ABSTRACT: We identified 5 patients with subnormal erythrocyte lactate transport plus symptoms and signs of muscle injury on exercise and heat exposure. All had transport rates below the 95% envelope for normals. Three cases had rates 40–50% of mean normal. One was found to have a missense mutation in monocarboxylate transporter 1 (MCT1), the gene for the red cell lactate transporter (also expressed in skeletal muscle), at a conserved site, which was not mutated in a cohort of 90 normal humans. The other 2 cases had a different missense mutation (at a nonconserved site), which was also not mutated in the normal cohort. All 3 patients were heterozygotes. We presume that these mutations are responsible for their subnormal lactate transport, and hence their muscle injury under environmental stress; homozygous patients should be more seriously compromised. The other 2 cases had lactate transport rates 60–65% of mean normal, and their MCT1 revealed a third mutation, which proved to be a common polymorphism in the normal cohort. These 2 patients may be physiologic outliers in lactate transport, with their muscle damage arising from some other genetic defect.

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MUTATIONS IN MCT1 cDNA IN PATIENTS WITH SYMPTOMATIC DEFICIENCY IN LACTATE TRANSPORT

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Some years ago we developed a clinically applicable assay for red cell lactate transport[9,10] and adapted our lactate–ammonia exercise ratio (LAER) test[6] to provide an estimate of muscle lactate level and its rate of decline after anoxic hand-grip exercise.[11] The premise was that lactate transporter defects in skeletal muscle (and red cells) might provide an explanation for some cases of muscle cramping or easy fatigue (or both) upon exercise, due to delayed removal of the lactic acid accumulated during (partially) anaerobic work. This would be an example of a perquisitory defect or disease; that is, one that compromises extreme performance, in the manner of myoadenylate deaminase deficiency,[5,8] rather than producing a sick patient at rest. After establishing normal ranges, we encountered, analyzed, and reported a symptomatic patient with defective red cell lactate transport plus a delayed decline in muscle lactate after exercise, thus satisfying our preset criteria.[7]

Over the subsequent years, we encountered several additional patients who presented similar findings, but lacked a demonstrable delayed decline in muscle lactate after exercise. Because these cases could be physiologic outliers, we postponed further reports until the gene for the lactate transporter had been found. In 1992, Kim-Garcia et al. cloned the first monocarboxylate transporter, MCT1, from Chinese hamster ovary cells[19] and showed that it is expressed, among other tissues, in erythrocytes, skeletal muscle, and heart.[20] Another monocarboxylate transporter, MCT2, expressed predominantly in hamster hepatocytes, was discovered soon thereafter,[22] and human MCT1 cDNA was also described.[21] This led us to retrieve the decade-old frozen muscle

Abbreviations: aa, amino acid; ATPase, adenosine triphosphatase; DAPI, 4′6-diamidino-2-phenylindole; EST, expressed sequence tag; IFET, ischemic forearm exercise test; LAER, lactate–ammonia exercise ratio; MCT, monocarboxylate transporter; RT-PCR, reverse transcriptase–polymerase chain reaction; SDS, sodium dodecylsulfate; SSCP, single-strand conformational polymorphism; UV, ultraviolet; x, any amino acid; φ, ile or leu or val

Key words: MCT1 mutations; lactate transport; muscle cramping; rhabdomyolysis; creatine kinase

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specimens and institute the current study to determine whether our patients with defective lactate transport had mutations in the MCT1 gene.

MATERIALS AND METHODS

Erythrocyte Lactate Transporter Assay. The lactate transporter assay has been described in detail elsewhere.[9,10] In brief, it involves freshly drawn anticoagulated blood, incubated at 37°C for 2–6 h without stirring to lactate-load the red cells. They are then packed, washed, and tenfold diluted into neutral buffer and stirred at 20°C (or 30°C). At six 1-min (or half-minute) intervals, aliquots are pipetted into iced buffer (pH 5), spun and washed, then spun and lysed in perchloric acid for enzymatic lactate assay.

LAER Test. The LAER test differs from the usual clinical ischemic forearm exercise test (IFET) by controlling blood flow through the exercised arm to permit diffusion equilibrium between blood and muscle lactate, and by quantifying energy expended and fatigue developed on squeezing a dynamometer wired to a recording integrator. The sphygmomanometer is locked at 20 mm Hg above resting systolic pressure while the patient (watching a seconds counter) squeezes every odd second and relaxes every even second for 120 s. The cuff pressure is then dropped to half-diastolic for 10 s and 7 mL of blood is withdrawn over a further 10 s. The cuff pressure is then dropped to 0 for a 2-min free-flow washout, then pumped back to half-diastolic for 10 s before the next blood sample is taken. This procedure is repeated for six samples covering a 10-min interval. Lactate and ammonia levels are measured on all samples, including an initial resting sample from the opposite arm (which does not exhibit postexercise elevations). The dynamometer trace is computer-converted to work and impulse (momentum) units, and fatigue factors are calculated from the decline of those parameters with time. A more detailed description, with examples, has been published.[11]

Isolation of mRNA and Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR). Frozen muscle biopsies were accessioned at the Armed Forces Institute of Pathology for diagnostic and research purposes, with informed consent, and were stored at −120° to −130°C. For mRNA, 30–40 sections of 40-μm thickness were cut from each biopsy in a cryostat at −20°C. The sections were thawed on ice and mRNA was immediately isolated using a QuickPrep Micro-mRNA purification kit (Pharmacia, Piscataway, NJ). The tissue was homogenized in 0.4 mL of extraction buffer in glass Duall tubes with eight to ten strokes and the subsequent steps were carried out as suggested by the manufacturer.

RT-PCR of MCT1 was performed using a First-Strand cDNA Synthesis Kit (Pharmacia) followed by PCR. The first-strand cDNA synthesis reaction was carried out using 20 μL (~200 ng) of mRNA and reverse primer RP7 (Table 1). An aliquot of cDNA was amplified by PCR using AmpliTaq polymerase (PE Applied Biosystems, Foster City, CA) with 2-μmol/L primers and 2 mmol/L $MgCl_2$. RP7 was

Table 1. Forward and reverse primer sets used in SSCP and sequencing of MCT1 cDNA.*

Primer pairs	Sequence	Amplimer total bp	Coding base
5′ > 3′			
P1	ACCACGTCACGCACACGCTCGG	266	(−88)–(−67)
P2	GCATTTCCCAAATCAATTACTGTCTTC	330	103–129
P3	GGAGGTCTTGGGCTTGCCTTCAACT	341	358–382
P4	GTCTAAAGCATCCCTTGAGAAAGCTG	287	627–652
P5	GGACTCTTTGCACCTTTGGTGT	332	826–847
P6	GGATTCTGTGTCTATGCGGGATTC	273	1063–1086
P7	ACACATACTGGGCATGTGGC	387	1262–1281
3′ > 5′			
RP1	ATGACACTTCGCTGGTGGTG		178–159
RP2	TGGTCGCCTCTTGTAGAAATACTTG		432–408
RP3	AGATCTGTATTTGCATCATGCAGATC		698–673
RP4	GAATGGAAAGAAGGAAGGCAGAC		913–891
RP5	CAACAAGGTCCATCAATGTTTCAA		1157–1134
RP6	ATAATTGATGCCCATGCCAATG		1335–1314
RP7	AAATCCCATCAATGAACAACTGGTATGATTTCCAC		(+148)–(+114)

**The correspondingly numbered P and RP were used as primer pairs for each of the seven amplimers. The coding bases start with the first nucleotide of the start codon and end with the last nucleotide of the last amino acid. In parentheses are nucleotides outside the coding region, either counting 5′ (−) before the first coding base, or counting 3′ (+) after the last coding base.*

used as the reverse primer, whereas the sequence for the 5′ forward primer, P1, was found in dbEST. Expressed sequence tag J0545F (GenBank acc. no. N84700) overlaps with the published MCT1 5′ sequence,[21] but has 76 extra nucleotides from the upstream region. P1 in Table 1 corresponds to nucleotides 1–22 from J0545F. The reaction mixture was overlaid with mineral oil and heat-denatured for 3 min at 95°C followed by 30 cycles of 1 min of denaturation (95°C), 1 min of annealing (55°C), and 3 min of elongation (72°C). After a final 10 min at 70°C, the PCR products were electrophoresed in agarose gels.

Single-Strand Conformational Polymorphism (SSCP) Analysis and Sequencing. For SSCP analysis, the full-length MCT1 cDNA was divided into seven overlapping fragments amplified by PCR using the primers shown in Table 1. PCR reactions were heat-denatured for 3 min at 95°C, followed by 35 cycles of 1 min of denaturation at 95°C, 1 min of annealing at 57.5°C, and 1 min of elongation at 72°C with a final 10-min period at 72°C. PCR products were analyzed by agarose gel electrophoresis and their concentrations were measured spectrofluorometrically using 4′,6-diamidino-2-phenylindole (DAPI) using ultraviolet (UV)-quantified herring sperm DNA as standard.[2] Two microliters of 10–20-ng/μL PCR products was added to 2 μL of denaturing solution (98% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, 2% glycerine), heated at 95°C for 3 min in the GeneAmp PCR System 9600 (PE Applied Biosystems), then cooled on ice for 2 min. Then, a 0.8-μL sample was loaded on each slot of a 1-μL application comb and run on 12.5% homogeneous polyacrylamide PhastGel with either native or SDS buffer strips at 14°C on PhastSystem electrophoresis (Pharmacia) for either 131 Vh (native) or 248 Vh (SDS). Gels were stained using a Pharmacia PhastGel DNA silver-staining kit.

PCR products (0.4 pmol) were sequenced using Sequenase 2.0 T7 DNA Polymerase (USB, Cleveland, OH) and ^{32}P-labeled dideoxy terminator nucleotides. Both forward and reverse PCR amplification primers were used for the sequencing reactions.

Probe Dot-Blots of Normal Samples. Ninety healthy volunteer blood donor samples were studied. Genomic DNA was extracted with kits supplied by Gentra Puregene (Research Triangle Park, NC), and fragments containing the mutation sites were amplified by PCR using the appropriate primer pairs from Table 1. Dot-blots were prepared on Nytran sheets with 1–2-μL aliquots of alkalinized amplimers by standard methods,[1] dried, and then hybridized at 42°C overnight in two separate chambers with complementary 14–16-mer probes centered on the mutation site (Table 2), one containing the mutant nucleotide, one containing the normal correspondent, and each end-labeled with [α^{32}P]CTP and terminal deoxynucleotidyl transferase.[35] After thorough rinsing of the excess isotope, the blots were incubated for 30 min at 56°C (for mutation III), 55°C (for mutation II), or 47°C (for mutation I) in 1% sodium dodecylsulfate (SDS)/450 mmol/L NaCl/45 mmol/L sodium citrate, and then drained, blotted, wrapped, and exposed to Kodak X-Omat AR film in a cassette with scintillator screen at −120°C for 3–24 h. Sequenced normal controls and mutant case(s) were used as standards. Dot-blots were graded by two independent observers and equivocal cases were repeated and omitted if again equivocal. Approximately equal reaction with the normal and the mutant probe indicated a heterozygote, whereas reaction with only the normal or with only the mutant indicated a normal or mutant homozygote, respectively.

RESULTS

Clinical and laboratory data on the 5 cases are summarized in Table 3. Case 1, the index case, was reported in detail earlier,[7] using red cell transport assays at 20°C. We later found that 30°C assays (also performed in case 1) gave somewhat narrower confidence limits,[10] as well as being closer to physiologic status; therefore, we utilized those data in this report. All of the patients were men, and all but case 5 had overt symptoms of muscle cramping or stiffness (or both) after exercise, especially in hot weather, often increasing in intensity over months to years. All had serum creatine kinase elevations documented on one or more occasions; the highest elevations were in case 5, who had no symptoms other than some malaise that he considered normal for the ex-

Table 2. Dot-blot hybridization probes used for the evaluation of normal blood donors' DNA for the three mutations found in MCT1.*

Name	Sequence	Coding base
Normal variant I	AGCCAACC**A**AGGCAG	602–616
Mutation I	GCCAACC**G**AGGCAG	603–616
Normal variant II	ATGTTGCT**G**GGAAGCC	1406–1421
Mutation II	GATGTTGCT**A**GGAAGCCA	1405–1422
Normal variant III	AGACACAGA**A**GGAGGGC	1461–1477
Mutation III	AGACACAGA**T**GGAGGGC	1461–1477

**See "Materials and Methods" for description of their use, and Table 4 for the mutation frequencies.*

Table 3. Clinicopathologic data of patients with deficient red cell lactate transport.

Case	Age, race, gender	Signs and symptoms	Maximum CK (IU)	Percent mean N RBC LT at 30°C mean ± SD (≠)	EMG	Muscle biopsy; % type 2 fiber area	Muscle CPT
1	26 BM	Three bouts of chest muscle cramping after exercise	14,000	42.4 ± 8.6 (9)	N	N; 77.1%	N
2	42 BM	Muscle cramping/stiffness increasing for 5 yr	800	49.2 ± 2.8 (5)	N	N; 41.0%	N
3	56 BM	Muscle cramping/stiffness increasing for 7 yr	900	42.1 ± 4.6 (9)	N	N; 67.6%	N
4	19 WM	Three-hour bouts of muscle cramps after 2-mile runs for 1 yr	500	66.3 ± 1.0 (4)	N	N; 83.0%	N
5	28 BM	Routine PE and blood chemistries after 2 PT workouts	15,000 25,700	59.3 ± 6.0 (4)	N	N; 76.8%	N

All cases were below the 95% envelope for lactate transport in normal volunteers as shown in Figure 1. The percent muscle area occupied by type 2 fibers was determined from the alkaline ATPase differential stain of type 1 and 2 fibers.[15] BM, black male; CK, serum creatine kinase; CPT, carnitine palmityltransferase; EMG, electromyogram; LT, lactate transport; N, normal; PE, physical examinations; PT, physical training; RBC, red blood cell; WM, white male; ≠, number of assays.

ercise level. Frozen muscle biopsies from the 5 patients displayed normal histologic architecture, and no significant abnormalities were detected using the standard battery of stains applied by the Armed Forces Institute of Pathology muscle laboratory (hematoxylin-eosin, nonspecific esterase, modified Gomori trichrome, NADH diaphorase, alkaline ATPase, myoadenylate deaminase, periodic acid Schiff reaction, alkaline phosphatase).[15] Because of the lack of an apparent etiology, all cases also had electromyograms that were within normal limits, and enzymatic assays for muscle carnitine palmityltransferase[4] that were also normal.

All cases underwent ischemic forearm exercise tests and had hand strength, work and impulse output, progressive fatigue, and lactate and ammonia elevations that were within normal limits. Case 1 alone had significantly delayed lactate decline in the exercised arm. All 5 cases had erythrocyte lactate transport below the 95% envelope for normal volunteers, as shown in Figure 1, although the percent of the normal mean transport rate, as shown in Table 3, suggested a carrier level of activity rather than a complete deficiency. The residual activity was not due to diffusional transport, because the addition of an organomercurial thiol reagent further reduced the transport to 10% of normal. Cases 1–3 had sufficient data over a broad lactate range to permit calculation of reasonably reliable rectangular hyperboles, which yielded K_m values about half of normal, and V_m values about 30% of normal. By direct ratios at each given substrate level, as used in Table 3 to accommodate all 5 patients, these cases had 40–50% normal transport, whereas cases 4 and 5 had significantly higher levels, near 60–65% normal.

Messenger RNA was isolated from each patient's frozen muscle biopsy and subjected to RT-PCR. The reverse primer for RT-PCR was based on the reported sequence of human MCT1, which included only 12 basepairs upstream from the initiation codon.[21] However, an overlapping expressed sequence tag (see "Materials and Methods") permitted use of a primer starting 88 bases upstream from the initiation codon. PCR yielded 1.7-kb PCR products corresponding to the patients' MCT1 cDNAs, which were screened for mutations by SSCP analysis of seven overlapping amplimers covering the full length of MCT1 cDNA (Fig. 2). SSCP was performed under both native and denaturing conditions (with SDS), which provides more than 95% mutation detectability.[23] Those PCR products that exhibited abnormal bands on silver-stained gels were sequenced in both directions for all of the patients. Later in the course of this study, an automated sequencer became available and, because of the vast improvement in speed, all cDNA fragments were then sequenced, but no additional mutations were found. The mutations are listed in Table 4, along with the dot-blot analyses of healthy blood donors, to estimate the population frequency of these mutations. An example of dot-blot preparation and interpretation is shown in Figure 3.

We identified three mutations in the 5 patients. Case 1 had A > G at coding base 610 in one of his two alleles, converting #204 lysine to glutamic acid; this is a conserved lysine in hamster, mouse, rat, and human models, and was not encountered in 90 normal donors, providing a significant difference at the 6% probability level. Cases 2 and 3 had G > A at coding base 1414, each in one of two alleles, converting #472 glycine to arginine. This glycine is not conserved, but no mutation was found in the normal donors, yielding a highly significant difference ($P < 0.01$). The third mutation involved an A > T at coding base 1470, converting #490 glutamic acid to aspartic acid. Patients 2 and 4 were homozygous for

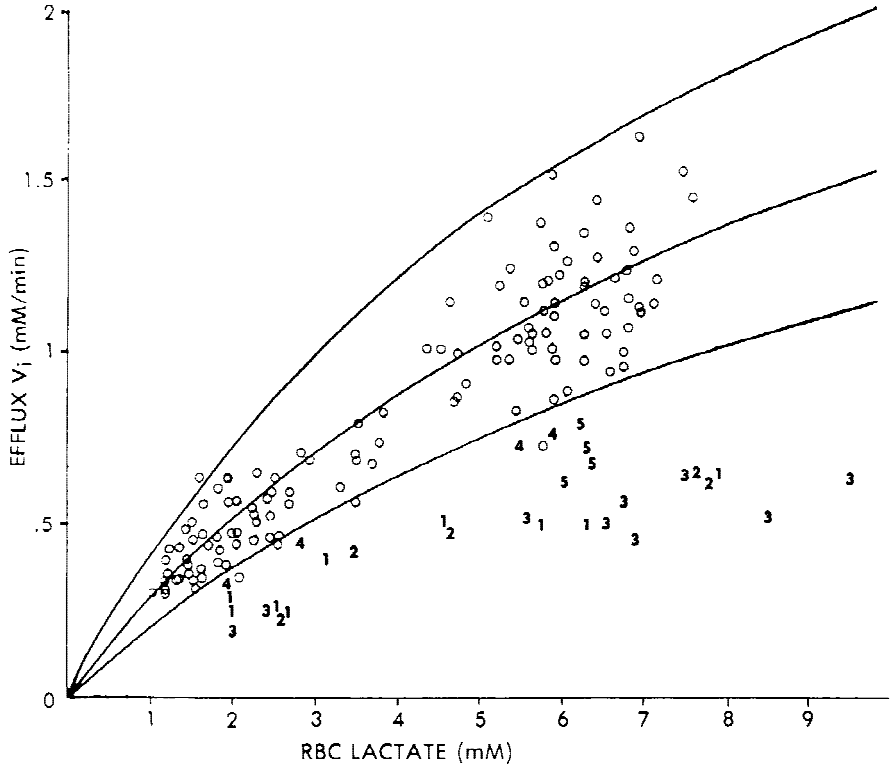

FIGURE 1. Substrate–velocity plot of the red blood cell lactate efflux at 30°C. The results of 130 assays for healthy volunteers (○) are shown. The hyperbolic curves show the least-squares best fit, based on grouped data to provide the most unbiased weighting function, and the 95% envelope, analogous to confidence limits, constructed from the standard errors of V_m and K_m by constant factor multiplication (see ref. 10 for details). The numbers mark the individual assay values for each of the 5 patients studied. The assay rates, as percent of the normal mean efflux velocity at the corresponding initial lactate concentration, were averaged for each patient in Table 3.

this mutation, whereas patient 5 was heterozygous for it (as were his mother and his daughter). The #490 glutamate is not conserved evolutionarily, and the mutant form of base #1470 was found in half the normal donors (see Table 4), indicating a very common polymorphism. The observed mutant gene frequency was 30.4%, and this yielded a distribution of phenotypes very close to that predicted by the Hardy–Weinberg equilibrium.[16]

An unrelated observation was that coding base #1438, in all patients and controls sequenced (total of 9), was a G and not an A, as reported by Garcia et al.,[21] so that amino acid (aa) #480 is alanine and not threonine.

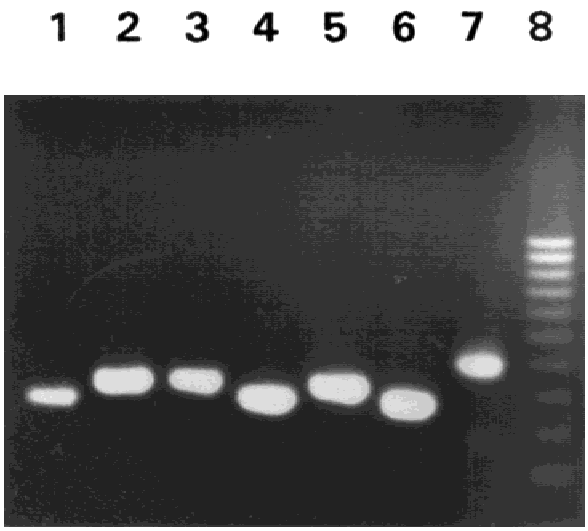

FIGURE 2. Agarose electrophoretic gel demonstrating overlapping PCR amplimers covering the entire human MCT1 coding sequence. These amplimers were used for SSCP and for manual sequencing. Lane 8 contains a 100-bp DNA stepladder series. Lanes 1–7 show the seven amplimers from a single control's cDNA. See "Materials and Methods" and Table 1 for the primer pairs used and the total basepairs in each amplimer.

DISCUSSION

Since our report of a patient with lactate transport deficiency,[7] there have been no further cases reported, to our knowledge. Although this could indicate the rarity of the entity, we believe it much more likely that it is due to the tedious nature of the physiologic assay[9] plus the lack of interest in a perquisitory disease. Our patient was strong and healthy, not

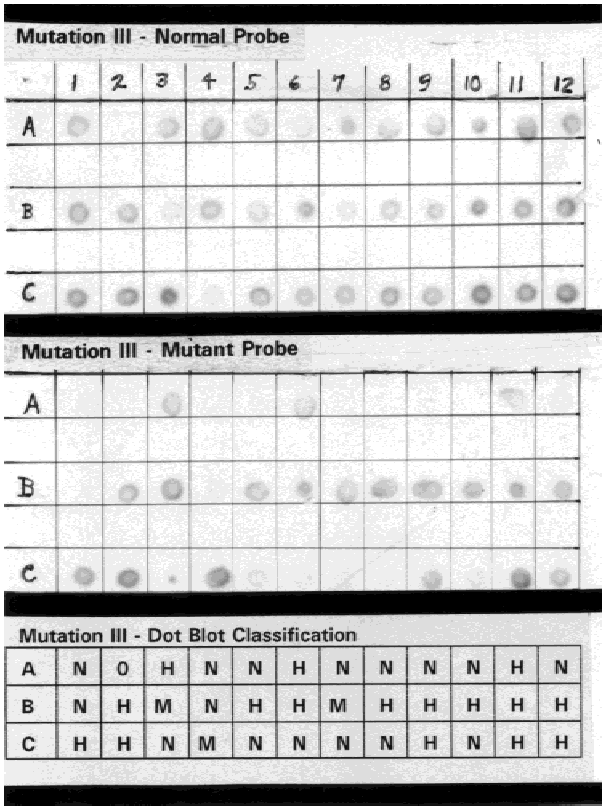

FIGURE 3. Dot-blots for mutation III and their interpretation. In the matrix, squares C1–C4 contain the standards, whose mutant or normal base has been verified by sequencing; that is, case 5, his mother, case 1, and case 4, respectively. A spot reacting approximately equally with the normal probe and with the mutant probe (e.g., squares C1 and C2) indicates a heterozygote (H). A spot that shows definite reaction with the normal probe, but negligible reaction with the mutant probe (e.g., square C3), is classified as homozygous normal (N), whereas a spot that shows definite reaction with the mutant probe, but negligible reaction with the normal probe (e.g., square C4), is classified as homozygous mutant (M). If neither probe reacts with the spot (as in square A2), the case is coded null (0) and not included, unless a response is obtained on a repeat test. The dot-blots are classified in the bottom matrix. See text for further details.

weak and sick; however, after heavy exercise, he developed severe chest (muscle) pain and elevated serum creatine kinase, which was mistakenly thought to represent coronary artery disease with angina. His red cell lactate transport rate was 40–50% normal, and his extrapolated maximal velocity at the highest lactate loads (after extreme exercise) would be about 30% normal. In addition, his LAER test indicated a much slower decline in muscle lactate from his exercised arm (as inferred by equilibration with local blood lactate) than in normal volunteers.[7] It seems reasonable, therefore, to presume that the heterozygous missense mutation that we have now found may explain his symptoms and muscle enzyme leak after heavy exercise.

Patients 2 and 3 had similar levels of deficiency to that of patient 1 in their red cell lactate transport. Their symptomatic episodes were less extreme and progressed in frequency over several years; and their muscle lactate decline after exercise was not significantly delayed in our LAER test. Both of these patients were heterozygous for the same missense mutation (but differing from that of patient 1), which was absent (or rare) in the normal blood donor population, and may well provide a genetic basis for the slow lactate transport observed.

Patients 4 and 5 had higher erythrocyte lactate transport rates (60–65% normal) and had a different missense mutation, which was so common in the normal population that we must presume it is a polymorphism without effect on the transport function. These cases may simply be physiologic outliers in lactate transport. Certainly, case 5, who had marked creatine kinase elevations documented on two occasions, is likely to have some other genetic explanation for his muscle membrane permeability on exercise. Case 2 was homozygous for this polymorphism as well, although it presumably did not contribute to any clinical effects of his mutation II. If cases 1–3 were indeed provoked by a single mutant allele, then homozygous mutants should have more serious problems than we would have anticipated. Screening for the two known mutations would represent a much simpler approach to locating deficient cases than applying the rather exacting physiologic testing we have used so far.

Using the red cell plasma membrane topology proposed for rat MCT1 on the basis of antibody reactivity,[31] we can assign all three human mutations to intracytoplasmic segments of the protein. Mutation I lies in the early part of the large central loop between transmembrane segments 6 and 7. Mutation II is halfway along the C-terminal chain, and mutation III is just ten amino acids from the C-terminal val (#500). This last mutation (#490) is in the signal sequence (asp–x–glu; aa 488–490) for release from the endoplasmic reticulum when downstream from a tyr–x–x–φ motif[28] (here, aa 445–448); a dibasic acid is important, but asp may be as effective as the preferred glu.

Evaluation has become complicated by the recent discovery of five additional mRNAs coding for lactate transporters, some of which seem tailored for highly specific cell or tissue destinations and functions.[17,24,32,36] Both mRNA and protein analyses revealed that each MCT has a specific pattern of tissue expression and that several transporters can be coexpressed in the same tissue.[18,32] In some cases, this is due to the utilization of separate MCTs by differ-

Table 4. Summary of the three mutations found in the 5 cases with deficient red cell lactate transport, and their frequency in a normal blood donor cohort.

Mutation	Coding base	aa change	Cases and status	Case frequency	Normal donor mutation frequency	Chi-square or Fisher Exact *P*-value
I	610 A > G	204 Lys > Glu	1 μN	1/5	0/90	0.053
II	1414 G > A	472 Gly > Arg	2 μN 3 μN	2/5	0/83	0.003
III	1470 A > T	490 Glu > Asp	2 μμ 4 μμ 5* μN	3/5	40/79	1.00
				μμ 2/5	μμ 8/79	
				μN 1/5	μN 32/79	(0.128)
				NN 2/5	NN 39/79	

N, one parental allele has the normal nucleotide rather than the specified mutation; NN, both parental alleles have the normal nucleotide rather than the specified mutation; μ, one parental allele has the specified mutation; μμ, both parental alleles have the specified mutation. In the last column, mutation incidence in the patients was compared with a normal blood donor cohort by Fisher's exact test, whereas the 2 × 3 way comparison of normals, mutants, and heterozygotes with respect to mutation III required a chi-square test for the P-value shown in parentheses.
**Blood samples available for the mother and daughter of case 5 were shown to be heterozygous for the same mutation, by sequencing amplimer 7.*

ent cells in the same organ. Thus, in rat skeletal muscle, MCT1 is expressed in fast-twitch oxidative glycolytic (or IIa) and slow-twitch oxidative (or I) fibers, but not in fast-twitch glycolytic (or IIb) fibers, which instead express MCT3.[36] This implies differential kinetics, directionality, or modulation of expression in the transporters, suited for improved metabolic efficiency. Thus, the transfer of lactate produced in white (IIa) fibers to red (types I and IIa) fibers for oxidation would provide a small but distinct improvement in energetics that might be crucial under conditions of extreme exercise. Indeed, the lactate transferred intercellularly may also induce the release of bound O_2 from the myoglobin of the red fibers, to further facilitate oxidative metabolism.[14,34] Again, in the nervous system, the astrocytes express MCT1 and MCT2, which may transport lactate to the neighboring neurons as a backup fuel.[12,13,33] In time of stress, that backup may enable more neurons to survive. These are what we have called perquisitory functions[7]: they are not essential, but they improve efficiency and performance, and their loss may produce disease or deficit only upon the extremes of activity or aging.

It is clear that MCT expression is subject to environmental and developmental modulation. The level of expression of MCT1 (and the lactate transport rate) in skeletal muscle was increased upon chronic electrical stimulation, whereas denervation reduced it.[25,27] Lactate transport kinetics differed between red cells and a tumor cell line, presumably because of altered membrane phospholipid composition,[3] while MCT1 and MCT2 expression has been shown to be developmentally regulated in mouse brain.[29] In some tissues, the MCT isoform expressed may vary from one species to another,[18] although, to date, only MCT1 has been found to be expressed in erythrocytes, the most facile medium for physiologic assay. Moreover, MCT1 is expressed in the skeletal muscle of all species examined, although its fiber-type distribution has varied.[20,26,27,30,36]

Special considerations are involved in the assessment of transport function after an amino acid substitution in a molecule that is not a simple soluble enzyme, but requires specific localization in a specific membrane to provide optimal function. Thus, the finding of transport activity in liposomes or some other membrane package would not suffice to exclude the significance of the mutations we have found. Indeed, MCT1 was discovered serendipitously, because a single base mutation, causing an amino acid change from phenylalanine to cysteine, converted the carrier from a lactate to a mevalonate transporter.[19] Only a long series of further studies on the family of lactate transporters will ultimately decipher their complete role in the tissues of humans and animals.

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