Five Candidate Genes for Hamster Cardiomyopathy Did not Map to the Cardiomyopathy Locus by FISH Analysis

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Abstract

The Syrian cardiomyopathic hamster (BIO14.6), that develops both muscular dystrophy and progressive cardiomyopathy, is widely used as an animal model of autosomal recessive cardiomyopathy mimicking human hypertrophic cardiomyopathy, and five genes have been proposed as strong candidates for the cause of cardiomyopathy. We recently mapped the cardiomyopathy locus of the hamster to the centromeric region of chromosome 9qa2.1-bl by construction of a genetic linkage map of the Syrian hamster. Thus, we analyzed the loci of the five candidate genes, α-tropomyosin, cardiac troponin T, adhalin, calpain 3 and cardiac myosin binding protein-C, by the FISH method, and found that these genes were mapped on the distal portion of chromosome 12qa5 and 4pa2 and the proximal portion of chromosomes 9qb7, 1qcl.1 and 1qb3, respectively. These results provide strong evidence that the five candidate genes previously proposed are not related to the hamster cardiomyopathy.

Key words: cardiomyopathic hamster (BIO14.6); hypertrophic cardiomyopathy; muscular dystrophy; FISH; chromosomal location

The Syrian cardiomyopathic hamster (BIO14.6), is an inbred strain with a hereditary abnormality in skeletal and cardiac muscles involving ventricular and atrial hypertrophy with subsequent development of congestive heart failure.1-4 This strain has been widely used as an animal model for human forms of hypertrophic cardiomyopathy and congestive heart failure.1-4 This strain has been widely used as an animal model for human forms of hypertrophic cardiomyopathy and congestive heart failure, in which cardiac cellular necrosis accompanied by a loss of sarcolemmal integrity begins 30-40 days after birth. It was first established by Homburger et al.1 in 1962 that the disease phenotype is inherited by a single locus in an autosomal recessive mode with complete penetrance. Several biochemical abnormalities in the cardiomyopathic hamster have been described,4,5 but the genetic defect of the BIO14.6 strain has not been identified.

Recently, we constructed a genetic linkage map of the Syrian hamster6 and mapped the cardiomyopathy (cm) locus to the centromeric region of hamster chromosome 9qa2.1-bl. This was expected to facilitate the genetic analysis of the causative gene for the cardiomyopathic hamster.

In this study, we used FISH analysis to evaluate five candidate genes which were previously strong candidate genes for cardiomyopathy or a certain type of muscular dystrophy. The five candidate genes are α-tropomyosin (α-TM),7 cardiac troponin T (TNT),7 cardiac myosin binding protein-C (MyBP-C),8,9 adhalin (ADH)10 and calpain 3 (CNP3)11 The first three genes have been reported to be the causative genes for human-type hypertrophic cardiomyopathies. ADH is reported to be specifically deficient in the BIO14.6 strain,12 and nonsense mutation of this gene causes a certain type of muscular dystrophy.10 Mutations in CNP3 also cause another type of muscular dystrophy. For these reasons, we chose these genes for further analyses.

1. Isolation of Syrian Hamster cDNA Clones and Genomic Clones for FISH Analysis

ADH and CNP3 cDNAs were isolated from a lambda ZAPII library (Stratagene Inc., La Jolla, Calif.), constructed from hamster muscle mRNA. A PCR prod-
uct used for screening ADH cDNA (accession number, L34355) was amplified using human placenta DNA with the primer pairs forward, CTG TAC GAC ACC TT, reverse, TGG TGG ACC ATC TGG ATG TC. Human CANP3 (accession number, X85030) exon 1 was amplified using forward-ex1a, CTT TCC TTG AAG GTA GCT GTA T, reverse-ex1m, GAG GTG CTG AGT GAG AG and used for screening hamster cDNA libraries. PCR conditions used for the amplification were denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, with a final extension at 72°C for 3 min. Probes were labeled by random priming to a specific activity of 10⁸ cpm/µg. Screening was performed using standard techniques, and clones were purified through tertiary screening. The identity of each clone was confirmed by one-pass sequence on both strands using an ABI 377 sequencer.

Since cDNA clones for α-TM, cardiac TNT and cardiac MyBP-C do not produce a clear signal in the FISH analysis, we used the corresponding phage and cosm genomic clones. A phage library was constructed using ImpactII vector (Stratagene) with partially MboI-digested hamster genomic DNA. A cosmIDlibrary was constructed using pWE15 vector (Stratagene) with MboI-digested hamster genomic DNA. Probes used for screening the library were amplified from ACN hamster genomic DNA using the primer pairs designed from the conserved region between human and rat: a-TM7 (accession number, M16433, J02689), forward329, CGT AAG CTG GTC ATC ATC GA, reverse721, CTT ATC CTC TGC AGC CAT TA.; cardiac TNT7 (accession number, M80829), forward15101, GCG TAT TCG CAA TGA CTG GTC ATC ATC GA, reverse18093, GAT CCT GTT TCG CAG AAC GT; PCR conditions used for the amplification were as described above. The amplified fragments were used to screen the genomic library. The cosmID clone corresponding to cardiac MyBP-C gene was screened using the Syrian hamster cDNA specific for cardiac MyBP-C. The probe used for screening the Syrian hamster cDNA library was amplified by PCR from reverse-transcribed (RT) human heart mRNA. The primers (accession number, X84075)9 used for the RT-PCR were forward1785, GGT CCC GAC AGC CGC ATA A; reverse2103, GCC TGG CTG GGG CCT TAT T. These clones were confirmed by one-pass sequence of both ends.

2. Mapping of Five Candidate Genes for Cardiomyopathic Hamster by Direct R- and G-Band FISH Analysis

α-TM, cardiac TNT and cardiac MyBP-C genes, which cause hypertrophic cardiomyopathy in humans, were subjected to FISH analysis. The direct R-banding FISH method used in this study has been described previously. The karyotyping of the Syrian hamster was determined by following the described nomenclature. As shown in Fig. 1, α-TM, cardiac TNT and cardiac MyBP-C genes were localized subregionally to the distal portion of the Syrian hamster chromosome 12qa5 (Fig. 1a and 1b), 4pa2 (Fig. 1c and 1d) and the proximal portion of chromosome 1qb3 (Fig. 1i and 1j), respectively. Thus, these genes could be excluded as candidate genes for the cm locus. Together with our previous results, which excluded the β-myosin heavy chain gene as a candidate by linkage analysis, our findings have eliminated all causative genes reported for human-type of hypertrophic cardiomyopathies as possible causes of hamster cardiomyopathy.

Roberds et al. reported that ADH (50 kDa dystrophin-associated glycoprotein) is specifically deficient in skeletal and cardiac muscles of the BIO14.6 strain and suggested it as a strong candidate for hamster cardiomyopathy. They reported that the cDNA sequence of BIO14.6 is normal but conducted no genetic analysis. We mapped the ADH gene onto Syrian hamster chromosome 9qb7 (Fig. 1e and 1f) by FISH, which completely excluded the ADH gene itself from responsibility for this cardiomyopathy. Thus, the deficiency of adhalin glycoprotein may be a secondary change of the disease.

All of these candidate genes are structural components of muscle tissue. Recently, a novel pathological mechanism leading to muscular dystrophy has been reported, in which the enzyme, CANP3 (named for calcium-activated neutral protease large polypeptide L3 or calpain 3), was eventually proved to cause limb-girdle muscular dystrophy type 2A. Although the precise pathological etiology is unknown, the mutations of the gene eventually resulted in degeneration of muscle fiber. Therefore, we also tried to determine whether CANP3 is the responsible gene. However, FISH analysis using hamster CANP3 cDNA showed its localization on chromosome 1qc1.1 (Fig. 1g and 1h), thus eliminating it as a candidate.

In conclusion, we determined the chromosomal localization of five candidate genes for hamster cardiomyopathy including α-TM, cardiac TNT, ADH, CANP3 and cardiac MyBP-C. These results strongly suggest that these five genes are not related to cm which is located on chromosome 9qa2.1-b1. Other structural components of skeletal or cardiac muscle, or other mechanisms yet to be reported should be considered to identify the defective gene in the BIO14.6 cardiomyopathic hamster.

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Figure 1. Chromosomal localization of five candidate genes for cardiomyopathic hamster. The hamster genomic DNA clones of α-TM (a,b), cardiac TNT (c, d), cardiac myosin binding protein C (i, j) and cDNA clones of ADH (e, f) and CANP (g, h) were used as probes for localization on R-banded chromosomes. α-TM, cardiac TNT, ADH, CANP and cardiac MyBP-C genes were localized subregionally to the distal portion of chromosome 12qa5 and 4pa2 and the proximal portion of chromosome 9qb7, 1q11.1 and 1q33, respectively. R-band patterns can be seen in a, c, e, g and i and G-band patterns in b, d, f, h and j. The direct R-banding FISH method was used. Preparation of R-banded chromosomes and FISH were performed as described previously.14,15 The hamster 1.4- and 2.5-kb cDNA fragments inserted in pBSII (SK+) for ADH and CANP, respectively, and 9-, 36- and 40-kb genomic DNA fragments inserted in λ-DASHII for α-TM and pWE18 for cardiac TNT and cardiac MyBP-C, were labeled by nick translation with biotin 16-dUTP (Boehringer Mannheim) following the manufacturer's protocol.
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