Deficiency*  

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Molecular Basis of Canine Muscle Type Phosphofructokinase Deficiency*  

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Muscle type phosphofructokinase (M-PFK) deficiency is a rare inherited glycogen storage disease in humans that causes exertional myopathy and hemolysis. The molecular basis of canine M-PFK deficiency, the only naturally occurring animal homologue, was investigated. Lack of M-PFK enzyme activity was caused by a nonsense mutation in the penultimate exon of the M-PFK gene, leading to rapid degradation of a truncated (40 amino acids) and therefore unstable M-PFK protein. A polymerase chain reaction-based test was devised to identify M-PFK-deficient and carrier animals. This represents one of only a few inborn errors of metabolism where the molecular defect has been identified in a large animal model which can now be used to develop and assess novel therapeutic strategies.

Phosphofructokinase deficiency, due to a lack of muscle type phosphofructokinase (M-PFK) subunits or activity, has been described in humans as a rare autosomal recessive trait. This disorder, also known as glycogenosis type VII or Tarui-Layzer syndrome, causes a metabolic myopathy and compensated hemolytic disorder (1). Mutations in the M-PFK cDNA have been described in Ashkenazi Jewish, Japanese, French Canadian, and Swiss patients, but little information is available regarding these defects at the protein level (2–4).

A naturally occurring animal model of M-PFK deficiency has been described in English springer spaniels (5, 6). The affected dogs have a chronic compensated hemolytic disorder and exertional myopathy, as is seen in human patients. However, these animals most often present with hemolytic crises due to the high capacity of the dog for aerobic work and the intrinsic alkaline fragility of erythrocytes (7). Dogs with M-PFK deficiency have 6–22% of normal erythrocyte PFK activity and 1–4% of normal muscle PFK activity (5–9). The normal canine M-PFK cDNA has been sequenced recently (10), making it possible to elucidate the underlying molecular mechanisms of canine M-PFK deficiency. We identified a single nonsense mutation that leads to rapid degradation of an unstable truncated M-PFK protein.

EXPERIMENTAL PROCEDURES  

Animals—Unless otherwise indicated, tissue and blood samples for PFK studies were obtained from a colony of English springer spaniels, founded and maintained at the School of Veterinary Medicine, the University of Pennsylvania. The M-PFK-deficient animals used to create this colony originated from Wisconsin, Maryland, and Indiana. The pedigrees of the three founding families have no common ancestor for at least six generations (11). All animals were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Muscle samples for PFK activity assays, protein studies, and RNA isolation were obtained by surgical biopsy of pentobarbital- and halothane-anesthetized dogs or by sterile excision post-mortem. These tissues were snap frozen in liquid nitrogen and stored at –70°C. Blood samples anticoagulated with EDTA were kept on ice until used for PFK enzyme activity assays and DNA extraction. Animals were considered to be PFK-deficient (affected), carriers (heterozygous asymptomatic), or normal based on erythrocyte and muscle PFK activities and pedigree analysis (9).

Immunoblots—Immunoblots of M-PFK were prepared as described previously (12). Briefly, 100 µg of total protein was run on a 7% denaturing polyacrylamide gel and transferred to a 0.45-µm nitrocellulose membrane. The membrane was blocked with 5% milk powder and was probed with guinea pig anti-rabbit M-PFK antiserum (13, 14). Metabolic Labeling—Primary canine myoblasts were obtained and cultured from a normal and an M-PFK-deficient dog, as described previously (13). The myoblasts were plated at high density (approximately 1.25 × 10³/mm²) and allowed to differentiate and form myotubes in Dulbecco’s modified Eagle’s medium supplemented with 10% horse serum. The medium was removed, and the adherent cells were washed with phosphate-buffered saline. Minimum essential medium (Eagle’s medium-deficient) (Sigma), supplemented with L-lysine, L-isoleucine, and sodium bicarbonate, but without methionine, was added for 30 min. The medium was removed and deficient medium (above) supplemented with [³⁵S]methionine (1000 Ci/mmol, Amersham Corp.) at 5.5 µCi/mL was added for 18 h. The cells were washed with phosphate-buffered saline and either harvested or Dulbecco’s modified Eagle’s medium was added for periods of up to 24 h. M-PFK was then immunoprecipitated with rabbit anti-dog M-PFK antibody as described and visualized by 7% SDS-polyacrylamide gel electrophoresis (PAGE) followed by autoradiography (12, 14).

RNA Blots—Total muscle RNA was prepared from a PFK-deficient English springer spaniel and a normal beagle by the method of Chomczynski and Sacchi and was subjected to formaldehyde-agarose gel electrophoresis (15). The RNA was transferred by capillary action onto a nylon membrane (Hybond, Amersham Corp.) and fixed to the membrane in a UV light cross-linking apparatus (Stratalinker, Strat-
agene, La jolla, CA). Blots of total muscle RNA were probed with the full-length canine M-PFK cDNA (PCR PFKd4) (10) for 18 h at 55 °C, washed at 60 °C in 1 × SSC, and exposed to x-ray film.

RT-PCR Cloning—First strand cDNA from an affected male dog was synthesized using 2 μg of total skeletal muscle RNA prepared as described for the Northern blot, or mRNA from the same preparation that was used to construct the cDNA library (15). Primers with the sequences gGCGGCGCGCCGCATTAGCCCAGCAGGAC (PFK Xho) containing the start codon (underlined, lower case, bold), and cogCTCGAGACACTGACCTACGTTGAGCAT (PFK Xho) were designed with homology to M-PFK (lower case, bold) and NotI or XhoI restriction sites (upper case). These primers were used to amplify oligo(dT)-primed first strand cDNA with a reaction profile of 40 cycles of 1 min at 95 °C, 1 min at 50 °C, and 3.5 min at 72 °C. The PCR product was purified by agarose gel electrophoresis, electroduted, serially digested with NotI and XhoI, and ligated into NotI/XhoI-di

gested Bluescript II phagemid (Stratagene).

**RESULTS**

SDS-PAGE of skeletal muscle extracts from normal, carrier, and M-PFK-deficient English springer spaniels, as determined by total erythrocyte PFK activity, were prepared and stained for total protein. A band of ~85 kDa, which was present in normal dogs, was absent in affected dogs (Fig. 1A, lanes 1 and 3). An 85-kDa band of reduced (approximately one-half) intensity was seen in muscle from carrier dogs (Fig. 1A, lane 2).

To determine whether the 85-kDa band corresponded to M-PFK, similar gels were transferred electrophoretically to nitrocellulose membranes and hybridized with a guinea pig anti-rabbit M-PFK antibody known to cross-react with M-PFK. The immunoprecipitated protein was then identified using the Lambda ZAP II vector following the manufacturer's protocol. Approximately 1 × 106 plaques constituting the entire primary plating of the M-PFK-deficient canine cDNA library were lifted on nitrocellulose membranes and screened using a full-length human M-PFK cDNA (HMPFK2, or the 5′ or 3′ EcoRI fragments of HMPFK2) (10). Positive clones were plaque purified and excised in vivo into the plasmid form, according to the manufacturer's instructions.

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DNA Sequencing—Sequencing reactions were performed on double-stranded and single-stranded DNA templates according to a modification of the Sanger dideoxy nucleotide chain termination method, both manually and with an automated sequencer (Applied Biosystems, model 373A automated sequencer at the DNA Facility, the School of Veterinary Medicine, of the University of Pennsylvania) (16). Both strands of M-PFK were completely sequenced and in many cases from multiple primers. In areas covered by PCR-derived clones, sequence from clones representing three separate PCR reactions was obtained.

Allele-specific PCR-based M-PFK Test—Genomic DNA was harvested from 100 μl of EDTA-anticoagulated blood as described (17). Amplification of 5–10% of this DNA was performed using primers with the sequence ccagcttcaggcac (PFKBan) which correspond to bases 2137–2161 of the coding sequence and bases 2229–2253 of the non-coding strand, respectively. The predicted size of the product of amplification with the PFK exon21/PFK Ban primer set was 326 base pairs, based on 116 base pairs in exons 21 and 22 and the rabbit muscle intron length of 210 base pairs (18, 19). Following a PCR amplification profile of 9 min at 95 °C, 5 cycles of 1 min at 95 °C and 1 min at 55 °C, and 35 cycles of 1 min at 95 °C and 1 min at 72 °C, the product of predicted size was purified by sequential phenol and chloroform:isoamyl alcohol (24:1) extractions. After ethanol precipitation, one-half of the product was digested with the restriction endonuclease BamHI and electrophoresed on a 5% acryl

results
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Fig. 2. Northern blot of total skeletal muscle RNA probed with full-length canine M-PFK cDNA. Lane 1, RNA from a normal beagle dog; lane 2, RNA from an affected English springer spaniel. Each lane was loaded with an equal amount (10 μg) of RNA as determined by the absorbance at 260 nm and confirmed by ethidium bromide staining. The positions of 18S and 28S rRNA and 2.4- and 4.4-kilobase RNA size markers are indicated. Representative results from two separate experiments.

Fig. 3. Sequencing gel demonstrating the mutation in canine M-PFK. The first lane in each pair is sequence from an affected and the second lane is sequence from a normal English springer spaniel. The mutation is a G to A substitution that leads to the tryptophan codon TGG being replaced by a stop codon TAG.

codon sequences.

The mutation in the affected dog neither created nor abolished a known restriction enzyme recognition sequence. For this reason, an allele-specific test was designed for the mutation using the normal base in combination with a PCR primer (PFKBan) that introduced a mismatch to create a restriction enzyme recognition site (Fig. 4B). Genomic DNA from dogs whose PFK status was known from enzyme assay was amplified with the primer set PFKexon21/PFKBan, digested with BanI, and evaluated by polyacrylamide gel electrophoresis. The PCR product from normal dogs was completely digested, and it migrated in a position (310 base pairs) ~25 nucleotides smaller than the undigested product (Fig. 4B, lane 2). In contrast, BanI-digested PCR product from affected individuals showed no change in electrophoretic mobility (Fig. 4B, lane 1). Carriers had bands of reduced intensity at both positions, when compared with normal or affected dogs (Fig. 4B, lanes 1, 3, 4, and 6). Thus, the PFKexon21/PFKBan assay allowed the number of normal alleles at position 2228 to be determined, thereby permitting carrier animals to be distinguished from normal and affected dogs.

Twenty-one of the dogs in our colony for which enzyme activities were available were evaluated utilizing the enzyme recognition site (Fig. 4B) that introduced a mismatch to create a restriction enzyme recognition sequence. For this reason, an allele-specific test was designed for the mutation using the normal base at the mutation site. B, a polyacrylamide gel of BanI-digested PCR products from dogs homozygous for the mutation at position 2228 (lane 2), dogs homozygous for the normal allele (lane 5), and heterozygous carriers (lanes 1, 3, 4, and 6). The pedigree indicates the relationship of the animals tested.

PFKexon21/PFKBan primer set. Since this colony was derived from three separate families of dogs with no relatives in common for the previous six generations, segregation of this mutation with the disease would strongly support its causative role. Five normal dogs, eight carrier dogs, and eight affected dogs were evaluated, and the correlation between enzyme activity and the number of normal alleles was complete. Fig. 4B shows PCR results from a typical mating between two carriers which produced one affected puppy, one normal puppy, and two carrier puppies.

Because M-PFK deficiency is a common inherited disorder in the English springer spaniel (5–7), we examined 35 samples from animals outside of the colony for M-PFK deficiency with the allele-specific PCR-based test. Of these dogs, 23 were English springer spaniels, 3 were American cocker spaniels, and 9 were breeds other than spaniels from which blood samples were submitted as normal controls for PFK enzyme activity assays. Twenty-two of these samples were submitted because the animal had been suffering from chronic or recurrent regenerative anemia and/or exercise intolerance, for breeding evaluation, or because the animal was related to an affected dog. Five English springer spaniels were shown to be homozygous, and three were heterozygous for the G to A mutation. An American cocker spaniel dog, with hemolytic anemia, was examined by this technique and found to have no normal M-PFK alleles (21). Subsequent sequencing confirmed that the disease in this American cocker spaniel was due to the same mutation that causes the disease in the English springer spaniel breed.

DISCUSSION

Canine M-PFK deficiency represents the only disease model of this autosomal recessive inborn error of metabolism in humans. The clinicopathological similarities of the exertional myopathy and erythroenzymopathy in both species have been reported (7, 9). Our studies characterize the molecular basis of M-PFK deficiency in dogs and suggest that canine PFK deficiency is an excellent disease homologue to further investigate pathogenesis and novel therapeutic approaches. When compared with the previously reported cDNA sequence from normal dogs (10), the coding sequence from affected dogs showed only a single base substitution of an A to G at position 2228, which changes a tryptophan codon to a stop codon, thereby truncating the M-PFK subunit by 40 amino acid residues. No other alterations were found in the protein-coding region compared to normal M-PFK. PCR testing of the region of the
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A mutation established an absolute correlation between the number of mutant alleles and normal, intermediate (carrier), and deficient PFK activity in all individuals tested, further supporting the causative nature of this mutation. Thus far, only very few canine and other large animal models have been defined at the molecular genetic level.

From among the >50 M-PFK-deficient humans, several mutations have been identified, but none have exactly the same genetic defect as in dogs. A splicing defect that results in recognition of a cryptic splice site within exon 15 and an in-frame 75-base pair deletion in the mRNA has been found in a Japanese family (2). In Ashkenazi Jews, the most commonly reported mutations are at the splice donor site of intron 5, leading to a precise deletion of exon 5, and a single base deletion resulting in a frameshift mutation in exon 22 and a premature stop codon (3), which is similar to the one we found in deficient dogs. In addition, missense mutations have been reported in French Canadian and Swiss patients (4).

The information regarding the enzyme defect at the protein level is very limited in human patients. Generally, M-PFK enzyme activity is nearly completely lacking in muscle tissue and reduced to one-half in erythrocytes, due to the contribution of L-PFK. The amino acid substitution in M-PFK found in a French Canadian patient resulted in a completely inactive protein, whereas the missense mutations in a double-heterozygote Swiss patient only modestly changed the enzyme activity and thermal stability as shown in a yeast expression system (4). No structure-function studies have, thus far, been performed with normal and mutant canine M-PFK.

Our data for the dog show that the 40-amino acid deletion caused by the mutation, representing only 5% of the protein, has a profound effect on the assembly and stability of the molecule. The lack of this region apparently leads to the rapid degradation of the entire subunit, since the half-life of the molecule. The lack of this region apparently leads to the rapid degradation of the entire subunit, since the half-life of the molecule. The lack of this region apparently leads to the rapid degradation of the entire subunit, since the half-life of the molecule. The lack of this region apparently leads to the rapid degradation of the entire subunit, since the half-life of the molecule. The lack of this region apparently leads to the rapid degradation of the entire subunit, since the half-life of the molecule. The lack of this region apparently leads to the rapid degradation of the entire subunit, since the half-life of the molecule. 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| Reference                                                                 | Journal                              | Pages                              |
|--------------------------------------------------------------------------|--------------------------------------|------------------------------------|
| 21. Trigun, S. K., and Singh, S. N. (1988)                               | Arch. Gerontol. Geriatr.             | 7, 239–247                         |
| 22. Anneren, G., and Epstein, C. J. (1988)                               | Upsala J. Med. Sci.                  | 93, 33–38                          |
| 23. Sharma, P. M., Reddy, G. R., Vora, S., Babior, B. M., and McLachlan, A. (1989) | Gene (Amst.)                        | 77, 177–183                        |
| 24. Le Bras, G., and Gardé, J. (1988)                                    | Biochemistry                        | 25, 2490–2493                      |
| 25. Levanon, D.; Danciger, E.; Dafni, N., and Groner, Y. (1987)           | Biochem. Biophys. Res. Commun.      | 147, 1182–1187                     |
| 26. Trigun, S. K., and Singh, S. N. (1987)                               | Cell. Mol. Biol.                     | 33, 767–774                        |
| 27. Lau, F. T.; Fersht, A. R.; Hellinga, H. W., and Evans, P. R. (1987)   | Biochemistry                        | 26, 4143–4148                      |
| 28. Buschmeier, B., Meyer, H. E., and Mayr, G. W. (1987)                  | J. Biol. Chem.                       | 262, 9454–9462                     |
| 29. Polymeropoulos, M. H., Rath, D. S., Xiao, H., and Merrill, C. R. (1991) | Nucleic Acids Res.                  | 19, 2537                           |
| 30. Morrison, N., Simpson, C., Fothergill Gilmore, L., Boyd, E., and Connor, J. M. (1992) | Hum. Genet.                          | 89, 105–106                        |
| 31. Rypniewski, W. R., and Evans, P. R. (1989)                           | J. Mol. Biol.                        | 207, 805–821                       |
| 32. Crepin, K. M., Darville, M. I., Hue, L., and Rousseau, G. G. (1989)   | Eur. J. Biochem.                     | 183, 433–440                       |
| 33. Noble, N. A., Xu, Q. P., and Ward, J. H. (1989)                      | Blood                               | 74, 475–481                        |
| 34. Mandarino, L. J., Wright, K. S., Verity, L. S., Nichols, J., Bell, J. M., Kolterman, O. G., and Beck Nielsen, H. (1987) | J. Clin. Invest.                     | 80, 655–663                        |
| 35. Gekakis, N., Gehnrich, S. C., and Sul, H. S. (1989)                   | J. Biol. Chem.                       | 264, 3658–3661                     |