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The gene encoding sarcoplasmic reticulum calcium ATPase-1 (*Atp2a1*) maps to distal mouse Chromosome 7

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Received: 25 January 1996 / Accepted: 29 May 1996

Species: Mouse

Locus name: ATPase, Ca²⁺ transporting, cardiac muscle fast twitch 1

Locus symbol: *Atp2a1*

Map position: Chr 7: cen–... *Atp2a1*–1.6 (0.2–5.7)–*Fgfr2*–9.1 (4.5–16.2)–*Fgf3*–

Method of mapping: Interspecific backcross of the type: (C57BL/6 × *Mus spretus*)F₁ × C57BL/6, N = 140 [1,2].

Molecular reagents: The mouse genomic DNA sequences corresponding to the *Atp2a1* and *Fgfr2* loci were identified by hybridizing Southern blots to ³²P-labeled probes. In the case of *Atp2a1*, we used a 648-bp cDNA fragment of the coding region [3] to probe the blots. In the case of *Fgfr2*, we used a 670-bp cDNA fragment also of the coding region [4]. The genomic sequence corresponding to the *Fgf3* gene was identified by amplification of DNA samples from the parental strains C57BL/6 and *Mus spretus* (SEG/Pas) by the polymerase chain reaction (PCR). Primers designed from the mouse cDNA sequence of *Fgf3* were as follows:

5' GTGACAATACATTCCTGCTGT 3' forward, and
5' CTCAGATCTTATCTCTAGCAC 3' reverse

Allele detection: Genomic DNA samples of the offspring of the interspecific backcross were digested with *Eco*RI, separated by electrophoresis in 0.8% Tris-borate/EDTA buffered agarose gels, blotted to nylon membranes (Amersham™), and probed with a radio-labeled *Atp2a1* cDNA probe under conditions of high stringency. Under such conditions, we found that the above-mentioned *Atp2a1* probe hybridizes to a 7.5-kb fragment with C57BL/6 DNA and to a 4.0-kb fragment with *Mus spretus* DNA. The *Fgfr2* alleles were identified by a method reported by Avraham and associates [5], where it was found that the *Fgfr2* probe hybridizes to a 6.4-kb fragment with C57BL/6 DNA and to a 4.7-kb fragment with *Mus spretus* DNA. The *Fgf3* locus was typed by comparing the size of the PCR products: 161 bp with C57BL/6 DNA versus 165 bp with *Mus spretus* DNA.

Discussion: Mammalian Ca²⁺-ATPases of the sarcoplasmic re-

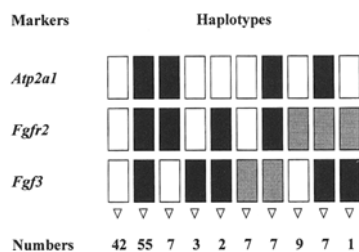


Fig. 1. Segregation data of *Atp2a1*, *Fgfr2*, and *Fgf3* shown as haplotype schemes. *Atp2a1* was placed on mouse Chr 7 with DNA samples prepared from the offspring of an interspecific backcross of the type: (C57BL/6 × *Mus spretus*)F₁ × C57BL/6. Each column represents the observed genotypes in a total of 140 mice. Black boxes represent homozygosity for the C57BL/6 allele, white boxes represent heterozygosity for the *Mus spretus* allele, and grey boxes indicate that the genotype is not known at the corresponding locus. The number of mice inheriting each genotype is indicated at the bottom of each column.

ticulum occur in different isoforms which are encoded by at least three different structural genes in human [6,7]. ATP2A1 encodes the adult and neonatal fast skeletal isoform, ATP2A2 encodes slow skeletal and cardiac or ubiquitous isoforms, and ATP2A3 encodes ATPases that are expressed in a great variety of muscular and nonmuscular cells. Two of these genes have been localized on different chromosomes in human: ATP2A1 maps to Chromosome (Chr) 16p11.2–12.1, while ATP2A2 maps to Chr 12q23–24.1 [8]. Given that, in human, the locus homologous to *Fgfr2* (FGFR2) maps to the q25.3–q26 region of Chr 10 [5] we confirm that the homology breakpoint between human Chrs 10 and 16 on mouse Chr 7 is centromeric and very close to the *Fgfr2* locus.

Technical comments: The PCR reactions were performed in a final reaction volume of 25 μl. Final concentrations were: 10 mM Tris pH 8.4; 50 mM KCl; 0.1% Tween 20; 200 μM dATP, dCTP, dGTP, dTTP, and 2 mM MgCl₂. Each reaction was performed with 100 ng of genomic DNA and 0.6 U of *Taq* DNA polymerase (Amersham™). 35 cycles of 40 s at 94°C, 40 s at 55°C were run. They were preceded by an initial denaturation step (3 min at 94°C) and followed by a final elongation step of 3 min at 72°C. PCR products were analyzed by 4% Tris-borate/EDTA-buffered agarose gel electrophoresis.

Acknowledgments: This work was supported in France by a grant from the Association Française contre les Myopathies (AFM), and in Germany by the Deutsche Forschungsgemeinschaft (SFB 223).

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