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BRIEF REPORT — MUTATION REPORT

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First case of missense mutation (LDH-H:R171P) in exon 4 of the lactate dehydrogenase gene detected in a Japanese patient

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Abstract Complete deficiency of lactate dehydrogenase (LDH) subunit H was identified in a 41-year-old woman with paralysis of her left lower limb. The propositus had extremely low LDH activity and five of her family members had levels of LDH activity that ranged from lower than normal to normal level. A transversion mutation at codon 171 (CGC→CCC), resulting in an Arg→Pro substitution was identified in her DNA sequence. A new *Nru*I restriction site was introduced into the polymerase chain reaction (PCR) product by PCR-primer introduced restriction analysis (PCR-PIRA) using a specific mismatched primer. Digestion with *Nru*I revealed that the propositus and her mother were, respectively, homozygous and heterozygous for this mutation.

Key words Lactate dehydrogenase · Missense mutation · RT-PCR

Introduction

Lactate dehydrogenase (LDH) is an enzyme that oxidizes pyruvate to lactate in the final step of the glycolytic pathway. This enzyme is formed as a tetramer by the combination of two different subunits, M (muscle, A) and H (heart, B), of lactate dehydrogenase.

These subunits give rise to five different isozymes of the tetrameric molecule, $LDH_1(H_4)$, $LDH_2(H_3M)$, $LDH_3(H_2M_2)$, $LDH_4(HM_3)$, and $LDH_5(M_4)$. The first case of a complete deficiency of LDH-H, in a male with slight diabetes, was reported as a phenotype by Kitamura et al. in 1971. Since then several similar cases of LDH-H subunit deficiencies have been reported (Houki et al. 1986; Kamata et al. 1992). Since the entire coding region of the *LDH-H* gene was reported by Takano et al. in 1989, seven genetic variants of LDH-H deficiency have been reported in the Japanese population (Maekawa et al. 1993, 1994; Sudo et al. 1990, 1992, 1994). In this article, we describe a new case of point mutation of the deficient type of LDH-H.

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Subjects and methods

Case report

The propositus, a 41-year-old woman, visited Yamaguchi Prefectural Central Hospital complaining of paralysis of her left lower limb. Physical examination showed no abnormal findings. Routine laboratory examination indicated no abnormalities, except for extremely low serum LDH activity, of 40 IU (normal range, 200–400 IU). Her parents had a consanguineous marriage. LDH studies carried out on her family members led to the suspicion that her parents and her two daughters might have the same LDH deficiency.

LDH activity and isozyme analysis

LDH activity was measured by spectrophotometric monitoring, according to the method of Wroblewski and Ladu (1955).

LDH isozymes were electrophoretically identified by the method of Shioya et al. (1971), with some modifications, and with an LDH reagent kit (LDH Isozyme Test; WAKO, Tokyo, Japan), used according to the manufacturer's instructions.

Total RNA extraction and cDNA analysis

Total RNA from whole blood was prepared using Catrimox-14 surfactant (Takara, Tokyo, Japan) according to the manufacturer's instructions. First-strand cDNA was generated from total RNA with a first-strand-cDNA Synthesis Kit (Clontech, Palo Alto, CA, USA) with Moloney murine leukemia virus reverse transcriptase (RT) and a random primer. Polymerase chain reaction (PCR) amplification of cDNA was performed for 30 cycles, consisting of denaturation at 90°C for 30s, annealing at 50°C for 30s, and extraction at 72°C for 1.5min.

The oligonucleotides used as the primers were 5′-CCGCACCGACTGTTACAGA-3′ for the sense and 5′-TGTTCAAGAGCTCAGATTGC-3′ for the antisense. One-tenth of the first PCR product was subjected to nested PCR amplification under the same conditions as those of the first PCR, but with a different primer set; that is, a sense primer of 5′-CGACTGTTACAGAGG-3′ and an antisense primer of 5′-AAGCATTAACCAAG-3′. The nucleotide sequence of the PCR product was determined with a Dye terminator cycle sequencing kit (Applied Biosystems (ABI), Foster City, CA, USA) and a DNA sequencer (model 373; ABI).

Genomic DNA analysis

Genomic DNA extraction from white blood cells (WBCs) was performed according to the method of Maniatis (1982). PCR amplification of exon 4 in the *LDH* gene was carried out according to the method of McGuire et al. (1989), using a primer set consisting of a sense 5′-AGTGGACATTCTTACGTATG-3′ and an antisense 5′-CATCCATGGCAGCTG-3′.

Polymerase chain reaction-primer introduced restriction analysis (PCR-PIRA)

Genomic DNA was amplified by the PCR method with a specific mismatched primer under the same conditions as those used with the DNA amplification method, except for the primers. The two oligonucleotide primers used were : sense 5′-AGTGGACATTCTTACGTATG-3′, which binds to the first position in exon 4 (nucleotides 420–439), and antisense 5′-TGAATGCCAAGTTTTTCAGCCATAAG-GTC*G-3′, which binds to the coding base in exon 4 (nucleotides 545–516) and introduces a single base change (A→C) at its penultimate position (asterisk) from the 3′ end.

Results and discussion

The LDH activity in the family members is shown in Table 1. The propositus (II-2) showed extremely low LDH activity. The LDH activity in five family members ranged from a level lower than normal to a normal level. A zymogram of LDH isozymes from the propositus's samples (serum, reticulocytes, erythrocytes, platelets, and saliva) showed only one band of $LDH_5(M_4)$ (data not shown). These results indicated that the propositus is a homozygote with complete deficiency of LDH subunit H. Zymograms of her five family members showed patterns different from those of a normal subject (data not shown). The H/M ratio in serum LDH, determined by calculation from the family members' zymograms, was 1.0–1.1, approximately half that in the normal subject. The H/M ratio for erythrocyte LDH was the same as that for serum LDH, as shown in Table 1. These results suggest that her family members are heterozygous for the mutant allele (Fig. 1A).

Table 1 Lactate dehydrogenase (LDH) activity and H/M ratio in sera and erythrocytes of the propositus (II-2) and her family members

	Serum		Erythrocytes	
Subject	LDH Activity	H/M	LDH Activity	H/M
I-1 Father	202	1.0	–	–
I-2 Mother	199	1.0	–	–
II-2 Propositus	40	–	11	–
II-3 Sister	236	1.0	160	1.9
III-1 Daughter	143	1.1	120	2.1
III-2 Daughter	210	1.0	–	–
Normal range	200–400 (IU/l)	1.5–2.5	166–203 (U/gHb)	2.8–4.4

The entire cDNA coding region of the LDH-H from the propositus (II-2) was amplified and sequenced. Sequence analysis verified that the *LDH-H* gene of the propositus had a transversion mutation of G to C in nucleotide 512, which changed codon 171 from CGC (Arg) to CCC (Pro), indicating the homozygous nature of this mutation. The propositus's mother (I-2) had two bases, G and C, at nucleotide 512, indicating she is heterozygous. The control subject showed one base, G only, at nucleotide 512, which is seen in the normal LDH-H DNA sequence. This mutation was reconfirmed by direct sequencing analysis of the PCR product from the genomic DNA of the propositus.

A PCR product (126bp) derived by the mismatched PCR method from the normal subject was completely digested into two fragments, of 96bp and 30bp, with *Nru*I. On the other hand, the PCR product of this mutation from the propositus (II-2) was found to be resistant to *Nru*I, and that of the propositus's mother (I-2) showed two fragments, of 126bp and 96bp (Fig. 1B).

The importance of the amino acid Arg at residue 171 in LDH-H is shown by its almost absolute conservation among six vertebrates (Takano et al. 1989; Hiraoka et al. 1990; Eventoff et al. 1977; Chung et al. 1985; Li et al. 1985; Taylor 1977) (Fig. 2).

These results suggest that the amino acid Arg at residue 171 is essential for the function of the LDH molecule. According to Grau et al. (1981) and Clarke et al. (1986) when pyruvate converts to lactate in the active center pocket of LDH-H, the enzyme-pyruvate-nicotinamide adenine dinucleotide, reduced (NADH) complex is created; that is, the carbonyl oxygen of pyruvate interacts with the

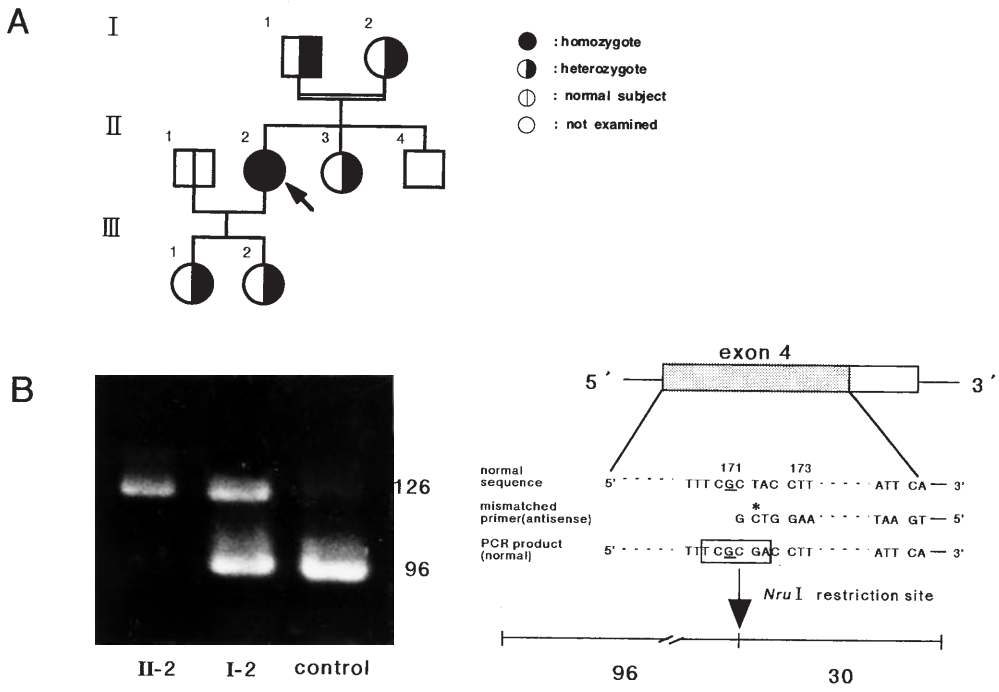

Fig. 1 **A** Family tree of subject with lactate dehydrogenase (LDH) deficiency. *Arrow* indicates the propositus. **B** *Right.* Scheme for detection of the Arg 171Pro mutation of the LDH-H variant. The specfic primer contains a mismatched single base (A→C) at the second position from the 3′ end (*asterisk*). A normal polymerase chain reaction (PCR) product of 126 bp derived from a normal subject at codon 171 (*underlined*) has a new *Nru*I restriction site (*rectangular box*) and can be digested into two fragments, of 30 bp and 96 bp (*arrowhead*), with *Nru*I. **B** *Left.* Separation of *the Nru*I restriction fragments of the PCR products from two individuals (*I-2* and *II-2*) on 4% agarose gel yielded fragments of (126 bp and 96 bp), as shown

				171				
human H	D	S	A	R	F	R	Y	L
mouse H	D	S	A	R	F	R	Y	L
pig H	D	S	A	R	F	R	Y	L
human M	D	S	A	R	F	R	Y	L
mouse M	D	S	A	R	F	R	Y	L
dogfish M	D	S	A	R	F	R	Y	L

The underlined residue represents the affected position

Fig. 2 Amino acid sequences of LDH-heart (H) and LDH-muscle (M) subunits of LDH in the vicinity of amino acid position 171

guanidinium group of Arg 109 and with the imidazole group of His 195, and the carbonyl carbon (C-2) of the pyruvate is linked to the hydrogen at C-4 of the NADH nicotinamide ring. Simultaneously, the carboxyl group of pyruvate forms an important salt bond with the guanidinium group of Arg 171 to stabilize the pyruvate in the active center. Therefore, the substitution of Arg to Pro at position 171 may prevent the formation of the former salt bond and lessen the stability of the pyruvate. This may explain, the finding that the zymogram for the propositus's LDH isozyme showed only one band of M_4 protein.

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