Supplementary Clinical Data on p.Trp3X Kindreds.

The proband in family 1 (patient 42970; ascertained in Utah) is now 67 years old. He first noted weakness at age 20 years, when he was running an obstacle course in the Navy, and was unable to do it in the allotted time. He was subsequently sent for remedial physical training during which he in fact became weaker, and he graduated to active duty without ever passing the test. By age 28, he was unable to walk up a flight of stairs while carrying a box of supplies. His weakness progressed through adulthood; although he walked with assistive devices, he never used a wheelchair until the age of 62 years, when he became functionally non-ambulant. In retrospect, he now recognizes symptoms in childhood, including the inability to run an entire 50-yard dash at age 12 and nocturnal muscle cramping when in elementary school. His younger brother (patient 43194) was examined by one of us (KMF) at age 58 years. At that time, although he noted occasional nocturnal post-exertional muscle cramps, his examination revealed only trace hip flexor and extensor weakness. Three years later, he reported mild difficulty in getting up from a sitting position or getting out of a car, but he walked up to three miles without difficulty, uses no assistive devices for walking, and has never used a wheelchair.

The proband in family 2 (patient 43043; from southern Michigan) presented with persistent stomach and back pain at age 3.5 years, associated with occasional vomiting and diarrhea. On examination, he had normal muscle strength, without use of a Gowers' maneuver. Laboratory testing demonstrated mildly elevated hepatic transaminase values, and a serum creatine kinase (CK) value, which was mildly elevated (on two different occasions: 523 IU/L and 1373 IU/L). Muscle biopsy revealed mildly diminished staining with three different antibodies (amino-terminal, rod domain, and carboxy-terminal), leading to a diagnosis of dystrophinopathy. His younger brother (patient 43640) had no muscle symptoms, but was determined to have an

elevated CK (5080 IU/L). Both the mother and maternal great grandmother tested positive as p.Trp3X carriers.

The proband of family 3 (patient 43800; from Kansas City, Missouri) presented at age 7 years with bilateral calf pain and elevated serum CK level (8,000-24,000 IU/L). The calf pain usually developed after prolonged running during soccer or basketball practice. It was relieved with rest. Examination at age 8 years showed mild deltoid weakness but normal lower extremity strength and gait, including toe walking and running. He had no contractures. Muscle biopsy showed degenerating and regenerating fibers and reduced N-terminal, rod, and C-terminal dystrophin antibody staining.

The proband of family 4 (patient 43676; also from Kansas City, but not known to be related to family 3) was incidentally found at age 4 years to have an elevated serum CK level (4558 IU/L and 14,559 IU/L on two separate occasions). Dystrophin immunostaining showed a normal and uniform pattern in the vast majority of myofibers regardless of diameter; occasional myofibers showed weak staining. Examination showed only minimal deltoid weakness, but no significant lower extremity weakness, and minimally tight heel cords.

The proband in family 5 (patient 43831); from Milwaukee, Wisconsin) presented in childhood with myalgias but no weakness; an elevated serum CK led to a muscle biopsy with decreased amino-terminal and rod domain dystrophin antibody staining. At age 14, he has had a single episode of exercise-induced rhabdomyolysis (CK of 149,462 IU/L, decreasing two days later to 19,189 IU/L) but has no significant weakness. A sibling pair of maternal cousins was included in this current analysis. One (patient 43738) is now 16 years old, and had onset of post-exertional muscle cramping and stiffness in his arms and legs at age 12, but has no significant weakness. His brother (patient 43741) is 4.5 years old and has no motor symptoms, but was

diagnosed with elevated serum CK at age 2.5 years; the mother tested positive as a p.Trp3X carrier.

Family 6 was ascertained in Philadelphia. The proband had no family history of muscle disorders; he presented at age 13 years for evaluation of "hyperCKemia" found incidentally during an evaluation of short stature. His serum CK ranged from 5877 IU/L when active to 712 IU/L when more sedentary. At age 13 he was playing soccer equal to his peers without weakness, but fatigued slightly after 40 to 60 minutes and complained of leg aching. Examination at age 14 was notable for short stature, normal cognition, mild knee flexion contractures, with normal muscle bulk, strength, and reflexes; and has one episode of myoglobinuria. Leg muscle ultrasound and MRI studies were normal, as was an echocardiogram. His maternal grandfather also carries the mutation, and at age 73 denies any current symptoms but recalls severe post-exertional myalgias in childhood; his examination shows a mild Trendelenberg gait but no weakness to manual muscle testing, and his serum CK was 308 IU/L (normal for lab <200 IU/L).

The proband of family 7, from Milan, Italy, had no family history of muscle disease. He was investigated at the age of 6 years for asymptomatic hyperCKemia; his parents reported only mild running difficulties and frequent falling. Neurological examination showed mild proximal weakness (quadriceps and iliopsoas 4+ on the MRC scale) with moderate calf hypertrophy and no heel cord contractures. His serum CK was 4975 IU/L. Four years later, his muscle involvement was stable. He was able to jump and run freely and to climb stairs without help, and Gowers' sign was absent. His CK was 2932 IU/L. When last examined at the age of 12 years, he was still ambulant and showed no cardiac and respiratory involvement, and had normal cognitive development. Muscle biopsy showed moderate chronic myopathic changes, and a mild

increase of connective tissue. Immunohistochemical analysis for dystrophin showed a reduced intensity with antibodies directed to three epitopes (with the weakest intensity seen with the antibody against the amino-terminal domain) and a mild reduction of α-sarcoglycan staining. Western blot analysis using both DYS1 and DYS2 antibodies showed a dystrophin product of reduced intensity and slightly smaller molecular weight compared to control muscle.