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Inherited Deficiency of Delta-Aminolevulinic Acid Dehydratase

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SUMMARY

Delta-aminolevulinic acid dehydratase (ALA-D) is the second enzyme in the porphyrin-heme pathway and converts delta-aminolevulinic acid (ALA) to porphobilinogen (PBG). A family is reported with an inherited deficiency of red cell ALA-D activity occurring over three generations in an autosomal dominant pattern. Initial experiments support the hypothesis that the mutation in this family may affect a regulatory gene, but enzyme purification and further study are required. Although no clinical manifestations of deficient ALA-D activity have been found in affected persons, families such as this may be at increased risk for the serious consequences of lead poisoning, which produces marked inhibition of ALA-D activity.

INTRODUCTION

Considerable progress has been made in the past decade in identifying enzyme deficiencies underlying hereditary disorders affecting the porphyrin-heme metabolic pathway. These studies have increased our understanding of porphyrin metabolism, led to early identification of mutant gene carriers, and elucidated clinically important gene-environment interactions. We report here a family showing autosomal dominant inheritance of ALA-D deficiency affecting this second enzyme in the porphyrin-heme pathway. Genetically determined low activity of ALA-D appears to produce no symptoms but could predispose gene carriers to the serious consequences of lead poisoning.

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MATERIALS AND METHODS

The index person, a woman, in figure 1 (IV-4) was fortuitously discovered during the accumulation of control data on erythrocyte uroporphyrinogen synthase I (URO-S-I) in a healthy population of 200 university students. The method assayed a two-step reaction (see fig. 2) using ALA as substrate and measuring porphyrin production, thus including both ALA-D and URO-S-I. This individual demonstrated low activity, but more specific testing proved that ALA-D activity was deficient, whereas URO-S-I was normal. Further biochemical studies were performed on this woman and other family members following full explanation of the experimental nature of the tests. Red cell ALA-D activity was determined by a previously reported method [1], both with and without zinc at 10^{-4} mol/liter in the final reaction [2]. ALA-D activity was determined in the presence of dithiothreitol (DTT) at 10^{-4} mol/liter to eliminate any possible inhibition by lead [3]. Red cell ALA-D activity was also measured in the presence of urine from the index person to test for any possible inhibitor substance as has been described in hereditary tyrosinemia [4]. Red cell URO-S-I activity was assayed by previously reported methods independently using either ALA or PBG as substrate [5].

The following laboratory studies were performed on blood obtained from available family members: hematocrit, total hemoglobin, hemoglobin electrophoresis, red blood cell indices, reticulocyte count, white blood cell count, protoporphyrin/heme ratio, serum zinc, iron, iron-binding capacity, iron saturation, and serum ferritin. The index family member (IV-4) had the following additional tests performed: 24-hr urine collection for determination of ALA, PBG, uroporphyrin, and coporphyrin, urine amino acid screen, and blood levels of lead and tyrosine.

RESULTS

The 18-year-old woman (IV-4) had red cell ALA-D activity of 0.56 μmol PBG formed/ml RBC per hr, which is about 22% of the mean value for 200 healthy adult controls (mean = 2.5, range 1.7–3.6). URO-S-I activity in her red cells was in the normal range (42 nmol porphyrins formed/ml RBC per hr) using PBG as substrate, but less than half normal (11 nmol porphyrins/ml RBC per hr) using ALA as substrate (the two-step reaction). Her hematocrit was 36%, hemoglobin 12.2 g/dl, and her hematologic profile indicated a mild iron deficiency anemia not unusual for her age. A 24-hr urine collection had normal levels of porphyrins as follows: ALA = 3 mg/24 hr (normal = 1.5–7.5 mg/24 hr), PBG = 0.35 mg/total volume (normal = less than 1 mg/total volume), uroporphyrin = 5.9 μg/total volume (normal = 10–50 μg/total volume), and coproporphyrin = 64.2 μg/total volume (normal = 50–200 μg/total volume). Blood level of lead was 16 μg/dl (normal is less than 30 μg/dl) and plasma tyrosine was 0.9 mg/dl (normal 0.2–4.0 mg/dl). Urine amino acid screen was negative. The index person was a healthy, athletic college freshman without history of medical problems.

Figure 1 shows that nine other members of her family (total of five males and five females) had low red cell ALA-D activity ranging from 22% to 41% of normal (somewhat higher levels in III-1 who is noted below). The mean ALA-D activity of these ten persons was 0.84 ± 0.22 μmol PBG formed/ml RBC per hr (range 0.56–1.22), which was 28% of the mean for 11 other blood relatives and spouses (3.00 ± 0.45, range 1.54–3.86). The difference between the means of these two groups was significant at the .01 level (*t* test). Three generations were affected with low ALA-D activity, with one instance of male to male transmission. Inheritance was most consistent with a simple autosomal dominant pattern.

We found no evidence for a plasma factor from affected persons that inhibited

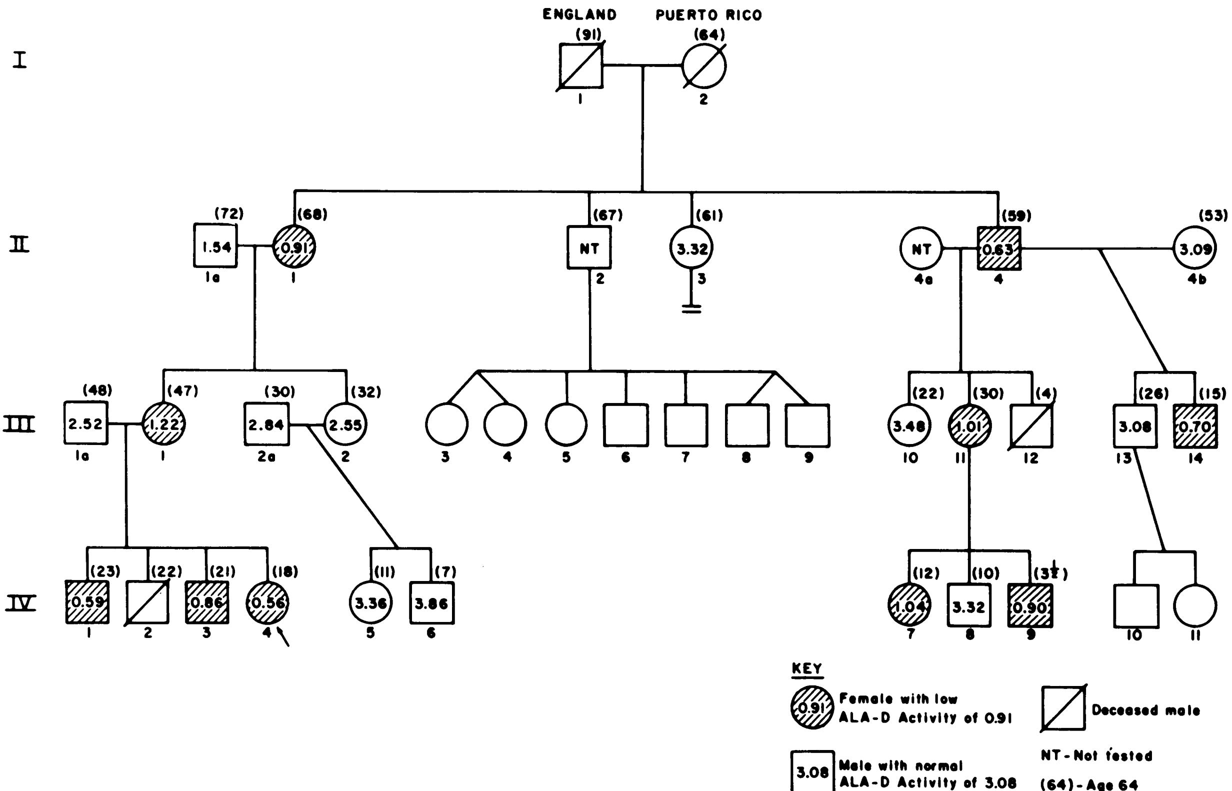

FIG. 1.—Pedigree of family demonstrating autosomal dominant inheritance of ALA-D deficiency. *Arrow* indicates index family member (IV-4). *Shaded symbols* indicate persons with low ALA-D activity. Ages are in parentheses. ALA-D activity was measured in presence of zinc and DTT as described under METHODS.

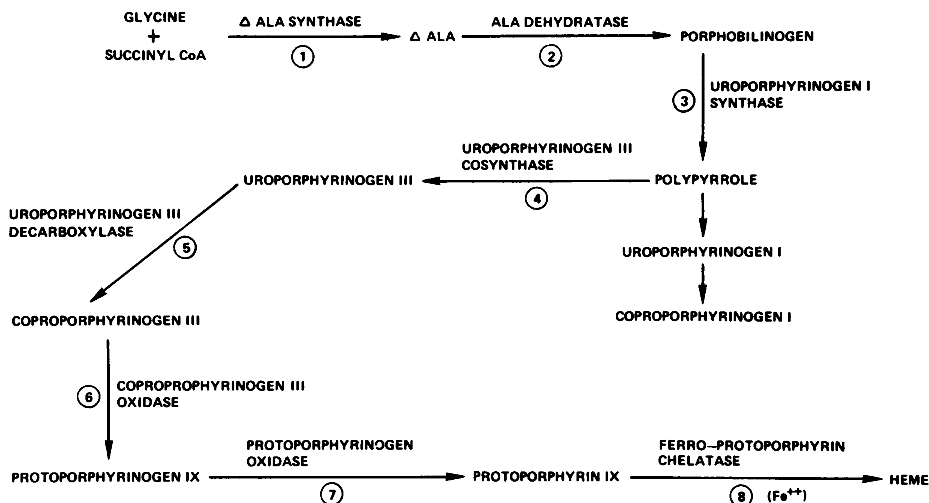

FIG. 2. —Schematic diagram of porphyrin metabolism. Enzymes at the following points in the pathway have been associated with specific diseases: ③ uroporphyrinogen I synthase —acute intermittent porphyria [19]; ④ uroporphyrinogen III cosynthase —congenital erythropoietic porphyria [6]; ⑤ uroporphyrinogen III decarboxylase —porphyria cutanea tarda [15, 16]; ⑥ coproporphyrinogen III oxidase —hereditary coproporphyria [18]; ⑧ ferro-protoporphyrin chelatase —hereditary protoporphyria [17]. Protoporphyrinogen oxidase ⑦ is speculated to be deficient in variegate porphyria [6]. Hereditary deficiency of ALA dehydratase ② is reported in this paper.

ALA-D activity in unaffected persons. Mixing blood with low ALA-D activity with normal blood produced a mean activity between the two separate values. Urine (up to 250 μl) from the index person had no inhibitory effect when added to the red cell ALA-D assay on control patients. Added zinc in the assay system stimulated enzyme activity to an equal degree (20% mean increase) in both affected and unaffected blood. K_m values, temperature, and pH optima for the reaction were similar for blood with low or normal ALA-D activity (see table 1).

The only individual in this family with low ALA-D activity and a symptomatic medical illness was III-1, a 47-year-old woman with typical iron deficiency anemia. She had a history of anemia and a current hematocrit = 26%, hemoglobin = 8.3 g/dl, serum ferritin = 7 ng/ml, serum iron = 15 μg/dl, total iron-binding capacity = 438 μg/dl, and iron saturation = 3%. She was not being treated for her anemia at the time of this study and declined therapeutic trials of iron replacement. No other family member had neurological or hematological disease nor any history of exposure to lead.

DISCUSSION

ALA-D catalyzes the conversion of two molecules of ALA to form PBG (fig. 2). This enzyme occurs in excess and is not generally considered a rate-limiting step in the porphyrin-heme pathway [6]. ALA-D is drastically inhibited by lead, which accounts for the elevated blood and urine ALA levels in patients with lead poisoning [7]. These

TABLE 1

CHARACTERISTICS OF NORMAL AND DEFICIENT ALA-DEHYDRATASE

Measurement	Normal	Family member IV-4
ALA-dehydratase activity (μmol PBG/ml RBC/hr)	3.0	0.56
K_m (ALA)	4.9×10^{-4}	4.8×10^{-4}
Zinc activation (% increase in activity)	21.8%	18.4%
Optimum pH range	6.3–6.5	6.3–6.5
Optimum reaction temperature	45°C	45°C

NOTE.—Results of typical experiments comparing ALA-D activity in red blood cells from family member IV-4 with activity in cells from normal individuals. K_m data is tentative. ALA-D activity is measured in the presence of DTT.

effects in turn may be partially responsible for the neurological and hematological manifestations of lead intoxication.

At least two mutations in the mouse are known to affect ALA-D activity. Two or more alleles at the *levulinate* (*Lv*) locus seem to be inherited in a codominant pattern; that is, a mouse heterozygous for high and low ALA-D activity genes will demonstrate intermediate activity [8]. Mutations at the *Lv* locus are not associated with anemia or other observable disease. There is biochemical evidence that the *Lv* locus in mice contains regulatory rather than structural genes for ALA-D [9]. Also, mutations at the *flexed-tail* (*f*) locus may have low ALA-D activity and a transitory siderocytic anemia [10].

Little is known about the inheritance of ALA-D activity in humans. Sassa and colleagues presented evidence in twins that the enzyme was under genetic control [3, 11]. The fourfold range of ALA-D activity in the normal population studied by these investigators makes further genetic interpretation of their data difficult. The normal range of ALA-D activity in our control population was about twofold. There has been no previous report of genetic transmission of ALA-D deficiency in humans.

Our present family represents inheritance of low red cell ALA-D activity in each of three successive generations best explained by a simple autosomal dominant pattern. There was no consistent association of low ALA-D activity with any illness. Indeed, low activity was usually found in perfectly healthy persons. One affected patient (III-1) had an uncomplicated iron deficiency anemia which is not likely to be causally related to the low ALA-D activity. However, it is of interest that her red cell ALA-D activity was the highest (1.22) of any affected family member and may represent an artificially elevated level of activity resulting from decreased negative feedback on ALA-D by heme. Elevation of ALA-D activity in experimental animals and humans with iron deficiency anemia has been described [12– 14]. The patient declined treatment for her anemia, so we do not know if her ALA-D activity would decrease as her anemia improved.

The deficiency of ALA-D activity in the present family is not similar to that reported in patients with hereditary tyrosinemia. Our index person had no tyrosinemia nor tyrosinuria, and there was no ALA-D inhibitor in her urine [4].

We found some evidence in this family that the mutation producing low ALA-D activity may affect a regulatory rather than a structural gene, consistent with *Lv* locus

mutations in the mouse. The evidence in our family included similar levels of zinc stimulation of the enzyme in normal and deficient blood and similar K_m values, temperature, and pH optima for the two conditions. However, mutation at a structural locus remains a possibility. Purification of the enzyme and further experiments are planned that will better define the precise nature of the mutation.

None of the individuals with low ALA-D activity had symptoms of porphyria, and the index patient did not excrete abnormal amounts of urinary porphyrins, including ALA, the substrate for the enzyme. No gene carrier had evidence of anemia other than that related to iron deficiency. These observations are consistent with previous studies showing that ALA-D occurs normally in considerable excess and is not rate limiting [5, 6]. Nevertheless, it should be noted that gene carriers of acute intermittent porphyria (AIP) may have no symptoms nor anemia, and normal urine porphyrin levels yet still show a reduction in red cell URO-S-I activity [5]. Although seemingly "normal," these "latent porphyrics" may develop serious illness when exposed to a wide range of environmental agents, such as phenobarbital or sulfonamides. Another example of deleterious gene-environment interactions affecting this metabolic pathway is the development of porphyria cutanea tarda in alcoholics who are also deficient in uroporphyrinogen decarboxylase [15, 16]. We speculate that persons with genetically low ALA-D activity may be especially sensitive to environmental lead exposure. However, a direct cause and effect relationship between very low red cell ALA-D activity and clinical symptoms remains to be proven. Longitudinal and family studies of patients with lead poisoning might uncover a subgroup predisposed to symptomatic intoxication by inherited ALA-D deficiency.

The porphyrin-heme pathway is a dramatic example of genetic control of successive steps in a clinically important metabolic system. Figure 2 shows that six of the eight enzymes in this pathway are now known to be affected by mutations in humans, and deficiencies of five of these enzymes either are definitely or probably related to specific diseases [6, 15, 17– 19]. (Protoporphyrinogen oxidase is speculated to be deficient in variegate porphyria, but this is not proven [6, 20]. ALA-synthase activity is secondarily increased in several porphyrias but not known to be primarily affected.) Four of these five disorders, variegate porphyria, and the present ALA-D deficiency occur as autosomal dominant traits. Only congenital erythropoietic porphyria is autosomal recessive. The mechanisms by which these mutations affect the final protein products remain to be determined. Why some affected individuals in these families [5, 15], including the present family, have enzyme activity less than the expected 50% of normal remains unclear. Many of these intriguing issues of the molecular genetics of the porphyrias have been critically reviewed by Romeo [21].

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