from the boy's parents.

Morphologic and Biochemical Studies

Histologic and histochemical studies were performed on muscle tissue obtained from the patient's left quadriceps muscle by standard techniques.⁶ Muscle enzymes were extracted and their activities determined as previously described.⁷

Enzyme activities and levels of ATP, 2,3-diphosphoglycerate, and reduced glutathione in red cells were determined according to the methods of the International Committee for Standardization in Haematology.^{8,9} Levels of erythrocyte metabolites, rates of glucose consumption and lactate formation, and glutathione stability were determined as previously described.¹⁰⁻¹² The metabolic studies included eight control subjects without any hematologic abnormalities and, as reticulocyte-rich controls, two patients with hereditary spherocytosis (reticulocyte counts, 11.8 and 18 percent). All control values are means \pm 2 SD.

The thermostability of aldolase activity was studied by incubation of hemolysates of red cells at 56°C for 30 minutes. The Michaelis constant (the substrate concentration yielding half-maximal activity) of aldolase for fructose-1,6-bisphosphate was determined by the conventional assay, with final substrate concentrations ranging from 0.1 to 50 μM . Electrophoresis and staining were carried out with partially purified aldolase (Sephacryl S-300, Pharmacia, Freiburg, Germany).¹³ All substrates and standard enzyme preparations were purchased commercially (Boehringer-Mannheim, Mannheim, Germany).

Molecular Studies

Total cellular RNA from muscle tissue and peripheral-blood mononuclear cells frozen in liquid nitrogen was purified by a single-step method.^{14,15} After reverse transcription, the entire coding sequence (positions 168 to 1262) of the aldolase A messenger RNA¹⁶ was amplified by a nested polymerase chain reaction (PCR) with a 5'GGATTCCAAGGAAGAATTCCTCTG3' sense primer (positions 113 to 138) and a 5'GGCAGGGCCCTGGAGTTGTTGG3' antisense primer (positions 1306 to 1286) during the first round and a 5'GCACCGGAACCTTGCTACTACCAG3' sense primer (positions 141 to 163) and a 5'GGCAGCCTGGGAACACCTCCG3' antisense primer (positions 1284 to 1264) during the second round. The second-round

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PCR primers carried recognition sites for the restriction enzymes *SacI* and *EcoRI* to facilitate cloning of the PCR products into the pBlue-script vector (Stratagene, La Jolla, Calif.). Plasmid preparations from single colonies were subjected to dideoxy sequencing¹⁷ with an automatic DNA sequencer (model ABI 373A, Taq DyeDeoxy Terminator cycle-sequencing kit, Perkin-Elmer, Applied Biosystems Division, Foster City, Calif.). From each sample two different PCR runs were carried out, followed by sequencing of three clones each in both directions. Apart from the universal M13 sequencing primers (Boehringer-Mannheim), six specific oligonucleotides (sense primer: positions 391 to 413, 671 to 694, and 977 to 998; antisense primer: positions 487 to 466, 764 to 747, and 1079 to 1058) were used to prime overlapping sequencing reactions. Solid-phase direct sequencing of PCR products was carried out as previously described in order to demonstrate heterozygosity of the patient's relatives.¹⁴ Sequence-homology scans were performed with PC/Gene computer software (IntelliGenetics, Geneva).

RESULTS

Morphologic and Biochemical Studies

On light microscopy, the patient's muscle tissue showed a well-preserved fascicular architecture but a remarkable range of diameters of both type 1 and type 2 fibers (Fig. 1A). Some fibers showed intracytoplasmic fiber splitting with increased activity of acid phosphatase (Fig. 1B). The results of staining with periodic acid-Schiff, oil red O, NADH dehydrogenase, cytochrome-*c*-oxidase, phosphorylase, and myosin ATPase were normal.

Electron microscopy revealed variable diameters of the myofibrils and dilated intermyofibrillar spaces containing fine, electron-dense accumulations of lipid; structures resembling myelin; and increased variation in the shape and size of mitochondria (Fig. 1C).

There was a profound reduction of aldolase activity in muscle tissue (9.8 U per gram of wet weight; mean [\pm SD] value in 11 controls, 92 \pm 46). The activities of myophosphorylase and distal glycolysis enzymes were as follows: phosphorylase, 7.6 U per gram of wet weight (normal value in 53 controls, 13.9 \pm 4.4); phosphoglycerate kinase, 160 U per gram of wet weight (normal value in 20 controls, 253 \pm 105); phosphoglycerate mutase, 375 U per gram of wet weight (normal value in 14 controls, 290 \pm 104); and lactate dehydrogenase, 304 U per gram of wet weight (normal value in 40 controls, 300 \pm 165).

In the patient's red cells, the residual aldolase activity was 4 percent of the normal level (Table 1). The activities of other enzymes were normal or even increased, mainly reflecting the young age of the erythrocytes. In order to eliminate the influence of reticulocytosis, the mean control values were corrected for the patient's reticulocyte count.¹⁸ The ratios of the patient's enzyme activities to the corrected control values exceeded 1 for the enzymes whose actions in the glycolytic pathway occur before those of aldolase (1.23 for hexokinase, 1.40 for glucose phosphate isomerase, and 1.26 for phosphofructokinase), but were less than 1 for the enzyme whose actions occur after those of aldolase (0.60 for pyruvate kinase). The aldolase activity of erythrocytes from the patient's parents and brother was about half the normal activity (Table 1).

In the patient's erythrocytes, the level of the aldolase

substrate fructose-1,6-bisphosphate was 75 nmol per milliliter of packed cells (normal, 41.0 \pm 10.2; values in the two patients with reticulocyte-rich blood, 49 and 57). The concentrations of the aldolase products dihydroxyacetone phosphate and glyceraldehyde-3-phosphate were 23 nmol per milliliter (normal, 30.8 \pm 7.4; in patients with reticulocyte-rich blood, 32 and 40) and 18 nmol per milliliter (normal, 22.5 \pm 6.3; in patients with reticulocyte-rich blood, 24 and 26), respectively. The ratios of fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate were 3.26 (normal, 1.38 \pm 0.22; in patients with reticulocyte-rich blood, 1.43 and 1.53) and 4.17 (normal, 1.99 \pm 0.85; in patients with reticulocyte-rich blood, 2.04 and 2.19), respectively. The level of 2,3-diphosphoglycerate in the patient's erythrocytes was 11.4 μ mol per gram of hemoglobin (normal, 12.9 \pm 3.10; in patients with reticulocyte-rich blood, 13.1 to 17.3).

The ATP content of fresh erythrocytes from the patient was lower than normal (4.13 μ mol per gram of hemoglobin; normal, 4.46 \pm 0.28). The glucose consumption of the patient's red cells was also lower than normal after incubation at 37°C for two hours (2.85 μ mol per milliliter of erythrocytes; normal, 4.32 \pm 1.30) and four hours (4.10 μ mol per milliliter of erythrocytes; normal, 6.93 \pm 2.05). The corresponding values for lactate formation were 6.15 μ mol per milliliter of erythrocytes (normal, 5.70 \pm 2.31) and 12.1 μ mol per milliliter of erythrocytes (normal, 11.4 \pm 3.42).

The level of reduced glutathione in the patient's red cells was 79.8 mg per deciliter (normal, 68.9 \pm 10.74). In the presence of acetylphenylhydrazine, the level of reduced glutathione decreased by 14.1 percent from baseline levels (normal decrease, 10.3 \pm 6.8 percent) after two hours of incubation and by 26.3 percent (normal decrease, 21.9 \pm 13.3 percent) after four hours of incubation at 37°C.

The aldolase activity in the patient's hemolysates was decreased to 10 percent of the base-line level after incubation for 30 minutes at 56°C. The activity of the enzyme was completely stable in hemolysates from the patient's parents and controls. The Michaelis constant of the patient's aldolase was 16.8 μ mol per liter (normal, 11.4 \pm 5.0). Because of the instability of the enzyme, the electrophoretic pattern of purified aldolase from the patient's erythrocytes could not be determined. The Michaelis constants and the electrophoretic patterns of the aldolase from the parents were normal, a finding that probably reflects the presence of wild-type aldolase A.

Molecular Studies

Within the coding region of aldolase A obtained both from the patient's peripheral-blood mononuclear cells and from muscle tissue, there was a single-base transversion from guanine to adenine at position 619, resulting in a change in the amino acid from glutamic acid (GAG) to lysine (AAG) at residue 206 (Glu206Lys). In contrast, peripheral-blood mononuclear cells from the

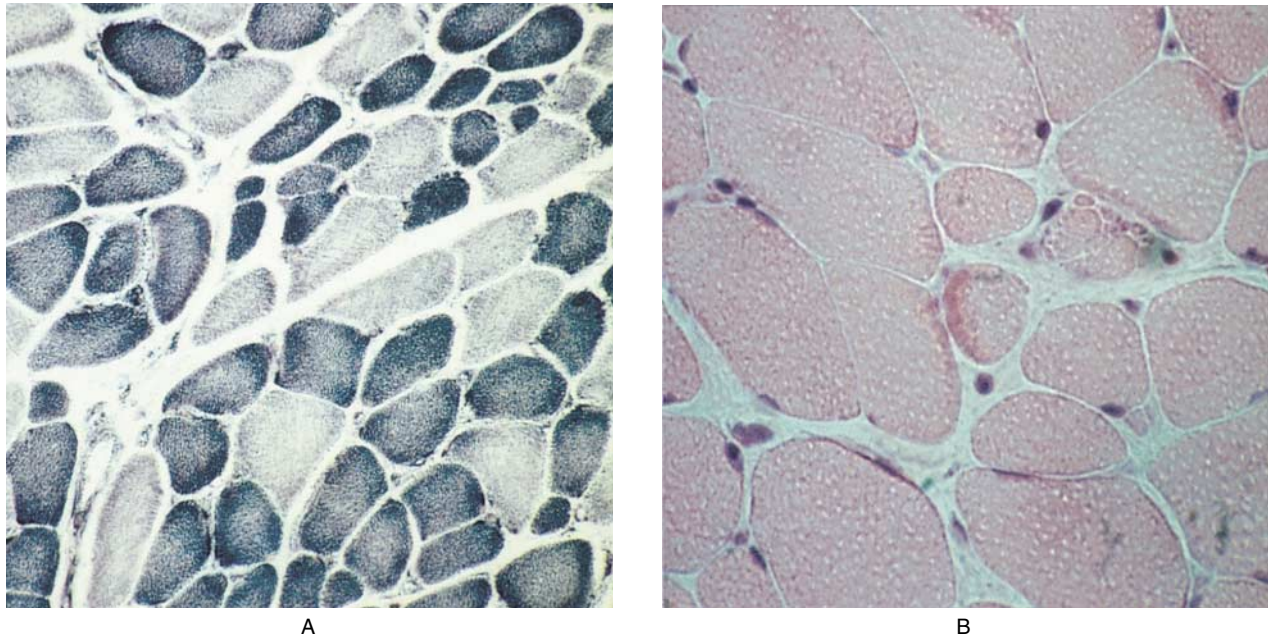


Figure 1. Light and Electron Photomicrographs of Aldolase A-Deficient Muscle Tissue.

A cryostat was used to section the muscle-biopsy specimen. In Panel A, staining with NADH dehydrogenase reveals marked variations in the diameters of both type 1 fibers (the strong reaction is indicated by dark staining) and type 2 fibers (weak reaction, indicated by light staining). The mosaic pattern of the fiber types is preserved ($\times 330$). In Panel B, staining with hematoxylin and eosin reveals irregularly distributed, atrophic muscle fibers, with splitting of one fiber in the upper-right-hand portion of the figure ($\times 500$). In Panel C, electron microscopy shows enlarged intermyofibrillar spaces containing electron-dense accumulations of lipid and mitochondria with distorted cristae and vacuolation ($\times 7700$).

patient's parents and his brother revealed a heterozygous pattern in which both guanine and adenine occurred at position 619, suggesting an autosomal recessive mode of inheritance.

DISCUSSION

Human aldolase A is composed of four identical subunits encoded by a single gene located on chromosome 16 (16q22-q24).^{3,19} Our patient carries a new homozygous germ-line mutation (Glu206Lys) in which the negatively charged glutamic acid is changed to the positively charged lysine at residue 206. This amino acid is conserved in all known aldolase isozymes from different species, including *Drosophila*.²⁰ Amino acid residues 196 to 218 represent helix E in the secondary structure of human aldolase and show complete homology with the corresponding enzymes in rats, rabbits, and mice. Helices E and F from adjacent subunits together form the more extensive of two subunit interfaces providing the tetrahedral-like configuration of the aldolase enzyme. Helix E interacts with its equivalent helix E in the other subunit virtually all along its length.²¹ The integrity of the quaternary structure has been suggested as the basis for the thermal stability of aldolase A.²²

Table 1. Enzyme Activities at 37°C in Erythrocytes from the Patient, His Parents, His Brother, and 200 Normal Control Subjects.*

ENZYME	PATIENT	BROTHER	MOTHER	FATHER	NORMAL CONTROLS
	<i>μmol of substrate used/g of hemoglobin/min</i>				
Hexokinase	1.9	1.8	1.2	1.3	1.0±0.1†
Glucose phosphate isomerase	70.0	61.5	70.2	63.6	44.7±4.8†
Phosphofructokinase	32.0	28.3	25.6	29.2	21.0±4.1†
Aldolase	0.3	3.4	3.0	3.2	7.9±1.4
Triose phosphate isomerase	2441	1965	1952	2034	2180±254
Phosphoglycerate kinase	254.3	159.7	146.4	165.3	138±23
Diphosphoglycerate mutase	5.0	4.6	4.3	4.4	4.6±0.8
Phosphoglycerate mutase	76.4	73.8	67.2	63.6	67.8±10.0
Enolase	24.2	19.7	18.3	16.5	16.1±2.1
Pyruvate kinase	24.7	22.1	21.4	19.7	20.2±2.2†
Glucose-6-phosphate dehydrogenase	14.3	13.5	12.8	12.7	11.0±1.6†
Glutathione reductase	10.5	10.4	12.2	10.8	9.3±1.1
Adenylate kinase	307.3	286.7	264.4	296.7	259.1±36.0

*Plus-minus values are means ± 2 SD.

†The following mean values (expressed as micromoles of substrate used per gram of hemoglobin per minute) were obtained after correction for the patient's reticulocyte count of 6.3 percent¹⁸: hexokinase, 1.54; glucose phosphate isomerase, 50.1; phosphofructokinase, 25.5; pyruvate kinase, 40.9; and glucose-6-phosphate dehydrogenase, 13.5.

Therefore, we assume that the lability of this essential subunit-interaction site is the main reason for the impaired thermostability of the Glu206Lys mutant. The finding of only a single nucleotide change in our patient and the biochemical importance of the resulting amino acid substitution in this highly conserved region of aldolase A clearly argue against the possibility of a DNA polymorphism.

The findings of impaired glucose consumption and reduced ATP levels suggest a severe disturbance of energy production as a cause of hemolysis and myopathy in our patient. These metabolic alterations become even more evident if the higher levels of glycolytic flux and ATP formation that occur in populations of young erythrocytes are taken into account.²³ The findings of normal glutathione levels and glutathione stability suggest that the activity of the hexose monophosphate pathway is unaffected, providing stability of the redox potential against oxidizing agents. Clinical and morphologic signs of myopathy reflect the occurrence of substantial damage to muscle tissue as a result of aldolase deficiency. Similar nonspecific myopathic signs have also been observed in patients with myopathy and other glycolytic defects in addition to more specific findings such as glycogen deposits.^{1,12} However, the aggravation of rhabdomyolysis by fever, as occurred in our patient, is quite an unusual finding in metabolic myopathies, possibly reflecting the thermolability of this mutant enzyme.

Erythrocyte aldolase deficiency has been described previously in three patients with chronic hemolytic anemia but no signs of myopathy.^{2,4,5} In one of these patients the underlying mutation was identified as a change from aspartic acid to glycine at position 128.²⁴ This mutation, affecting the less extensive interaction site between aldolase A subunits, also causes thermolability of the enzyme.^{4,21,22,24} The absence of myopathic

symptoms in this patient, however, was assumed to be due to ongoing protein synthesis in muscle tissue.^{2,4,24} In our patient, the extent of impairment of the main subunit interface of the aldolase tetramer probably exceeds the capacity of transcriptional factors to compensate for the muscular enzyme deficiency and may explain the myopathy.

Glycolytic enzyme deficiencies may cause multisystem disease with predominant neurologic and myopathic abnormalities, including a variable degree of hemolysis as one component. Even in cases without marked alteration of red-cell function or life span, erythrocyte-enzyme assays may still provide a simple means of detection and diagnosis of these multisystem disorders.^{2,25} Our findings suggest that deficiency of fructose-1,6-bisphosphate aldolase A in muscles should be included in the diagnostic spectrum of inherited metabolic myopathies characterized by exercise intolerance and rhabdomyolysis.

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REFERENCES

- DiMauro S, Tsujino S. Nonyl-sosomal glycogenoses. In: Engel AG, Franzini-Armstrong C, eds. *Myology*. 2nd ed. New York: McGraw-Hill, 1994:1554-76.
- Tanaka KR, Paglia DE. Pyruvate kinase and other enzymopathies of the erythrocyte. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease*. 7th ed. Vol. 3. New York: McGraw-Hill, 1995:3485-511.
- Gamblin SJ, Davies GJ, Grimes JM, Jackson RM, Littlechild JA, Watson HC. Activity and specificity of human aldolases. *J Mol Biol* 1991;219:573-6.
- Miwa S, Fujii H, Tani K, et al. Two cases of red cell aldolase deficiency associated with hereditary hemolytic anemia in a Japanese family. *Am J Hematol* 1981;11:425-37.
- Beutler E, Scott S, Bishop A, Margolis N, Matsumoto F, Kuhl W. Red cell aldolase deficiency and hemolytic anemia: a new syndrome. *Trans Assoc Am Physicians* 1973;86:154-66.
- Dubowitz V. *Muscle biopsy: a practical approach*. 2nd ed. London: Baillière Tindall, 1985:28-40.
- Reichmann H, Srihari T, Pette D. Ipsi- and contralateral fibre transformations by cross-reinnervation: a principle of symmetry. *Pflügers Arch* 1983; 397:202-8.
- Beutler E, Blume KG, Kaplan JC, Löhr GW, Ramot B, Valentine WN. International Committee for Standardization in Haematology: recommended methods for red-cell enzyme analysis. *Br J Haematol* 1977;35:331-40.
- Miwa S, Luzzatto L, Rosa R, et al. International Committee for Standardization in Haematology: recommended methods for an additional red cell enzyme (pyrimidine 5'-nucleotidase) assay and the determination of red cell adenosine-5'-triphosphate, 2,3-diphosphoglycerate and reduced glutathione. *Clin Lab Haematol* 1989;11:131-8.
- Beutler E. The glutathione instability of drug-sensitive red cells: a new method for the in vitro detection of drug sensitivity. *J Lab Clin Med* 1957; 49:84-95.
- Schröter W, Brittinger G, Zimmerschmitt E, König E, Schrader D. Combined glucosephosphate isomerase and glucose-6-phosphate dehydrogenase deficiency of the erythrocytes: a new haemolytic syndrome. *Br J Haematol* 1971;20:249-61.
- Rosa R, George C, Fardeau M, Calvin MC, Rapin M, Rosa J. A new case of phosphoglycerate kinase deficiency: PGK Creteil associated with rhabdomyolysis and lacking hemolytic anemia. *Blood* 1982;60:84-91.
- Susor WA, Penhoet E, Rutter WJ. Fructose-diphosphate aldolase, pyruvate kinase, and pyridine nucleotide-linked activities after electrophoresis. *Methods Enzymol* 1975;41:66-73.

14. Borkhardt A, Repp R, Haupt E, et al. Molecular analysis of MLL-1/AF4 recombination in infant acute lymphoblastic leukemia. *Leukemia* 1994;8:549-53.
15. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
16. Sakakibara M, Mukai T, Hori K. Nucleotide sequence of a cDNA clone for human aldolase: a messenger RNA in the liver. *Biochem Biophys Res Commun* 1985;131:413-20.
17. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A* 1977;74:5463-7.
18. Lakomek M, Schröter W, De Maeyer D, Winkler H. On the diagnosis of erythrocyte enzyme defects in the presence of high reticulocyte counts. *Br J Haematol* 1989;72:445-51.
19. Kukita A, Yoshida MC, Fukushige S, et al. Molecular gene mapping of human aldolase A (ALDOA) gene to chromosome 16. *Hum Genet* 1987;76:20-6.
20. Malek AA, Hy M, Honegger A, Rose K, Brenner-Holzach O. Fructose-1,6-bisphosphate from *Drosophila melanogaster*: primary structure analysis, secondary structure prediction, and comparison with vertebrate aldolases. *Arch Biochem Biophys* 1988;266:10-31.
21. Gamblin SJ, Cooper B, Millar JR, Davies GJ, Littlechild JA, Watson HC. The crystal structure of human muscle aldolase at 3.0 Å resolution. *FEBS Lett* 1990;262:282-6. [Erratum, *FEBS Lett* 1990;264:159.]
22. Beernink PT, Tolan DR. Subunit interface mutants of rabbit muscle aldolase form active dimers. *Protein Sci* 1994;3:1383-91.
23. Miller DR. Hemolytic anemias: metabolic defects. In: Miller DR, ed. *Blood diseases of infancy and childhood: in the tradition of C.H. Smith*. 6th ed. St. Louis: C.V. Mosby, 1989:294-357.
24. Kishi H, Mukai T, Hirono A, Fujii H, Miwa S, Hori K. Human aldolase A deficiency associated with a hemolytic anemia: thermolabile aldolase due to a single base mutation. *Proc Natl Acad Sci U S A* 1987;84:8623-7.
25. Valentine WN, Paglia DE. Erythrocyte enzymopathies, hemolytic anemia, and multisystem disease: an annotated review. *Blood* 1984;64:583-91.

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