Dinucleotide repeat polymorphism at the RYR1 locus (19q13.1)

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Source/Description: A HaeIII fragment from a cosmid containing a portion of the human skeletal Ryanodine receptor gene (RYR1) (1) was selected by hybridisation to poly(dC-dA).poly(dG-dT) and was designated Wis1. Sequencing was performed for determination of primer sequences for the polymerase chain reaction. The predicted length of the amplified fragment was 227 bp.

Primer Sequences:
5'GCATCACGGTCTGCAATTCAT 3' (CA strand);
5'GCAATGGCATAATCTCAGCT 3' (GT strand).

Frequency: Estimated from 160 chromosomes of the CEPH parents.
Observed heterozygosity = 0.43, PIC = 0.38

Allele (bp)	Frequency	Allele (bp)	Frequency
A1 233	0.0125	A5 225	0.0250
A2 231	0.0125	A6 223	0.0313
A3 229	0.1000	A7 221	0.0500
A4 227	0.7688		

Chromosomal Localisation: The skeletal muscle Ryanodine receptor gene (RYR1) has been mapped to 19q13.1 (2).

Mendelian Inheritance: Co-dominant segregation was observed in 40 two generation families.

PCR Conditions: Amplification reactions were as follows, denaturation at 94°C (3 min), 32 cycles of denaturation at 94°C (1 min), annealing at 54°C (45 sec) and extension at 72°C (1 min) using 0.5 U Amplitaq, 1×GeneAmp buffer and 200 μM dNTPs (Perkin Elmer-Cetus), 100 ng genomic DNA and 10 pmol of each primer in a volume of 15 μl. Allele sizes were determined on 8% denaturing polyacrylamide gels with autoradiography by comparison to the cloned sequence. The sequenced repeat was (CA)17. The sequence has been submitted to Genbank. Accession number = X60187 Human RYR1 DNA.

Acknowledgements: This work was supported by a grant from the Department of Veterans Affairs (K.H.), and Eolas, the Irish Science and Technology board (F.C., T.V.M.). Special thanks to Dr. J. Mulley for assistance with CEPH pedigree typing.

References: 1) Zorzato,F. *et al.* (1990) *J. Biol. Chem.* **265**, 2244–2256. 2) MacKenzie,A.E. *et al.* (1990) *Am. J. Hum. Genet.* **46**, 1082–1089.

Microsatellite polymorphism in human insulin receptor gene (*INSR*) on chromosome 19

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Primers/Description: Two primers (INSR E3–2B, 5'-ATTGC-TGCATATGCAGACAG-3' and INSR E3-rC3, 5'-TGC-AGCCGTGTGACTTACAG-3') were used to amplify a 138–156 bp ATTT and CT repeat-rich region in intron 2 of the human *INSR* gene (1).

Frequency: Nine alleles were observed in 48 unrelated Caucasians. The heterozygosity was 58%.

Allele	bp	Frequency	Allele	bp	Frequency
AA1	156	0.01	AA2	154	0.01
AA3	152	0.01	AA4	150	0.10
AA5	148	0.65	AA6	146	0.05
AA7	144	0.03	AA8	142	0.12
AA9	138	0.02			

Chromosomal Localization: *INSR* was assigned to chromosome 19p13.3-p13.2 (2).

Mendelian Inheritance: Codominant inheritance was observed in four nuclear families.

Other Comments: The PCR was performed using ^{32}P-labeled INSR E3–2B and unlabeled INSR E3-rC3 for 30 cycles: denaturation at 94°C for 1 min; annealing at 56°C for 2 min; and extension at 72°C for 2.5 min. The PCR products were analyzed on a 5% denaturing polyacrylamide gel (Fig. 1). The microsatellite sequence in intron 2 was of the form $(ATTT)_{11}CC(CT)_{10}$.

References: 1) Seino,S. *et al.* (1990) *Diabetes* **39**, 123. 2) Yang-Feng,T.L. *et al.* (1985) *Science* **228**, 728.

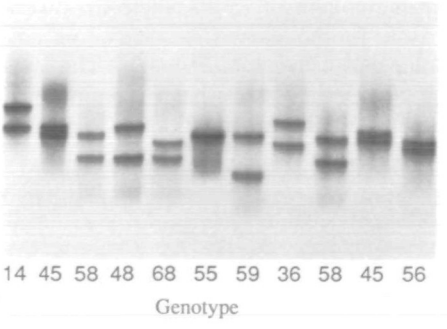

Figure 1. PCR amplification of microsatellite repeat polymorphism in *INSR* gene. The genotypes are noted at the bottom of the figure.

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