

SHORT COMMUNICATION

Chromosome Mapping of Five Human Cardiac and Skeletal Muscle Sarcoplasmic Reticulum Protein Genes

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Fluorescence *in situ* hybridization (FISH) experiments were performed using genomic and complementary DNA probes in order to determine the location on human chromosomes for five genes expressed in cardiac and skeletal muscle sarcoplasmic reticulum. The chromosome location of each gene was determined in terms of both cytogenetic bands and fractional chromosome length. The *ATP2A2* gene, expressing the SERCA2 isoform of the Ca²⁺ pump, maps to bands 12q23–q24.1, the phospholamban gene (*PLN*) to 6q22.1, the human skeletal muscle calsequestrin gene (*CASQ1*) to band 1q21, the cardiac calsequestrin gene (*CASQ2*) to bands 1p11–p13.3, and the cardiac calcium release channel gene (*RYR2*) to the interval between band 1q42.1 (distal) and band 1q43 (proximal). © 1993

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The sarcoplasmic reticulum in skeletal and cardiac muscles regulates intracellular Ca²⁺ concentrations, thereby regulating muscle contraction (1). There are four major proteins associated with the functions of the sarcoplasmic reticulum membrane. The family of 110-kDa Ca²⁺ pumps transports Ca²⁺ into the membrane lumen, thereby initiating muscle relaxation. Phospholamban, a 6-kDa pentameric protein, regulates Ca²⁺ pumps in cardiac and slow-twitch skeletal muscle. The calsequestrins, a family of 45-kDa Ca²⁺ binding proteins, concentrate Ca²⁺ near its release site in the lumen of the junctional terminal cisternae. The 565-kDa release channel (ryanodine receptor) proteins associate as tetramers to form elaborate channels and release Ca²⁺ to the sarcoplasm to initiate muscle contraction. With the exception of phospholamban, each protein has more than one isoform. Fast-twitch skeletal muscle sarcoplasmic reticulum contains fast-twitch isoforms of the Ca²⁺

pump, calsequestrin, and the ryanodine receptor; cardiac muscle sarcoplasmic reticulum contains cardiac isoforms of these proteins, plus phospholamban; slow-twitch skeletal muscle sarcoplasmic reticulum is a hybrid, containing a cardiac Ca²⁺ pump, phospholamban, skeletal muscle ryanodine receptor, and a mixture of cardiac and skeletal muscle calsequestrins.

In earlier studies, we localized the skeletal and cardiac muscle Ca²⁺ pump genes, *ATP2A1* (2, 3) and *ATP2A2* (2), to human chromosomes 16p12.1 and 12, respectively; the skeletal and cardiac ryanodine receptor genes, *RYR1* (4) and *RYR2* (5) to human chromosomes 19q13.1 and 1, respectively; the single phospholamban gene, *PLN*, to human chromosome 6 (6), and the skeletal calsequestrin gene, *CASQ1*, to human chromosome 1 (7). In this study, the sublocalization of *ATP2A2*, *RYR2*, *PLN*, *CASQ1*, and *CASQ2* was determined by fluorescence *in situ* hybridization using a combination of FLpter measurements (defined as the percentage fractional length from the terminal end of the short arm) and G-banded chromosome preparations. The 6-kb human *ATP2A2* clone was localized to the q arm of chromosome 12 at the border of bands 12q23–q24.1; this probe gave FLpter measurements spanning the range 80.5 to 84.7%, which correlates well with the cytogenetic bands (see Fig. 1). Of the three clones isolated for the human *PLN* gene (1.6, 2.3, and 2.6 kb in size), only the 2.3-kb clone gave a hybridization signal strong enough for visualization. This clone hybridized to chromosome 6 and gave a fractional length distribution between 67.8 and 73.9%, corresponding to band 6q22.1. The 7-kb human *CASQ2* clone hybridized to the p arm of chromosome 1 (42.4–48.6%) corresponding to bands 1p11–p13.3. The 15-kb human *CASQ1* clone mapped to bands 1q21.3 to 1q23, which is just distal to the heteromorphic band on chromosome 1, showing that the two *CASQ* genes are not in tandem. Only the 2.7-kb rabbit *RYR2* cDNA clone hybridized successfully, indicating its localization to the q arm of chromosome 1 (93.4–95.8%), from the distal quarter of band 1q42.1 to the proximal border of band 1q43.

An important consequence of identifying the chromosomal locus of genes is the potential for demonstrating their association with human disease loci by linkage

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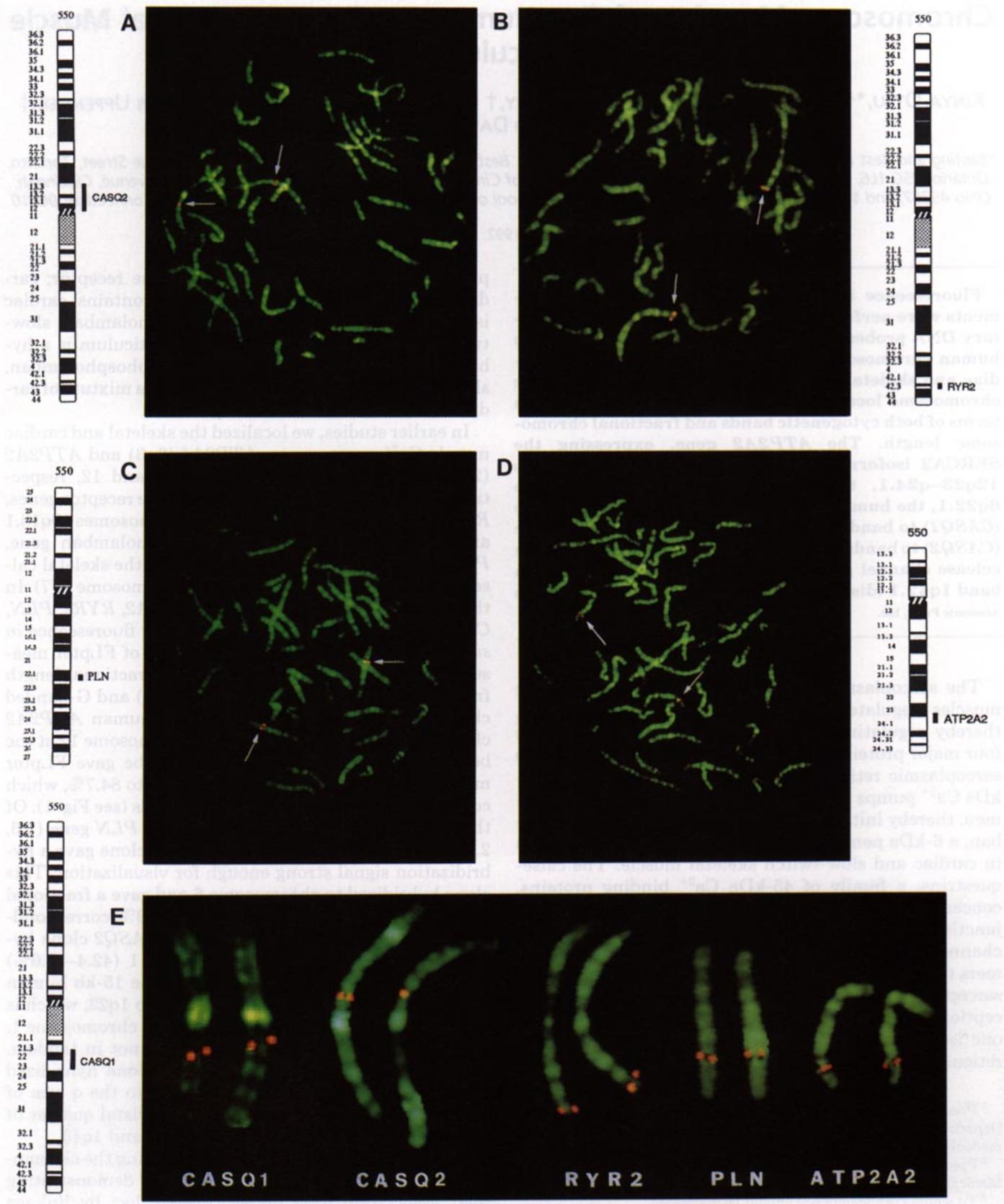


FIG. 1. Metaphase spreads displaying FISH mapping of genes *CASQ2* (A), *RYR2* (B), *PLN* (C), and *ATP2A2* (D). (E) Hybridization signals on representatives of each chromosome at higher magnification. All probes were labeled with biotin-11-dUTP by nick-translation and purified over Sephadex G-50 spin columns. All of the human genomic clones were reannealed with 1 μ g human Cot 1 DNA to suppress any repetitive sequences (i.e., *Alu*); the rabbit cDNA clones did not require any competitor DNA to suppress cross-hybridizing sequences. FISH was

analysis. We have linked the *RYR1* gene to malignant hyperthermia in humans (4) and in swine (11). Because there is no evidence of direct cardiac involvement in malignant hyperthermia, it is unlikely that this disease will be associated with *RYR2* on chromosome 1. The *ATP2A1* gene has been implicated in Brody disease (12), a disease characterized by difficulty in relaxing skeletal muscle (13). Although neither linkage nor mutational analysis has been completed, such studies are in progress.

The *RYR2* gene is expressed in brain as well as in cardiac tissue (5). The *ATP2A2* gene is expressed throughout the body. In this case, an alternatively spliced isoform, SERCA2b, with an extended COOH-terminus containing an additional transmembrane sequence, is the form of the protein that is ubiquitous, whereas the SERCA2a isoform is restricted to cardiac and slow-twitch muscle. The expression of *PLN* and *CASQ2* genes appears to be restricted to cardiac and slow-twitch skeletal muscle (6, 10), the expression of *CASQ1* to fast- and slow-twitch skeletal muscle (1), and the expression of *ATP2A1* to fast-twitch skeletal muscle (1). It would appear that mutation of either *RYR2* or *ATP2A2* would result in systemic problems that would be lethal, whereas mutation of *ATP2A1*, *PLN*, *CASQ1*, or *CASQ2* could be restricted to myopathies. Localization of these muscle genes sets the stage for potential linkage analysis to myopathies.

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performed following the procedure of Lichter *et al.* (8) and detection of the probes was achieved using avidin-FITC (pseudocolored red). Fluorescent signals were digitally imaged on a Zeiss Axioskop epifluorescence microscope coupled to a cooled charge coupled device camera (Photometrics CH220). The chromosomes were counterstained with DAPI (pseudocolored green for better photographic resolution), yielding a G-banding pattern for easy chromosome identification and band assignment. Enhancement and merging of the raw images were performed using Enhance 1.0.1 (MicroFrontier Inc.) and Gene Join MaxPix (Tim Rand-Ward lab) as described (9). Fractional length measurements (8) were taken from the p-terminal end of the chromosomes (FLpter) and the full range of FLpter values observed (13 measurements/probe) is displayed on the chromosome idiograms. The merged images were displayed using Pixel Paint Professional (SuperMac Technologies) and photographed off of the computer monitor using Kodak 100 HC color film. Isolation of probes: Two rabbit cardiac ryanodine receptor (*RYR2*) cDNA sequences (5) corresponding to nucleotides 2780-5443 and 5443-8290 were subcloned into an *EcoRI* site in the Bluescript vector. These subcloned DNA fragments were purified by equilibrium centrifugation in CsCl-ethidium bromide gradients and used for mapping on chromosomes by fluorescence *in situ* hybridization. The human cardiac Ca^{2+} -ATPase gene (*ATP2A2*) was isolated by screening of a human genomic library, constructed in the Lorist B vector by Dr. Henry Klamut, Hospital for Sick Children, Toronto, with a human cardiac cDNA sequence (2). A 6-kb *EcoRI* fragment of the human Ca^{2+} -ATPase gene was subcloned into the plasmid vector PTZ 19R (Pharmacia LKB Biotechnology). The human phospholamban *PLN* and the two human calsequestrin genes (*CASQ1* and *CASQ2*) were isolated by screening a human genomic library obtained from the Japanese Cancer Research Resources Bank, constructed in the λ EMBL3 vector by Dr. Y. Sasaki, Kyushu University, with a human phospholamban cDNA fragment (6), a rabbit skeletal muscle calsequestrin fragment (7), and a rabbit cardiac calsequestrin fragment (10), respectively. Three *HindIII* fragments (1.6, 2.3, and 2.5 kb) of the human *PLN* gene, as well as a 7-kb *EcoRI* fragment of the human *CASQ2* gene, were subcloned into the Bluescript vector (Stratagene). The 15-kb human *CASQ1* gene was not subcloned from the λ EMBL3 vector.