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Three New Mutations in Patients with Myophosphorylase Deficiency (McArdle Disease)

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Summary

We report three new mutations in patients with myophosphorylase deficiency (McArdle disease). A splice-junction mutation (G-to-A transition at the 5′ end of intron 14) and a missense mutation (CTG to CCG at codon 291, changing an encoded leucine to a proline) were identified in Caucasian patients who were heterozygous for a common mutation reported elsewhere (CGA [Arg] to TGA [stop]) at codon 49. The splice-junction mutation destroyed the consensus sequence at the 5′ splice site, and a cryptic splice site 67 bp upstream was recognized instead. As a result, there was a 67-bp deletion in the 3′-terminal region of exon 14 in the transcript, resulting in a frameshift with premature translation termination. A deletion of a single codon, 708/709 (TTC, specifying phenylalanine) was identified in Japanese patients. Two affected siblings were homozygotes, and their parents were heterozygotes. A third, unrelated patient was heterozygous for the same mutation, while the myophosphorylase gene on the other allele was only faintly expressed.

Introduction

Glycogen phosphorylase (α-1,4-glucan orthophosphate glycosyl transferase; E.C.2.4.1.1) initiates glycogen breakdown by removing α-1,4-glucosyl residues phosphorylytically from the outer branches of glycogen, with liberation of glucose-1-phosphate. There are three mammalian isozymes (muscle, liver, and brain type), and genetic defects of the muscle-specific isozyme (myophosphorylase) cause a metabolic myopathy known as glycogenosis type V, or McArdle disease (McArdle 1951; Mommaerts et al. 1959; Schmidt and Mahler 1959). Typical patients with this disorder have exercise intolerance, with premature fatigue, myalgia, cramps in exercising muscles, and recurrent myoglobinuria.

The gene for myophosphorylase has been cloned, sequenced (Burke et al. 1987), and assigned to chromosome 11q13 (Lebo et al. 1984). In a previous report (Tsujino et al. 1993), we described three mutations that cause myophosphorylase deficiency. One of those, a nonsense mutation (CGA[Arg] to TGA[stop]) at codon 49 (Mut49), appears to be the most common cause of McArdle disease, because 30 of 40 patients studied had Mut49 on at least one allele, as confirmed by Bartram et al. (1993).

In the present paper, we describe three novel mutations in the myophosphorylase gene—a splice-junction mutation and a missense mutation in Caucasian patients who were heterozygous for the common Mut49 and a single-codon deletion in Japanese patients.

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Material and Methods

Patients and Controls

Patient 1.—A 27-year-old woman complained of episodic myalgia and weakness, usually after exercise. Neurological examination was normal. Resting levels of serum creatine kinase (CK) were as high as 11,000 IU/liter (normal = less than 50 IU/liter), but there was no

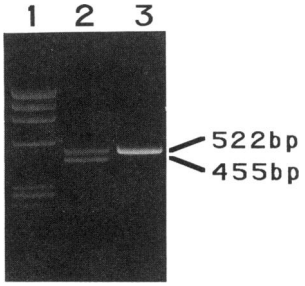

Figure 1 Electrophoresis on 2% NuSieve agarose of the fifth PCR fragment from the 5′ end, obtained from RNAs of patient 1 (lane 2) and a control (lane 3), by using primers 5′-GTTTGCTGCCTACCTAGAGAG and 5′-ACATCCTCCACCCGCATGCCA. Lane 1, DNA molecular marker ΦX174/*Hae*III.

pigmenturia. A forearm ischemic exercise caused no increase of venous lactate. Myophosphorylase deficiency was documented both histochemically and biochemically in a muscle biopsy.

Patient 2.—A 42-year-old woman had episodic muscle cramps and weakness, but no pigmenturia. Resting level of serum CK was 1,053 IU/liter (normal = less than 210 IU/liter). A forearm ischemic exercise caused no increase of venous lactate. Myophosphorylase deficiency was documented biochemically in a muscle biopsy.

Patients 3 and 4.—Two Japanese siblings, a 14-year-old boy and a 9-year-old girl, have been described elsewhere (Maekawa et al. 1984). In that report, substantial residual phosphorylase activity was found (about 50% of normal) in muscle from patient 3 but, when we analyzed it, the activity in the same muscle was undetectable. The reason for this discrepancy is not clear, but lack of activity is likely, because only a very little amount of myophosphorylase protein was detected by SDS-polyacrylamide gel in the original report.

Patient 5.—A 33-year-old Japanese man had muscle cramps and pigmenturia since childhood and experienced acute renal failure secondary to rhabdomyolysis. A forearm ischemic exercise caused no increase of venous lactate. Myophosphorylase deficiency was documented both histochemically and biochemically in a muscle biopsy.

Control muscle samples consisted of biopsies obtained for diagnostic purposes from individuals ultimately deemed to be free of neuromuscular diseases. Control genomic DNAs were extracted as described below from muscle or white blood cells of 91 normal individuals and nine patients with various metabolic myopathies: two with myoclonic epilepsy and ragged-red muscle fibers; two with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; two with muscle phosphofructokinase deficiency; one with phosphoglycerate kinase deficiency; and two with muscle phosphoglycerate mutase deficiency.

Genomic DNA Extraction and Screening for Three Known Mutations

Genomic DNAs were extracted from biopsied muscle tissues of cases 1, 2, 3, and 5 and of both parents of cases 3 and 4, and from white blood cells of case 4 (Shanske et al. 1987). Genomic DNAs from cases 1, 2, and 5 were screened for the three known mutations by using RFLP as described elsewhere (Tsujino et al. 1993).

RNA Extraction, PCR Amplification, and Sequencing

Extraction of mRNA from muscle was performed using the Micro Fast Track™ mRNA isolation kit (Invitrogen, San Diego) in patient 1, while total RNA was

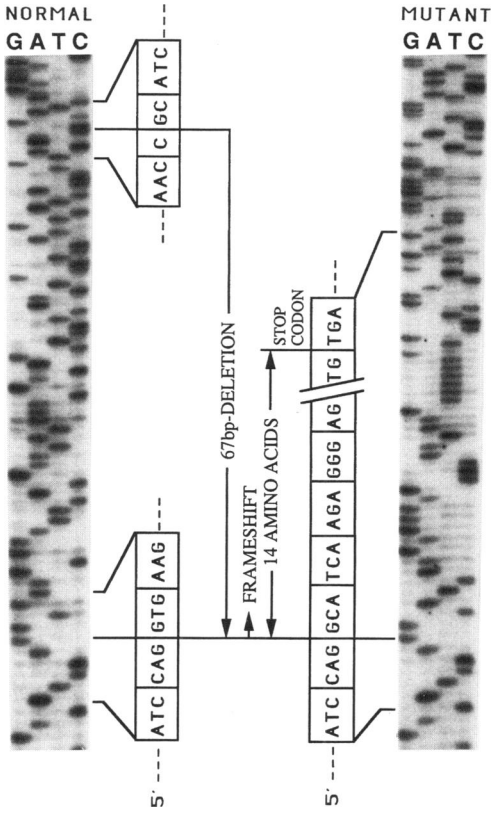

Figure 2 Mutant cDNA sequence with a 67-bp deletion and a frameshift, generating a premature stop codon. Boxed triplets are recognized as codons.

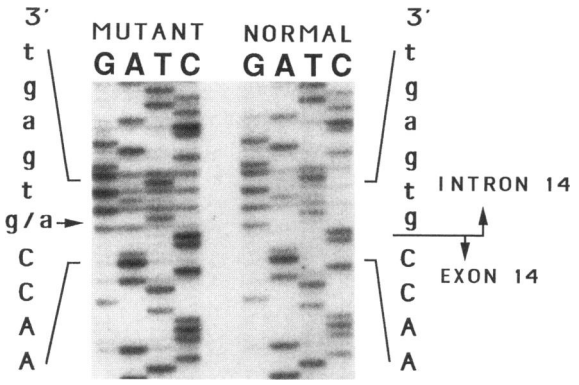

Figure 3 Sequence of the mutant DNA, showing a heterozygous mutation—a G-to-A substitution—at the 5′ end of intron 14 of the myophosphorylase gene in patient 1.

extracted with a modified cesium chloride centrifugation method (Glisin et al. 1974) in patients 2, 3, and 5. Six DNA fragments encompassing the entire coding region of the myophosphorylase cDNA were directly amplified from mRNA or total RNA by using primers described elsewhere (Tsujino et al. 1993) and the Gene Amp Thermostable rTth Reverse Transcriptase RNA PCR kit (Perkin Elmer Cetus, Norwalk, CT) according to the manufacturer's specifications. The PCR products were electrophoresed through 2% NuSieve agarose gels (FMC Bioproducts, Rockland, ME), purified by Geneclean kit (BIO101, La Jolla, CA), and sequenced using the same primers and the dsDNA Cycle Sequencing System kit (GIBCO BRL Life Technologies, Gaithersburg, MD) according to the manufacturer's specifications. The sequences were compared with those of PCR fragments obtained from a normal control. DNA fragments were subjected to a cycle-sequencing PCR program in the presence of 2 pmol of [γ-^{32}P]ATP 5′ end-labeled primer. The PCR products were electrophoresed through a 6% polyacrylamide/7-M urea gel. The gel was vacuum dried for 30 min and exposed to Kodak XAR film for 12 h.

We found several discrepancies from the sequence reported elsewhere: CGC (our data) instead of CGG (Burke et al. 1987) at codon 16, GCC instead of GCT at codon 153, GGC instead of GGT at codon 454, GCG instead of GCC at codon 456, TCC instead of TCG at codon 460, CAG instead of CAA at codon 754, ACC instead of ACT at codon 807, CCTC instead of TCC starting from the second nucleotide after the termination codon in the 3′ UTR, and G instead of T at the 25th nucleotide after the termination codon in the 3′ UTR. These differences may have been misreadings in the previous report (Burke et al. 1987), or they may represent neutral polymorphisms, since both controls and all our patients shared these changes and since they do not result in amino acid substitutions.

PCR Amplification of Genomic DNA and Sequencing

A PCR fragment that included the entire intron 14 was amplified from genomic DNA of case 1 by using the following primers: primer 1 (5′-GTTTGCTGCCTA-CCTAGAGAG-3′) in exon 14 and primer 2 (5′-CTC-CAATCATCACAGTCCGA-3′) in exon 15. Two more PCR fragments encompassing the 5′-flanking region (up

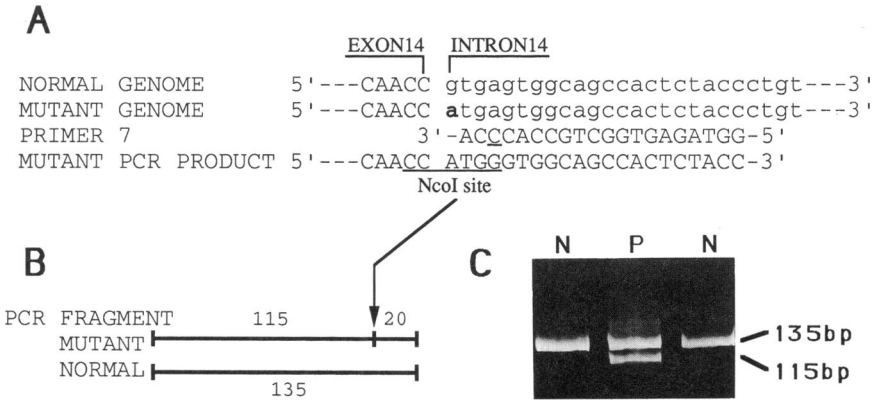

Figure 4 *A, top to bottom,* Sequences, around the mutation (bold letter), of normal and mutant genomic DNAs, primer 7 (the mismatched base is underlined), and mutant PCR product. *B,* Schematic representation of the mutant PCR fragment and of the normal PCR fragment. Numbers indicate length (in bp) of segments cleaved by *Nco*I. *C,* Electrophoresis on a 15% acrylamide gel of PCR products obtained using primers 1 and 7 (see Material and Methods) in a normal control (lane N) and in patient 1 (lane P) after digestion with *Nco*I.

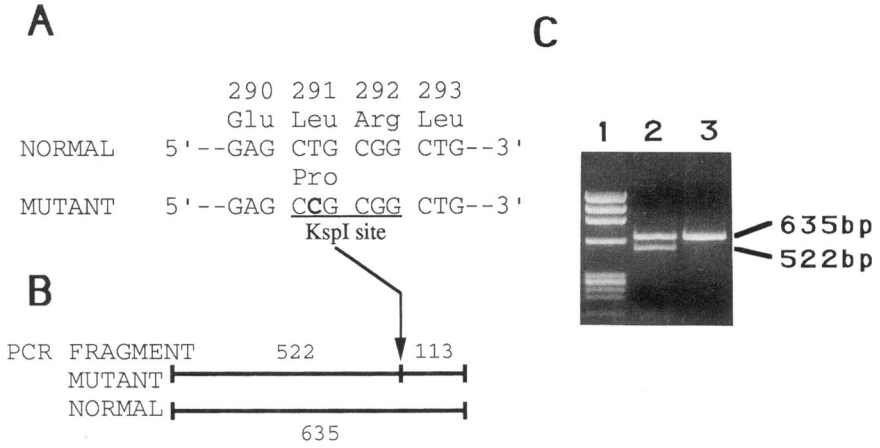

Figure 5 *A*, Nucleotide sequence and deduced amino acids around the missense mutation in patient 2. The mutation is indicated by the bold letter. The *Kspl* site is underlined. *B*, Schematic representation of the mutant PCR fragment and of the normal PCR fragment. Numbers indicate lengths (in bp) of segments cleaved by *Kspl*. *C*, Electrophoresis on a 2% NuSieve agarose gel of PCR products obtained using primers 8 and 9 (see Material and Methods) in patient 2 (lane 2) and in a normal control (lane 3) after digestion with *Kspl*. Lane 1, DNA molecular marker ΦX174/*Hae*III.

to 649 bp upstream from the transcription start site) were amplified from genomic DNA of case 5 by using primer 3 (5′-CCCGTGCTGCAGGCTAGCGG-3′) and primer 4 (5′-TCAGGGAGCTATTTTGAGGGC-3′), for an upstream fragment, and primer 5 (5′-AAGTGGAGC-TCACCTCTCCCT-3′) and primer 6 (5′-CATTGCGGT-CCTTTACGAGTG-3′), for a downstream fragment. *Taq* polymerase and reagents were purchased from Boehringer-Mannheim (Indianapolis). PCR conditions were 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. The PCR product was sequenced by the method described above and compared with a PCR product obtained from a normal control.

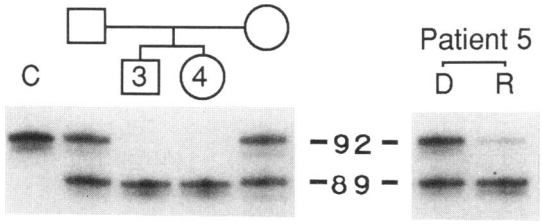

Figure 6 Electrophoresis on a 7% acrylamide/7-M urea gel of end-labeled single-strand DNA of PCR fragment. The PCR fragment from the allele containing the single-codon deletion is 89 bases, and the fragment from a normal allele is 92 bases. Patient numbers are indicated within the symbols. Lane C, Normal control. In patient 5, lanes D and R are PCR fragments from genomic DNA and RNA, respectively.

Digestion of PCR Fragment from Mismatch Primer with NcoI

To simplify the method of detection of the G-to-A mutation at the 5′ splice junction of intron 14, we designed an oligonucleotide (primer 7) with one base mismatch (5′-GGTAGAGTGGCTGCCACCCA-3′) (fig. 4*A*). A PCR fragment was amplified from genomic DNA using primers 1 and 7 under the conditions described above. In the presence of the G-to-A mutation, the PCR product was cleaved by the restriction endonuclease *NcoI* (Boehringer-Mannheim) (fig. 4*A* and *B*). Restriction digests were analyzed on 15% acrylamide gels.

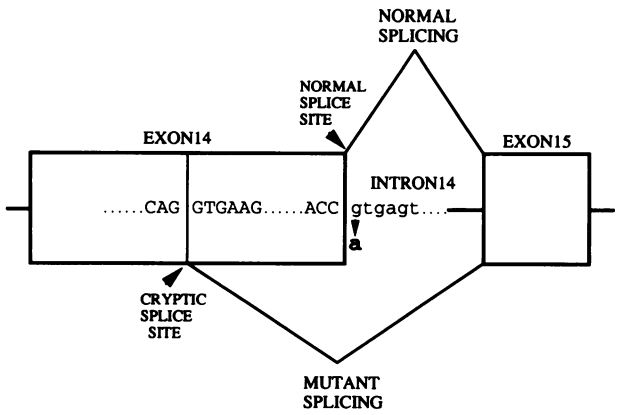

Figure 7 Schematic illustration of the splicing error in patient 1. The mutation is indicated by a boldface "**a**."

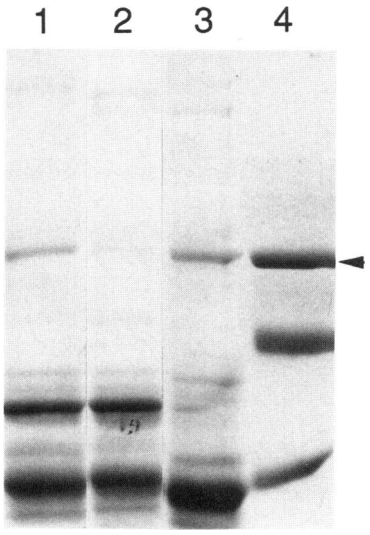

Figure 8 SDS-PAGE of muscle homogenates from patient 2 (lane 2) and from normal controls (lanes 1 and 3), as described by Laemmli (1970). Lane 4, Molecular weight standards (from top to bottom: myophosphorylase, serum albumin, and ovalbumin) (Bio-Rad, Hercules, CA). The arrowhead indicates myophosphorylase.

Digestion of PCR Fragment with KspI

To simplify the method of detection of the missense mutation at codon 291, a PCR fragment was amplified from genomic DNA by using primer 8 (5′-AAGGCATCGAAGTTCGTGCG-3′ in exon 7) and primer 9 (5′-AGGCTGTGTTGGACCGAAACC-3′ in exon 8, one of the primers used for PCRs from RNA) under the conditions described above. In the presence of the missense mutation, the PCR product was cleaved by the restriction endonuclease *Ksp*I (Boehringer-Mannheim) (fig. 5). Restriction digests were analyzed on a 2% NuSieve agarose gel.

PAGE of End-labeled Single-Strand DNA of PCR Fragment

To detect the single-codon deletion, a short PCR fragment was amplified from genomic DNA using primer 10 (5′-ACCATTGGCACCATGGACGGG-3′) and primer 11 (5′-ACATCCTCCACCCGCATGCCA-3′), the same primers used for PCRs from RNA. One (primer 10) of the two primers was end-labeled with [γ-^{32}P]ATP by polynucleotide kinase. The PCR was conducted in a final volume of 10 μl containing approximately 500 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.25 mM $MgCl_2$, 200 μM dNTP, 0.5 unit of *Taq* polymerase, and 1 pmol of each primer, under the following conditions: two cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, followed by 38 cycles of denaturation at 91°C for 1 min, annealing at 65°C for 1 min, and extension at 72°C for 1 min. For PCR amplification from RNA in patient 5, one of the PCR fragments (the fifth fragment from the 5′ end), first amplified from RNA, was used as a template. After addition of 5 μl of stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), the PCR products were denatured at 90°C for 2 min and electrophoresed through a 7% polyacrylamide/7-M urea gel. The gel was vacuum dried for 30 min and exposed to Kodak XAR film for 30 min.

Results

RFLP screening of two Caucasian patients (cases 1 and 2) with typical McArdle disease showed that they were heterozygous for Mut49, one of three mutations reported elsewhere (Tsujino et al. 1993). To identify the genetic errors in the other alleles of these patients and in Japanese patients who did not have any of the three known mutations, we extracted RNAs from muscle, and, using the combined reverse transcription–PCR method, we amplified six partially overlapping DNA fragments encompassing the entire coding region (2,523 bp) of the myophosphorylase cDNA.

A Splice-Junction Mutation

In patient 1, electrophoresis of these DNA fragments through a 2% NuSieve agarose gel showed that the fifth fragment from the 5′ end resolved into a normal and a truncated band (fig. 1). Each fragment was purified, sequenced, and compared with the corresponding control fragment. The normal size fragment had the same sequence as a control, but the truncated fragment harbored a 67-bp deletion in the 3′ terminal region of exon 14 (fig. 2). All other fragments, except for the heterozygous Mut49, showed no differences from normal. No other abnormal size transcript could be detected by our method.

Next, we amplified, from genomic DNA of case 1, a PCR fragment containing the entire intron 14. Analysis on a 2% NuSieve agarose gel showed that this fragment was of normal size (455 bp), implying that there was no deletion in genomic DNA. However, thorough sequence analysis of this fragment identified a heterozygous G-to-A substitution in the first nucleotide at the 5′ end of intron 14 (fig. 3), while the rest of the sequence in intron 14 was identical to that of our normal control. In both the patient and a control, however, we found

Table 1

3-bp Deletions Reported in the Literature

Gene	Sequence	Reference
Myophosphorylase	N F F I AAC *TTC* *TTC* ATC	Present report
Cystic fibrosis	N I I F G AAT *ATC* *ATC* TTT GGT	Kerem et al. 1989
Alpha 1-antitrypsin	N F F S P ATC *TTC* *TTC* TCC CCA	Fraizer et al. 1989; Curiel et al. 1989
Hypoxanthine phosphorybosyl transferase	D F V G F GAC T*TT* *GTT* *G*GA TTT GAC T*TT* *GTT* *G*GA TTT	Davidson et al. 1989 Gibbs et al. 1989
Pyruvate dehydrogenase E1 alpha	E V R S K S *GAA* *GTA* *AGA* *AGT* *AAG* AGT	Hansen et al. 1991
Beta-hexosaminidase A	T F F L ACA *TTC* *TTC* TTA	Akli et al. 1991
Beta-propionyl-CoA carboxylase	G I I R GGC *ATC* *ATC* CGG	Tahara et al. 1990; Lamhonwah et al. 1990
Acid sphingomyelinase	L C R H CTG T*GC* *CGC* *C*AC	Levran et al. 1991
Rhodopsin	V I I M GTC *ATC* *ATC* ATG	Inglehearn et al. 1991

NOTE.—*Italic* letters indicate a repeat sequence, and underlined triplets are deleted.

one more difference from the sequence reported elsewhere (Burke et al. 1987): at the 5′ end of intron 14, the sequence in the patient (the pathogenic mutation described above is in parentheses) and in a control was (g/a)tgagtggca, while the sequence reported elsewhere was gtga*tggca.

To confirm the splice-junction mutation and to develop a simple screening procedure, we designed an oligonucleotide with one base mismatch (fig. 4A). In the presence of the mutation, the mismatch primer created a new *Nco*I site in the 135-bp PCR fragment that was cleaved by *Nco*I into two fragments of 115 and 20 bp (fig. 4B). We looked for this mutation in 14 other patients with McArdle disease, 9 of whom were heterozygous for known mutations and 5 of whom had unknown mutations (Tsujino et al. 1993). None had this splice-junction mutation.

A Missense Mutation

In patient 2, a heterozygous T-to-C transition that changed CTG (Leu) to CCG (Pro) at codon 291 in exon 8 was identified (fig. 5; sequence data not shown). As the nucleotide change creates a restriction-enzyme *Ksp*I site, an appropriate PCR fragment (635 bp) containing the mutation was amplified from genomic DNA of case 2 by using primers 8 and 9 and was cleaved with *Ksp*I (fig. 5). In the presence of this missense mutation, the PCR fragment was cleaved by *Ksp*I into two fragments of 522 and 113 bp. Sequencing of this PCR fragment containing intron 7 revealed that the length of the intron in both patient and control was 439 bp, instead of 368 bp, as reported elsewhere (Burke et al. 1987). Screening of the 14 McArdle disease patients described above and of 91 normal controls and 9 disease controls showed that none of them had this missense mutation.

A Single-Codon Deletion

In patient 3, we identified a 3-bp deletion comprising a single codon (TTC at codon 708 or 709) encoding phenylalanine in exon 17 (sequence data not shown). The patient was homozygous, and the rest of the sequence of the coding region was identical to that of controls.

To detect this deletion, we amplified from genomic DNA a short PCR fragment including codon 708/709 and containing one end-labeled primer, and we studied the denatured PCR product by electrophoresis on a polyacrylamide gel. This method showed that the pa-

Table 2

Genotypes of 43 Patients with McArdle Disease

No. of Patients	Genotype[a]	
	Allele 1	Allele 2
18	1	1
3	1	2
2	2	3
1	1	3
1[b]	1	4
1[b]	1	5
6	1	?
3	2	?
2[b]	6	6
1[b]	6	?
5	?	?

[a] 1 = nonsense mutation at codon 49 (Tsujino et al. 1993; Bartram et al. 1993); 2 = missense mutation at codon 204 (Tsujino et al. 1993); 3 = missense mutation at codon 542 (Tsujino et al. 1993); 4 = splice-junction mutation (present report); 5 = missense mutation at codon 291 (present report); and 6 = deletion of codon 708/709 (present report).

[b] Patients in the present report. Others are from Tsujino et al. (1993).

tient's affected sister (case 4) was also homozygous for the deletion and that the parents were heterozygous (fig. 6). Screening of 5 non-Oriental patients with unknown mutations, as well as 91 normal controls and 9 disease controls by this method showed that they did not have this deletion.

In patient 5, sequencing of the PCR fragment from RNA showed that he was apparently homozygous for the deletion (data not shown). In contrast, the PCR fragment from genomic DNA indicated that he was heterozygous for the deletion, and the PCR fragment from RNA showed that the myophosphorylase gene on the other allele was faintly expressed (fig. 6). However, sequencing of all PCR fragments from RNA could not clarify the nature of the other mutation. In addition, the sequence of the 5′-flanking region up to 620 bp upstream from the transcription start site was identical to that reported elsewhere (Lockyer and McCracken 1991) and to the sequence of our control.

Discussion

We have identified three new molecular genetic defects in patients with myophosphorylase deficiency (McArdle disease)—a splice-junction mutation and a missense mutation in Caucasian patients who were heterozygous for the common nonsense mutation (Mut49) and a single-codon deletion in Japanese patients.

All introns contain consensus nucleotides (gt at the 5′ end and ag at the 3′ end [Breathnach and Chambon 1981; Mount 1982]), which are thought to be important for RNA splicing (Green 1986; Sharp 1987). The G-to-A mutation in patient 1 destroys the highly conserved consensus nucleotides gt at the 5′ end of intron 14 by changing AACC/gtgagtg to AACC/atgagtg. As a result, the sequence CCAG/GTGAAG in exon 14 is recognized instead and spliced, which results in a 67-bp deletion in the transcript (figs. 2 and 3). The splicing error is schematically illustrated in figure 7. The deletion leads to a frameshift containing a premature stop codon (fig. 2). It is likely that the resulting truncated peptide, with 14 mistranslated amino acids in the COOH-terminal, would lack glycogen phosphorylase activity. It also appears that the mutant peptide may be rapidly degraded, since immunoblot analysis failed to show any immunoreactive material in the muscle homogenate from the patient (data not shown). As the gene on the other allele contains a nonsense mutation (i.e., Mut49) and cannot make active enzyme protein, the combination of these two mutations explains the complete lack of myophosphorylase activity observed in the patient.

Mutations in the consensus sequence at the 5′ splice site of introns are known to cause two types of splicing errors. The first is due to recognition of the 5′ splice site of the intron upstream of the mutation and causes the upstream exon to be skipped. The second is due to activation of cryptic splice sites either in the upstream exon or in the downstream intron. As a consequence, either part of the upstream exon is deleted or part of the downstream intron is inserted in the transcript. Both types of splicing errors have been described in another glycogenosis, phosphofructokinase deficiency (glycogenosis type VII; Tarui disease). The first type was described in an Ashkenazi Jewish patient (Raben et al. 1993), and the second type, in a Japanese patient (Nakajima et al. 1990).

The leucine residue at codon 291, which is changed to a proline by the missense mutation in patient 2, and the phenylalanine residue at 708/709, which is deleted in the Japanese patients, do not appear to be in active sites (Sprang and Fletterick 1979). However, both mutations are likely to be the cause of myophosphorylase deficiency, because (1) they represented the only nucleotide changes in the coding region of myophosphorylase cDNA, except for the heterozygous Mut49 in patient 2; (2) two affected siblings (patients 3 and 4) were

homozygous, and their asymptomatic parents were heterozygous, for the single-codon deletion; and (3) 91 normal controls and 9 disease controls did not have any of these mutations in their 200 alleles. These mutations may cause structural changes, and, especially in the case of the deletion, the difference in length of the resulting peptide could be crucial. The abnormal enzyme proteins may be more prone to degradation, since SDS-PAGE showed a marked reduction of myophosphorylase protein in muscle from patient 2 (fig. 8) and from patients 3 and 4 (Maekawa et al. 1984).

Patient 5 was heterozygous for the single-codon deletion, but we were unable to identify the mutation on the other allele, even after sequencing the entire coding region in PCR fragments obtained from RNA. One reason for this failure may be that the myophosphorylase gene on the other allele is not expressed normally (fig. 6). Not only were all three mutations reported elsewhere negative in this patient by RFLP (Tsujino et al. 1993), but we also failed to identify any mutation that might reduce the level of mRNA in the 5′ flanking regulatory region of the myophosphorylase gene, which contains tissue-specific enhancers (Lockyer and McCracken 1991). Those authors also proved that a transcriptional repressor in the region upstream to the tissue-specific enhancers was strong enough to mask the effect of the enhancers. This observation led them to conclude that additional positive *cis*-acting regulatory elements may also be present (Lockyer and McCracken 1991). It is, therefore, possible that the still-unidentified mutation in patient 5 may alter an additional enhancer either further upstream or downstream of the myophosphorylase gene.

All single-codon deletions or 3-bp deletions reported so far, including the one described here, have been adjacent to short repeat sequences (table 1). As pointed out in several reports (Gibbs et al. 1989; Lamhonwah et al. 1990; Akli et al. 1991; Inglehearn et al. 1991), the mechanism for this kind of deletion may involve strand slippage during replication.

We have reported elsewhere that the nonsense mutation Mut49 is observed in most patients with myophosphorylase deficiency but that there is considerable genetic heterogeneity (Tsujino et al. 1993). The three new mutations described in the present study provide further evidence of genetic heterogeneity in McArdle disease. A summary of the mutations and of the genotypes seen in 43 patients that we studied so far is shown in table 2. The splice-junction mutation and missense mutation must be rare, since each mutation was present in only 1 of 80 alleles of the 40 non-Oriental patients studied by us (Tsujino et al. 1993). However, the single-codon deletion may be common in Japanese or Oriental patients with McArdle disease, because three Japanese patients from two unrelated families had this mutation. However, the presence of a different and still-unidentified mutation in case 5 suggests that molecular genetic heterogeneity also exists in the Japanese population.

Acknowledgments

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