correspondence

A common nonsense mutation results in α-actinin-3 deficiency in the general population

The α-actinins are actin-binding proteins encoded by a multigene family. In skeletal muscle, they are a major structural component of the Z-lines that anchor the actin-containing thin filaments and maintain the spatial relationship between myofilaments[1]. In humans, two genes (*ACTN2* and *ACTN3*) encode the closely related α-actinin-2 and α-actinin-3 skeletal muscle isoforms[2]. *ACTN2* is expressed in all skeletal muscle fibres, whereas expression of *ACTN3* is limited to a subset of type 2 (fast) fibres[3]. We have previously demonstrated absence of α-actinin-3 in muscle biopsies from several patients with muscular dystrophy[3]. A follow-up study identified additional α-actinin-3–negative biopsies from neuromuscular patients with other known diseases, suggesting that this deficiency was not the primary cause of muscle weakness[4]. Subsequently, we screened muscle specimens with dystrophic (118 specimens), myopathic (74), neurogenic (20) and normal (55) features (Fig. 1*a–d*). Although these biopsies contained normal α-actinin-2 expression, deficiency of α-actinin-3 was identified by immunocytochemistry and western blot in 51 of 267 cases (19%), a finding not associated with any particular histopathological or clinical phenotype. To ascertain whether α-actinin-3 deficiency was associated with mutations of *ACTN3*, we used an RT-PCR approach to amplify mRNA isolated from diagnostic muscle biopsies. Using primer pairs AB16/AB9 (5′–GATGGTTATGCAGCCCGAGG–3′ and 5′–AGCAACGCCCGCACCTCCT–3′) and AB8/AB1 (5′–TGCACGAAGCCTG-GACCC–3′ and 5′–AGAGAGGGATCTT-TATTCAG–3′), we PCR-amplified two overlapping fragments encompassing bases 24–2,852 of *ACTN3* mRNA (ref. 2). Initially, we focused on one family with two affected male siblings with congenital muscular dystrophy and complete deficiency of α-actinin-3. Sequencing of *ACTN3* cDNA from the proband identified two changes relative to controls and the previously determined sequence M86407. These were an A→G transition at nt 1,586 in exon 15, changing a glutamine (CAG) to an arginine (CGG) at residue 523 (Q523R), and a C→T transversion at position 1,747 in exon 16, converting an arginine to a stop codon at residue 577 (R577X; Fig. 1*e–g*). Direct sequencing of genomic DNA from the proband and the affected sibling confirmed homozygosity for both point mutations. Subsequent testing of the parents and two unaffected siblings revealed that these phenotypically normal individuals had the same genotype as the proband and were thus homozygous for the *ACTN3* 577X nonsense mutation.

The R577X change creates a novel *Dde*I site (Fig. 1*h*). An additional 125 biopsies for which matched DNA samples were available were tested for α-actinin-3 expression and *ACTN3* genotype (48 α-actinin-3–deficient and 77 α-actinin-3–positive biopsies with a mixture of histological and clinical phenotypes). Homozygosity for the stop codon at position 577 was identified in 46 of 48 (96%) cases in which α-actinin-3 staining was negative. In the two remaining cases, the genotype was 577R/577X; however, fibre typing of both muscle biopsies demonstrated a type 1 fibre predominance, with less than 5% type 2 fibres. Thus α-actinin-3 deficiency in these two discordant cases is likely a secondary phenomenon due to loss of type 2 fibres[5]. There was no significant difference in the frequency of homozygous null genotypes among patients with dystrophic, myopathic, neurogenic or normal biopsies, and α-actinin-3 deficiency did not alter
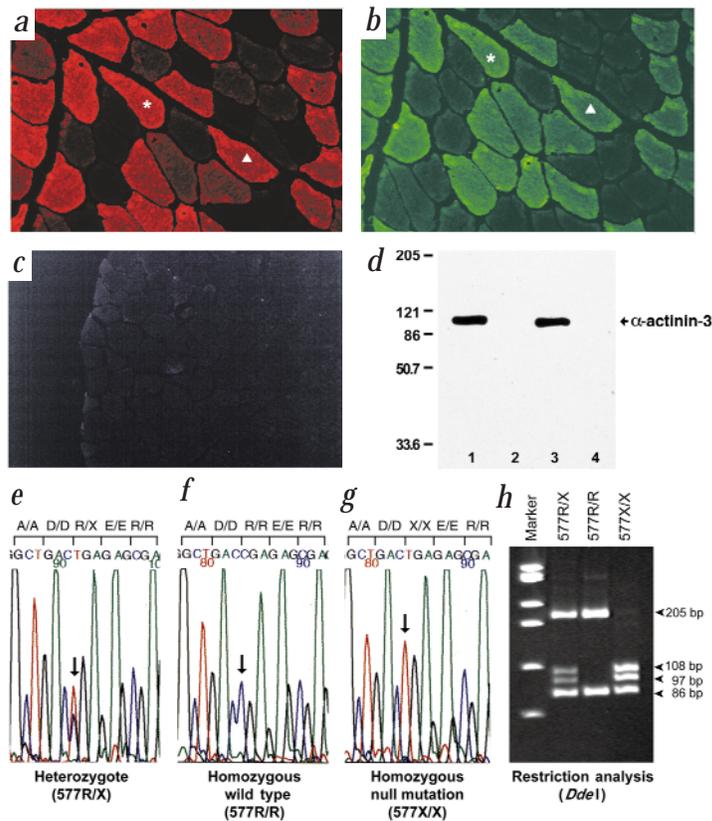

Fig. 1 Molecular analysis of α-actinin-3 genes and proteins. Indirect immunofluorescence (*a–c*) and western-blot analysis (*d*) of human skeletal muscle (quadriceps muscle biopsy with normal histology) using affinity purified antibodies (5B) specific for α-actinin-3 (*a,c*; refs 3,8) and mouse myosin heavy chain (fast, MY32) specific for type 2 fibres (*b*). Methodology as described in North and Beggs[3]. Normal expression of α-actinin-3 is restricted to type 2 (fast) fibres (*a*) as indicated by double staining with MY32 (*b*). Corresponding fibres in each section are indicated by the same symbol. *c*, Complete deficiency of α-actinin-3 in a patient homozygous for the stop codon in exon 16 (577X). *d*, Western-blot analysis of α-actinin-3 in skeletal muscle from individuals with normal α-actinin-3 expression (genotype 577R/577X; lanes 1,3) and α-actinin-3 deficiency (genotype 577X/577X; lanes 2,4). α-actinin-3 migrates at approximately 100 kD. The *ACTN3* 577X allele encodes a truncated 66-kD protein, which is thought to be incapable of dimerization[10]. Since the anti-α-actinin-3 5B antibody is directed towards the amino terminus of the protein[3,8], it should detect the truncated protein if it is stable. All individuals homozygous for the stop codon demonstrated complete absence of detectable α-actinin-3 by immunocytochemistry, and there was no evidence of the truncated protein in 577R/X or 577X/X individuals on western blots. *e,f,g,h*, DNA sequence and restriction endonuclease analysis of *ACTN3* exon 16 demonstrating the three possible *ACTN3* genotypes at position 577. Products were either directly sequenced (*e–g*) or subjected to *Dde*I digestion and agarose gel electrophoresis (*h*).

Table 1 • Sequence conservation of α-actinin genes

Gene	chk Actn2	hum ACTN2	hum ACTN3	hum ACTN4
chk Actn2		2.9±0.4	14.2±0.9	14.9±0.9
hum ACTN2	117.6±10.2		13.6±0.9	14.8±0.9
hum ACTN3	261.2±88.1	143.7±19.5		17.3±1.0
hum ACTN4	230.1±53.3	146.4±20.2	98.0±10.6	

Numbers of nucleotide substitutions, and their standard errors, per 100 synonymous sites (above diagonal) and per 100 nonsynonymous sites (below diagonal) between indicated α-actinin genes (estimated as described[11], with correction for multiple substitution using the two parameter method[12]). chk Actn2=X13874; hum ACTN2=M86406; hum ACTN3=M86407; hum ACTN4=D89980. The substitution rate per nonsynonymous site[11] between human (hum) ACTN2 and ACTN3 is 1.43, whereas the rate per synonymous site is only 0.14. Similar results are obtained for the comparison between both human and chicken (chk) ACTN2 genes. This implies that the proteins encoded by both genes have evolved very slowly since their divergence.

fibre-type distribution in control muscle. None of the 77 α-actinin-3–positive biopsies were from 577X homozygotes (53 were heterozygous and the remaining 24 individuals were homozygous 'wild type' 577R/577R). These data suggest that hereditary α-actinin-3 deficiency is common and may not be associated with an abnormal neuromuscular phenotype.

To determine the frequency and ethnic distribution of the ACTN3 577X allele in the general population, we genotyped an additional 485 DNA samples. The relative allele frequency of 577X ranged from 0.22±0.05 to 0.52±0.04 in ethnic populations from Asia and the Americas, Australasia, Africa and Europe (data not shown). Approximately 16% of the world population are predicted to have congenital deficiency of α-actinin-3, suggesting that other factor(s) likely compensate for its absence at the Z lines of skeletal muscle fast fibres.

Individuals genotyped for R577X were also genotyped for Q523R (which creates a novel MspI site). Fifteen 523Q/577X and thirteen 523R/577R halotypes were detected among the 674 alleles from 337 subjects who were homozygous for at least one of the loci. The remaining ACTN3 haplotypes were all either 523Q/577R or 523R/577X. Tests for linkage disequilibrium[6], using maximum likelihood estimates of haplotype frequencies, were significant in all populations ($P<10^{-5}$, except in a small Bantu sample where $P<0.05$). Thus, 577X likely results from a single mutational event and not from multiple independent mutations in different chromosomal backgrounds. Although we can not rule out some subtle selective pressure keeping these alleles together, it appears unlikely that the 577X protects against a deleterious effect of 523R, as all 13 individuals with 523R/577R haplotypes (including one of the authors) were phenotypically normal.

The high allelic frequency of ACTN3 577X in the general population demonstrates that this stop codon is a non-pathogenic polymorphism in humans. Absence of a structural protein caused by homozygosity for a null mutation cannot be assumed to be disease-related without additional family and population data. These findings should also prompt re-evaluation of previous studies describing apparent loss of fast-fibre α-actinin in patients with Duchenne muscular dystrophy[5]. The high frequency of α-actinin-3 deficiency and absence of an obvious associated disease phenotype suggests that α-actinin-3 is functionally redundant in humans. Although mouse studies have identified a number of genes whose homozygous null mutant phenotypes are apparently normal, genetic redundancy is not a well-characterized phenomenon in humans[7]. We propose that α-actinin-2, which is structurally and functionally highly similar to α-actinin-3 (Table 1; refs 2,8), is able to compensate for α-actinin-3 absence in type 2 (fast) fibres. On the other hand, ACTN3 has been highly conserved over a long period of evolutionary time, implying a constraint on evolutionary rate imposed by continued functioning of the gene (Table 1). The force-generating capacity of type-2 muscle fibres at high velocity, the speed and tempo of movements and the capacity of an individual to adapt to exercise training are all genetically influenced[9]. Although we have not yet identified any subtle phenotypes associated with α-actinin-3 deficiency, ACTN3 genotype may be one of the factors that influence normal variation in muscle function, both within patient groups (that is, as a disease-modifying locus) and in the general population.

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