Am J Hum Genet 36:1204–1214, 1984

Estimation of the Gene Frequency of Lactate Dehydrogenase Subunit Deficiencies

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SUMMARY

To detect the frequency of lactate dehydrogenase (LDH) subunit deficiency, screening for LDH subunit deficiency was performed on 3,776 blood samples from healthy individuals in Shizuoka Prefecture by means of electrophoresis. The frequency of heterozygote with LDH-A subunit deficiency was found to be 0.185%, and with LDH-B subunit deficiency, 0.159%. The frequencies of both subunit deficiencies were not significantly different. Gene frequencies of LDH subunit deficiencies were calculated by the simple counting procedure, and the results are as follows: gene frequency of LDH-A subunit deficiency was 11.9×10^{-4}, and that of LDH-B subunit deficiency, 7.9×10^{-4}.

In addition, the second case in the world of a homozygous individual with LDH-A subunit deficiency was detected by this screening. This case with regard to the characteristics of LDH-A subunit deficiency are summarized herein.

INTRODUCTION

Lactate dehydrogenase (E.C.1.1.1.27, LDH) can be separated into five isozymes in human tissues [1]. These isozymes are formed by the random combination of two different subunits into tetramers. Each subunit, A(M) and B(H), is a product of a distinct gene locus. The locus of LDH-A subunit is located on chromosome 11 [2], and that of B subunit, on chromosome 12 [3]. A case of a complete deficiency of LDH-B subunit was first reported by Kitamura et al. [4] in 1971, and that of a complete LDH-A subunit deficiency, by Kanno et al. [5] in 1980.

Received December 28, 1983; revised May 30, 1984.

This study was supported in part by a research grant for intractable diseases from the Ministry of Health and Welfare of Japan and by a Kato Memorial research grant of Keio University.

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These disorders are considered to be autosomal recessive traits [4, 5], and the detection of these hereditary disorders is very important in illuminating potentially misinterpretable hereditary disorders in clinical laboratories.

Here, criteria for the detection of LDH subunit deficiencies are established and their gene frequencies in Japan are estimated. In addition, the second case discovered worldwide of LDH-A subunit deficiency is characterized.

MATERIALS AND METHODS

Screening

Screening for LDH subunit deficiency was performed on 3,776 blood samples received from the Health Care Center of Hamamatsu Seirei Hospital and Shizuoka Health Care Center. (Shizuoka and Hamamatsu Cities are both located in Shizuoka Prefecture, the middle part of Honshu Island.) EDTA venous blood was used for analysis.

Erythrocyte Analysis

Erythrocytes prepared from EDTA venous blood were washed with saline three times and disrupted by the addition of an equal volume of distilled water. LDH activity was measured by a spectrophotometric continuous monitoring method using a JEOL 6 channel reaction rate analyzer (model H6R, Japan Electron Optics Laboratory, Akishima, Tokyo, Japan). The assay condition was largely based on the method described by Wroblewski and LaDue [6]. Final reagent concentrations of the assay mixture were as follows: 0.68 mmol/l, pyruvate; 0.17 mmol/l, NADH; and 100 mmol/l, sodium-sodium phosphate, pH 7.4. The activity of the enzyme was expressed as IU/l and was converted to IU/10^9 erythrocytes at 37°C.

LDH isozymes of the erythrocyte hemolysates were electrophoretically fractionated by the slightly modified method described by Shioya et al. [7] using Cellogel membrane (Chemetron, Milan, Italy) with spot application. Activity bands were visualized using D,L-lactate as a substrate, NAD, and tetrazolium salt (MTT) as the final hydrogen accepter. The final concentrations of reagents were as follows: D,L-lactate, 0.13 mmol/1; NAD, 1.0 mmol/1; MTT, 0.4 mmol/1; PMS, 0.1 mmol/1; Tris-HCl, pH 7.4, 60 mmol/1. After observation of spot zymograms, previously doubted samples of subunit deficiencies were fractionated by the method originated by Shioya et al. [7], and the activities of the individual isozymes were densitometrically determined. The ratio between B and A subunits (B/A ratio) was calculated [4, 5].

Family Studies

A family analysis was performed in as many instances as possible in which a positive screening revealed the presence of LDH subunit deficiencies. A pedigree was obtained from as many members of the family as possible. Using fresh samples of the proband and family members, B/A ratios and enzyme activities of erythrocytes were measured. In this way, individuals from 14 families were studied with respect to B/A ratios and LDH activities per 10^9 erythrocytes.

RESULTS

Criteria for the Detection of Heterozygous Individuals with LDH Subunit Deficiencies

On reviewing all heterozygous individuals ever detected in our hospital, two parameters—B/A (H/M) ratios of erythrocytes and LDH activities (IU/10^9 erythrocytes)—were found to be criteria. The range of B/A ratios (mean ± 2 standard

deviations) obtained from 156 cases from the normal control group was 2.3–3.5, and that of LDH activities were 5.5–8.2 IU/10^9 erythrocytes. The distributions of these two parameters obtained from heterozygous individuals are compared with those from the normal control group. Both distributions of B/A ratios and LDH activities appear in figure 1*a* and *b*.

As shown in figure 1*a*, a few overlaps are seen in B/A ratios in cases of heterozygous individuals with LDH-B subunit deficiency. However, B/A ratios are useful to detect heterozygous individuals with either LDH-A or LDH-B subunit deficiency. On the other hand, levels of LDH activity in red blood cells are useful to detect only heterozygous individuals with LDH-B subunit deficiency (fig. 1*b*). Therefore, B/A ratios might be a first choice in detecting heterozygous individuals with either LDH subunit deficiency, and LDH activities are helpful and should be added in detecting heterozygous individuals with LDH-B subunit deficiencies.

Frequencies of Heterozygous Individuals with LDH Subunit Deficiencies

We have studied LDH isozyme patterns of erythrocytes from 3,776 healthy persons. Typical zymograms obtained from spot electrophoresis are exemplified in figure 2. Thirteen heterozygous individuals with LDH subunit deficiencies and one homozygous individual with LDH-A subunit deficiency were detected by applying the criteria established above (table 1). Table 2 shows gene frequencies of LDH subunit deficiencies calculated by the simple counting procedure.

Family analyses were performed on the kindreds of four out of the 13 individuals with LDH subunit deficiencies. Figure 3 and table 3 present the data obtained from the 13 subjects including the four kindred studies of LDH subunit deficiencies

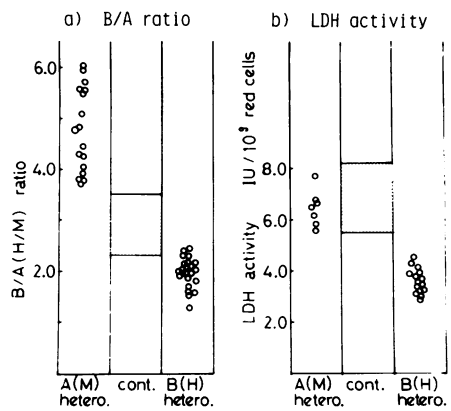

FIG. 1.—The discriminant criteria for the detection of heterozygous individuals with LDH subunit deficiencies. *a*) B/A subunit ratios in erythrocytes. B/A subunit ratio are calculated from densitometric analysis of erythrocyte isozyme patterns. *To the left* are shown the heterozygous individuals with LDH-A subunit deficiency. The B/A ratios are higher than those obtained from normal controls (*shaded*). In contrast, the B/A ratios of heterozygous individuals with LDH-B subunit deficiency are significantly lower than those of normal controls. *b*) LDH activity in erythrocytes. Total LDH activities in erythrocytes are demonstrated in this figure. *To the left* are shown the heterozygous individuals with LDH-A subunit deficiency, and *to the right*, the heterozygous individuals with LDH-B subunit deficiency. This parameter is useful only for the detection of LDH-B subunit deficiency.

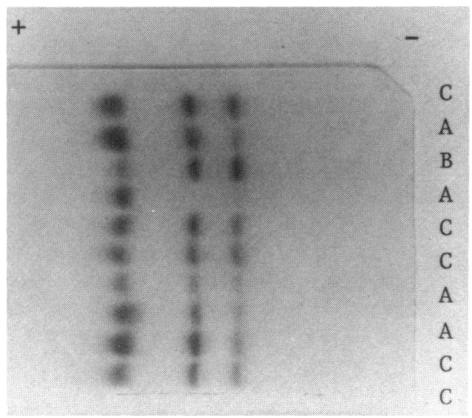

FIG. 2.—Zymograms of LDH isozymes in erythrocytes used for screening. *Anode is left side*, and *cathode is right side*. Therefore, LDH isozymes are *from left to right:* B_4, B_3A_1, and B_2A_2. *Letters—A, B, C—on the right side of figure* show types of LDH subunit deficiencies; *A:* LDH-A subunit deficiency, *B:* LDH-B subunit deficiency, *C:* normal control. *The fourth sample from the top* is of a homozygous individual with LDH-A subunit deficiency. In the case of a heterozygous individual with LDH-A subunit deficiency, the densities of spots are gradually diminished from anode to cathode in their activity stainings. Conversely, they are diminished from cathode to anode in the case of an individual with LDH-B subunit deficiency.

encountered in the course of the survey. As shown in figure 3, the column labeled "pedigree position" refers to the position of each individual in the pedigrees of the four kindreds concerned. In every kindred studied, other members of the kindreds were found to be carriers.

Estimation of the Frequencies of Homozygous Individuals of LDH Subunit Deficiencies

Calculating the frequencies of homozygotes with LDH subunit deficiency from these gene frequencies (squared number of gene frequencies), homozygotes with LDH-A subunit deficiency were estimated as 14.20×10^{-7}, and with LDH-B subunit deficiency, as 6.31×10^{-7}—in short, about one person per one million. However, these frequencies are only frequencies of occasions when there are no consanguineous marriages. In practice, there are many cases of consanguineous marriages, and their frequency in Japan was estimated to be about .004 [8]. When

TABLE 1

FREQUENCY OF LDH SUBUNIT DEFICIENCY BY SCREENING

Type of subunit deficiency	Screening nos.	Putative deficiencies	Frequency (percent)
A subunit deficiency:			
Homozygote	3,776	1	0.026
Heterozygote	3,776	7	0.185
B subunit deficiency:			
Homozygote	3,776	0	0
Heterozygote	3,776	6	0.159

TABLE 2

FREQUENCY DISTRIBUTION OF PHENOTYPES

SUBJECTS	No.	PHENOTYPE			ALLELE FREQUENCY	
		Normal	Heterozygote	Homozygote		
LDH-A	3,776	3,768	7	1	.9988	.00119
LDH-B	3,776	3,770	6	0	.9992	.00079

considering them, the frequencies of homozygotes and heterozygotes are calculated as follows: homozygotes = $(1 - f)q^2 + fq$; heterozygotes = $2q(1 - q)(1 - f)$, where q = the frequency of the gene responsible for LDH subunit deficiency and f = the coefficient inbreeding for the population, assumed from previous studies to be .004 [8]. When each gene frequency of LDH subunit deficiencies—.00119, .00079—is substituted in q, we can obtain frequencies of LDH subunit deficiencies (table 4). The use of this formula is based on assumption that all

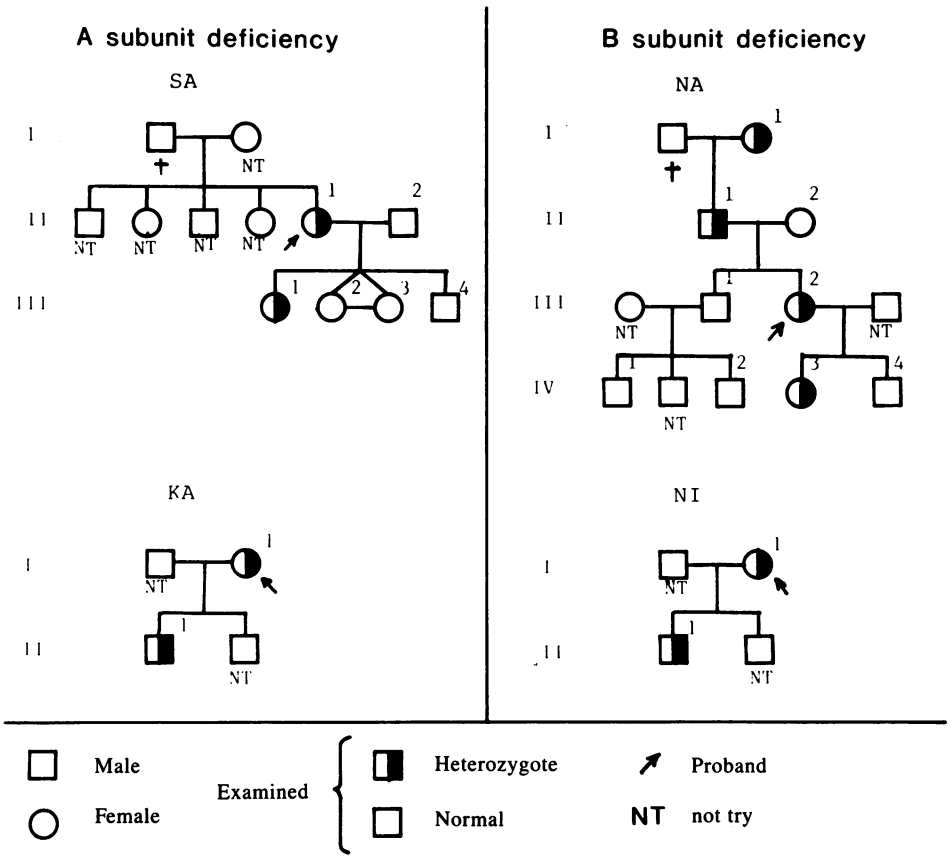

FIG. 3.—The results of family analysis on heterozygous individuals with LDH subunit deficiency found in the course of the screening.

TABLE 3

B/A RATIO AND LDH ACTIVITY IN ERYTHROCYTES FOR MEMBERS OF KINDREDS WITH LDH SUBUNIT DEFICIENCY

LDH-A SUBUNIT DEFICIENCY				LDH-B SUBUNIT DEFICIENCY			
Family	Pedigree position	B/A ratio	Activity IU/10^9	Family	Pedigree position	B/A ratio	Activity IU/10^9
A. Individuals confirmed by family analysis							
SA	II-1	4.29*	5.95	NA	I-1	1.91	4.59
	II-2	3.25	6.92		II-1	2.01	4.17
	III-1	4.34	5.56		II-2	2.85	7.58
	III-2	2.84	6.13		III-1	2.63	7.01
	III-3	2.88	5.82		III-2	2.08*	3.47
	III-4	3.19	5.68		IV-1	2.68	6.32
					IV-2	2.79	7.53
					IV-3	1.87	4.30
					IV-4	3.24	6.92
KA	I-1	5.58*	5.64	NI	I-1	2.19*	2.68
	I-1	3.77	6.47		II-1	1.95	3.83
B. Other heterozygous individuals							
HA		4.06	6.02	IK		2.04	3.87
KI		4.87	5.60	KU		2.07	···
FU		5.34	···	MA		1.67	···
IW		4.98	···	OO		2.20	···
SI		5.28	···				

* Propositus.

LDH subunit deficiencies are due to a gene change at the same genetic locus, and on the further assumption of the gene being present throughout Japan.

The Second Case of a Homozygous Individual with LDH-A Subunit Deficiency

The homozygous individual with LDH-A subunit deficiency discovered in this screening is the second case in the world and is from a different family from the first case found in Hamamatsu [5]. Family analysis showed that both families are not related. The second case was found, by means of family analysis, to be a consanguineous marriage. Figure 4 shows a pedigree chart of the second case. Parents of the propositus are heterozygous individuals with LDH-A subunit deficiency and are first cousins. The propositus and her sister are both homozygous individuals. Since the propositus of the first case showed interesting results from ischemic work of the forearm [5], it was thought that ischemic work should be tried with the second case. The propositus of the second case is now in the third month of pregnancy, and, therefore, ischemic work was tried with her sister instead of her. Figure 5 shows the responses of blood lactate and pyruvate in four normal subjects and homozygous individuals with LDH-A subunit deficiency. The usual increase of lactate after ischemic work was not observed, but, on the other hand, a marked increase of pyruvate was observed. In the ratio between lactate and pyruvate (Lac/Pyr ratio), a reciprocal relation was shown between control subjects and homozygous individuals with LDH-A subunit deficiency.

TABLE 4

COMPARISON OF THE CONSEQUENCES OF CONSANGUINEOUS MARRIAGES

	HOMOZYGOTES		HETEROZYGOTES	
	$(1 - f)q^2 + fq$	$n[(1 - f)q^2 + fq]$	$2q(1 - q)(1 - f)$	$2nq(1 - q)(1 - f)$
LDH-A subunit deficiency:				
Without consanguineous marriages	14.16×10^{-7}	166	2.38×10^{-3}	278,120
With consanguineous marriages	6.17×10^{-6}	722	2.37×10^{-3}	277,010
LDH-B subunit deficiency:				
Without consanguineous marriages	6.24×10^{-7}	73	1.58×10^{-3}	184,710
With consanguineous marriages	3.78×10^{-6}	442	1.57×10^{-3}	183,970

NOTE: q = gene frequency; f = the coefficient inbreeding for the population (without consanguineous marriages: 0; with consanguineous marriages: .004); n = Japanese population, ca. 117,000,000.

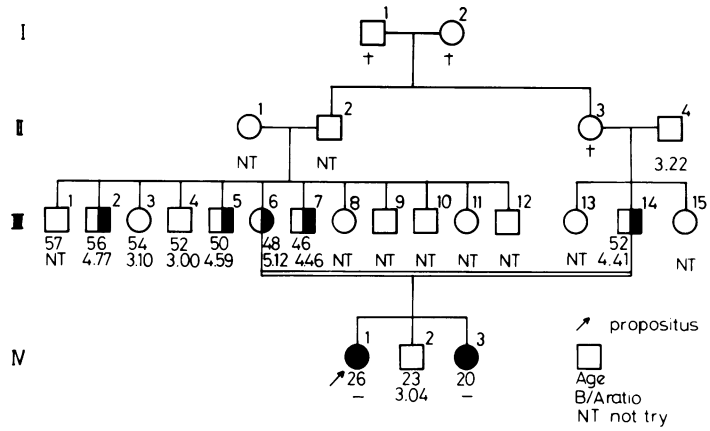

FIG. 4.—Pedigree chart of the second case in the world of a homozygous individual with LDH-A subunit deficiency detected in the course of the screening. There is consanguineous marriage and a first-cousin marriage in the family.

Both cases of homozygous individuals with LDH-A subunit deficiency showed similar responses. Determination of the Lac-Pyr ratio may be a more useful index in distinguishing complete LDH-A subunit deficiencies.

DISCUSSION

The incidence of heterozygotes of LDH-B subunit deficiency was in the past reported to be 1/7,000 [9]. However, this incidence was obtained from studies in which hospitalized individuals with low serum LDH activity were selectively analyzed, and serum LDH activities of hospitalized individuals tend to skew higher; that is, individuals with naturally low serum LDH activity did not actually show low serum LDH activity. In our study, the incidences of both LDH subunit deficiencies were obtained from the screening of healthy persons and they were about 1/500, higher than in past reports. On reviewing all heterozygous individuals with LDH-B subunit deficiency ever detected in our hospital, serum LDH activities were skewed lower, but not always abnormally low. Therefore, a 10-fold difference in the two sets of incidence data are understandable. As for LDH-A subunit deficiency, individuals with low serum LDH activity were rare.

The average frequency of individuals with enzyme deficiencies was, in a survey in the United States, defined to be 0.24% of nine enzymes of newborn infants, while for LDH, no enzyme deficiency variants were identified [10]. However, the number of samples in the U.S. study was 702, and it seems likely that no LDH deficiency variants were observed. In Japan, the frequency of heterozygous individuals varied from 0.0% (adenylate kinase, 6-phosphogluconate dehydrogenase) to 1.38% (pyruvate kinase), with an average of 0.24%, and the frequency for LDH was 0.06% [11]. However, in contrast to the present study, the samples with low activity were detected only as heterozygous individuals.

The second case of a homozygous individual with LDH-A subunit deficiency was discovered in this study. However, as for LDH-B subunit deficiency, Tanis

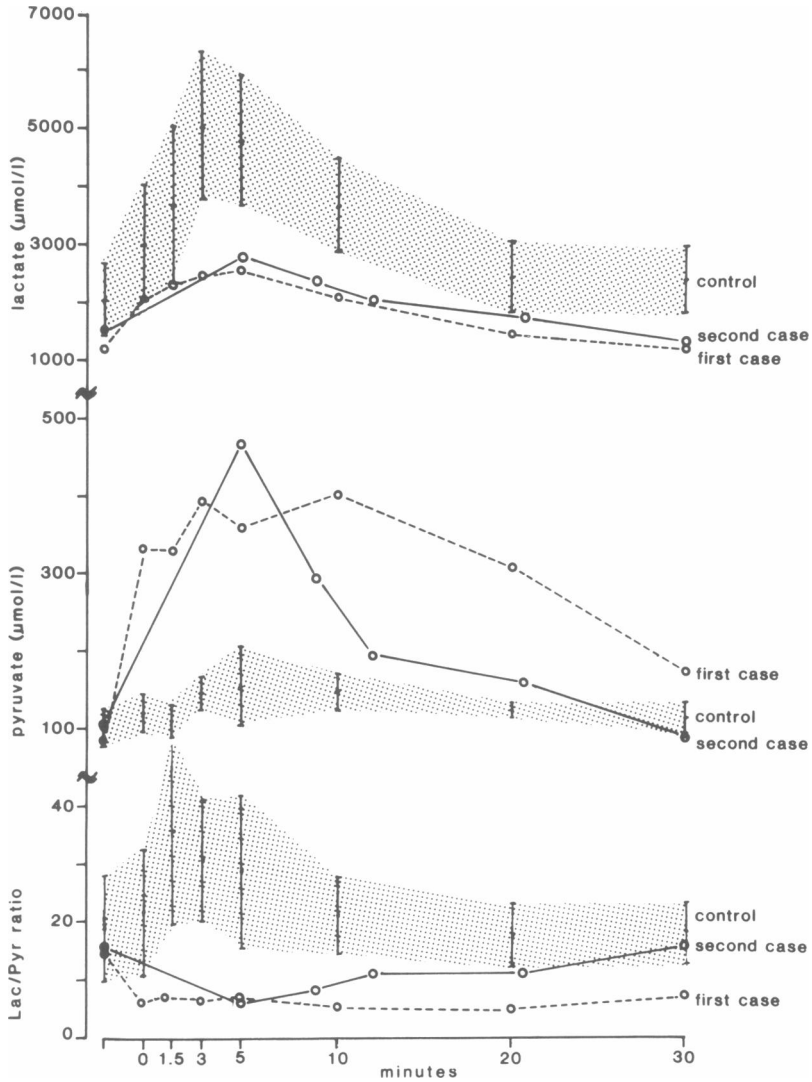

FIG. 5.—The variations of lactate and pyruvate, and the ratio between lactate and pyruvate (Lac/Pyr ratio) in venous blood after ischemic work in four control subjects and both the first and the second cases of homozygous individuals with LDH-A subunit deficiency. *Shadowed ranges* indicate mean ± standard deviation in the four control group subjects.

et al. [12] and Mohrenweiser et al. [13] reported the existence of a human LDH-B variant named LDH_B GUA-1. The variant is an electrophoretic one that is enzymatically inactive. It is only detectable because of its ability to form heterotetramers with A and/or active B subunits and to alter the electrophoretic pattern, although B-GUA-1 subunits are always enzymatically inactive. All tetrameric combinations of active plus inactive subunits, including either an A or an active B plus three inactive B subunits, possess enzymatic activity. On the

other hand, LDH-B subunit deficiency as reported by Kitamura et al. [4] has only one isozyme, LDH 5, in an active form. These two cases are of different types of LDH-B subunit abnormalities. The latter case includes the possibility that B subunit is not only enzymatically but also antigenic inactive, that is, having a complete deficiency of LDH-B subunit. In the present study, in addition to examining the complete deficiency of LDH-A subunit, we also set our sight on the latter type of LDH-B subunit abnormalities. The frequencies of two kinds of homozygous individuals with LDH subunit deficiency are not significantly different. However, homozygous individuals with LDH-B subunit deficiency have not been detected for 12 years since the first case was detected in 1971 [4].

From a clinical viewpoint, homozygous individuals with LDH-B subunit deficiency have only mild diabetes [4], and homozygous individuals with LDH-A subunit deficiency have episodes of myoglobinuria only after strenuous exercise [5]. However, this second case of a homozygous individual with LDH-A subunit deficiency is a female who does not complain that she does not like strenuous exercise. The differences in the two cases suggest that homozygous individuals with LDH-A subunit deficiency are naturally symptomless in daily life, but that after strenuous exercise there is a possibility of falling into acute renal failure due to myoglobinuria. In short, LDH-A subunit deficiency is a latent disease. LDH subunit deficiencies may not be clinically significant under healthy conditions except in the case of LDH-A subunit deficiency after strenuous exercise. However, in diseased states, which are diagnosed by using serum LDH activity such as myocardial infarction and liver damage, these symptom-poor hereditary diseases are easily misdiagnosed because of the lessened release of LDH from affected organs. Consequently, it is important to recognize the LDH subunit deficiency as a potentially misinterpretable hereditary state and as an important factor in the study of human genetics.

ACKNOWLEDGMENTS

We thank Drs. Inomata, Kamata, and Suzuki and Mr. Kasezawa for supplying blood samples used in this screening study.

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MEETINGS: Tissue Culture Association Annual Meeting, New Orleans, La., June 2–6, 1985. Genetics Society of America Annual Meeting, Boston, Mass., August 25–28, 1985. American Society of Human Genetics Annual Meeting, Salt Lake City, Utah, October 9–12, 1985.