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Letter to the Editor

Nonsense Mutation of the Alpha-Actinin-3 Gene Is Not Associated With Dystrophinopathy

To the Editor:

Single nucleotide polymorphisms (SNPs) are currently attracting much attention for their association with morbidity [Cargill et al., 1999]. It has recently been reported that a single nucleotide change from C to T at nucleotide position 1747 (C1747T) of the alpha-actinin-3 gene (*ACTN3*), which converts arginine (CGA) to a stop codon (TGA) at the 577th amino acid residue (R577X) of alpha-actinin-3, is present in more than half (58.8%) of normal Australian chromosomes [North et al., 1999]. In homozygotes for the mutation, alpha-actinin-3 protein is deficient, while it is present in type 2 muscle fibers of homozygotes for the wild-type allele [North et al., 1999]. However, individuals with congenital deficiency of alpha-actinin-3 showed no discriminating phenotype.

Severe and milder dystrophinopathies, i.e., Duchenne (DMD) and Becker (BMD) muscular dystrophies, respectively, are caused by mutations of the dystrophin gene which extends over 3,000 kb on Xp21 [Nishio et al., 1994]. Immunohistochemical studies on the skeletal muscle disclose that dystrophin is absent or abnormal in the plasma membranes of all muscle fibers from DMD or BMD patients, respectively. DMD and BMD have been determined by whether deletion mutations of the dystrophin gene disrupt or maintain the translational reading frame of the dystrophin mRNA (frame-shift hypothesis). However, there is a variety of clinical severities among patients with DMD or BMD [Emery, 1993]. The severity of dystrophinopathy is supposed to be determined by unclarified modifiers, such as degeneration and/or regeneration of damaged muscle fibers, cellular responses to different hormones, or cytoskeletal proteins, including beta-spectrins, utrophin, and alpha-actinins.

Recently, alpha-actinin-2, a member of the alpha-actinin family, was shown to bind to the C-terminal region of the dystrophin protein [Hance et al., 1999]. We therefore hypothesized that deficient alpha-actinin-3 that is caused by the homozygous mutation of C1747T of *ACTN3* may modify the clinical phenotype of dystrophinopathies. The C1747T, which creates a novel *Dde*I restriction enzyme recognition site, was analyzed in 119 Japanese patients with dystrophinopathy (87 DMD and 32 BMD cases) and in 30 normal Japanese controls. After obtaining informed consents, genomic DNA was extracted from their peripheral blood leukocytes. A 489-bp fragment of *ACTN3*, consisting of exons 15 and 16, was amplified by PCR using primers ACTN3-E15F (5′-CGCCCTTCAACAACTGGCTGGA-3′) and ACTN3-E16R (5′-GGGTGATGTAGGGATTG-GTGGAG-3′). PCR was performed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 3 min, and a final extension step of 10 min at 72°C. The amplified fragment subsequently underwent digestion by *Dde*I (Boehringer-Mannheim, Germany) in a condition recommended by the supplier. The digested products were then electrophoresed in a 3% agarose gel. The following three electrophoretic patterns of the digests were observed: 1) a homozygous, single band pattern for the

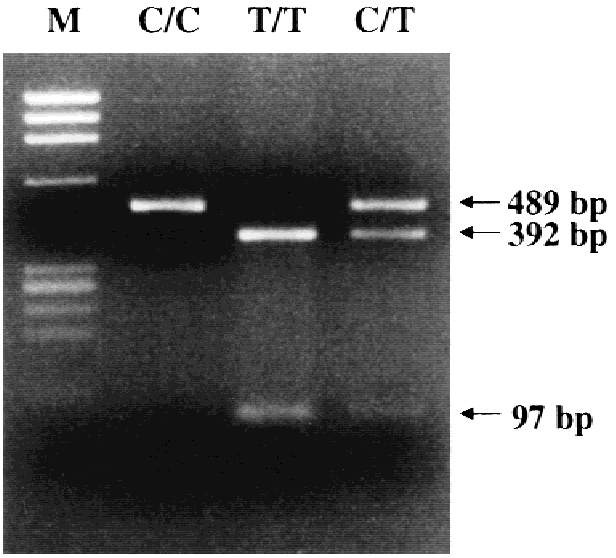

Fig. 1. PCR-restriction analysis. A 489-bp fragment of the alpha-actinin-3 gene (*ACTN3*) consisting of exons 15 and 16 was PCR-amplified and the products were subsequently subjected to *Dde*I digestion. Lane C/C depicts a homozygote for the wild-type allele (489-bp band, 1747C/C), lane T/T a homozygote for the mutant 1747T allele (392-bp and 97-bp bands, 1747C/C), and lane C/T shows a heterozygote for the wild-type and mutant alleles (1747C/T). M refers to a DNA size marker.

*Correspondence to: Masafumi Matsuo, M.D., Ph.D., Division of Genetics, International Center for Medical Research, Kobe University School of Medicine, 7-5-1 Kusunokicho, Chuo, Kobe 650-0017, Japan. E-mail: matsuo@kobe-u.ac.jp/

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TABLE I. Incidence of *ANTN3* Mutations Among Japanese Dystrophinopathy Patients and Normal Control Individuals

Persons	No. of persons	No. of alleles (%)		No. of genotypes (%)		
		1747C	1747T	1747C/C	1747C/T	1747T/T
DMD	87	95 (54.6)	79 (45.4)	26 (29.9)	43 (49.4)	18 (20.7)
BMD	32	30 (46.9)	34 (53.1)	7 (21.9)	16 (50.0)	9 (28.1)
Subtotal	119	125 (52.5)	113 (47.5)	33 (26.4)	59 (49.6)	27 (22.7)
Control	30	26 (43.3)	34 (56.7)	5 (16.7)	16 (53.3)	9 (30.0)
Total	149	151 (50.7)	147 (49.3)	38 (25.5)	75 (50.3)	36 (24.2)

489-bp wild-type allele (Fig. 1, lane C/C); 2) a homozygous pattern showing two bands of 392 bp and 97 bp for the C1747T allele (lane T/T); and 3) a heterozygous, three-band pattern consisting of both wild-type and mutated alleles (lane C/T). Homozygosity for C1747T was identified in 27 (22.7%) of 119 dystrophinopathy patients and in 9 (30.0%) of the 30 controls. In total, 24.2% of the Japanese population had congenital alpha-actinin-3 deficiency. Heterozygosity was identified in 59 (49.6%) and 16 (53.3%) dystrophinopathy and control groups, respectively (Table I). As the allele frequency of C1747T was 0.48 in the dystrophinopathy cases and 0.57 in the control individuals, its frequency in the Japanese population is computed to be 0.49. No significant statistical difference regarding the C1747T frequency was found between the dystrophinopathy and the normal control groups. Furthermore, both groups satisfied the Hardy-Weinberg equilibrium.

Although the incidence (24.2%) of congenital deficiency of alpha-actinin-3 seems high among the Japanese, it is found to be higher (36.8%) in Australians [North et al., 1999]. North et al. [1999] reported that the allele frequency of C1747T ranges from 0.22 ± 0.05 to 0.52 ± 0.04 in each ethnic group of Asia, Australia, Africa, and Europe. Therefore, the Japanese rank as a group with higher frequency (0.49) of C1747T.

We then examined whether congenital alpha-actinin-3 deficiency is associated with the DMD/BMD phenotype. Eighteen (20.7%) of the 87 DMD patients and 9 (28.1%) of the 32 BMD patients were found to be homozygous for C1747T. Although it seems that the homozygotes are more frequent in BMD, there was no statistically significant difference between the two patient groups. We subsequently examined the DMD/BMD patients regarding the age when they first walked, age at diagnosis, age when they became wheelchair bound, and age when ventilation therapy was started. Unfortunately, the data sufficient for statistical analysis were available only with regard to the age at the first walk. In 36 DMD cases, the average age when the patients started to walk was calculated to be 18.1 ± 2.8, 16.4 ± 3.1, and 16.2 ± 3.6 months for genotypes 1747C/C (8 cases), 1747C/T (23 cases), and 1747T/T (5 cases), respectively. DMD patients with alpha-actinin-3 deficiency seemed to start unsupported walk early, but the difference was not statistically significant. These results indicate that the C1747T mutation of *ACTN3* is highly observed among the Japanese and that the mutation is not a modifier of dystrophinopathy.

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Ryo Suminaga
Masafumi Matsuo*
Division of Genetics
International Center for Medical Research
Kobe, Japan

Yasuhiro Takeshima
Hajime Nakamura
Department of Pediatrics
Kobe University School of Medicine
Kobe, Japan

Hiroko Wada
Sakura Ryoikuen Hospital
Hyogo, Japan