# Localization of the Aquaporin 1 (AQP1) Gene within a YAC Contig Containing the Polymorphic Markers D7S632 and D7S526

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The aquaporin protein acts as a water selective, transmembrane channel (5). It is expressed in a wide range of tissues and organs and is especially abundant in the anterior segment of the eye (6, 7). Studies have shown that there is only a single AQP1 gene locus, which has been localized by *in situ* hybridization to chromosome 7p14 (1, 5). A study by Deen *et al.* (1) failed to identify any RFLP at the AQP1 locus. A poly(CA) sequence 400 bp upstream of the transcription start site also proved to be nonpolymorphic (1). The same authors identified six other sequence-tagged sites from a single cosmid containing the entire AQP1 gene. These all contained repeat motifs, but none was investigated for possible size variation at that time.

In an attempt to obtain a more accurate genetic localization, we designed primers to amplify the longest and least interrupted of these repeat motifs (EMBL Accession No. Z21985). A forward primer GGCACTGAGAGGTGTGGC and a reverse primer ATGCTGGCAAACACATGCAC gave a product size of 86 bp containing the repeat. PCR analysis in 20 individuals revealed no size variation. However, amplification of this STS from DNA of YAC clones known to localize to the 7p14–p15 region (2) revealed that it was located on a contig of three YACs also containing the microsatellite markers D7S632 and D7S526 (Fig. 1). It was possible to deduce from the contig that AQP1 is proximal to D7S632, but its position relative to D7S526 is unknown. The published genetic distance between D7S632 and D7S526 is only 1 cM (2). Therefore, despite AQP1 not having a polymorphism directly associated with it, these two highly informative microsatellites are within 370 (D7S526) and 400 (D7S632) kb of the gene and therefore will allow its involvement in inherited disease to be determined.

Aquaporin is known to be expressed in a diverse range of secretory and absorptive epithelia, including many in the eye (6), and it has been proposed as a possible candidate for conditions that involve an imbalance of ocular fluid movement (7). Two eye disease loci have recently been localized to this chromosomal region. This lab has defined the region containing a locus for autosomal dominant retinitis pigmentosa (adRP) to lie in an interval flanked by D7S484 to D7S526 (3). Subsequent haplotype analysis in an affected family has allowed us to place D7S690, which is absent from the YACs in Fig. 1, proximal to D7S526 and the disease region proximal to D7S690 (data not shown). Thus, aquaporin is excluded as a candidate gene in this family. Another group has mapped dominant cystoid macular dystrophy (DCMD) to the interval D7S526–D7S493 (4), which overlaps our physical placement of AQP1. DCMD is characterized by an early-onset cystoid macular oedema, and it has been suggested that the disease involves a dysfunction of the retinal pigment epithelium. On the basis of the localization described here and its role in fluid movement, the aquaporin gene should therefore be considered a candidate for DCMD.

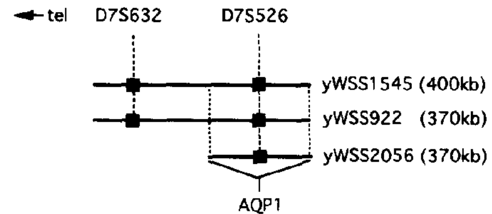


**FIG. 1.** Schematic diagram showing the three YACs containing the AQP1 locus and D7S526; two of the YACs also contain D7S632 (YAC nomenclature as in Green *et al.* (2)).

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# Dinucleotide Repeat Polymorphism at the Human Chromosome 11p Telomere (D11S2071)

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The recent publication of detailed PCR-based linkage maps for all human chromosomes (3, 5, 9) has greatly facilitated linkage mapping of disease genes. However, most of the markers on these maps have been isolated using approaches based on random screening of genomic libraries. As a consequence, the completeness of coverage of the map in the telomeric chromosomal regions is uncertain. To remove this uncertainty and to aid in positional cloning of disease genes in telomeric regions, it is necessary to develop polymorphic markers that are physically linked to the telomeres of all chromosomes. The need for telomeric markers is highlighted by the discovery that the highest gene concentrations in the human genome are in subtelomeric regions (11).

The successful cloning of human chromosomal telomeres in modified YAC vectors (10) has paved the way for the development of such markers. Thus, polymorphic markers have been developed from YAC clones containing the telomeres of human chromosomes 7q (6), 2p, 10p, 12p, 13q, and 14q (12). Here, we report the isolation and characterization of a highly polymorphic $(CA)_n$ microsatellite marker from a YAC containing the telomere of human chromosome 11p.

yRM2209 is a 140-kb YAC from a human genomic library enriched for telomere sequences (10). yRM2209 hybridized with a probe for the subterminal repeat element TelBam3.4 (2), and when used as a probe for FISH, it gave a strong signal at the 11p telomere, but also gave a weak signal on chromosome 10p14. Cosmid 194B is a partial *Sau*3A subclone of yRM2209. This cosmid gave a strong and highly specific signal on 11pter with FISH, with no trace of any secondary signal on chromosome 10 (data not shown).

Probing Southern blots of various restriction digests with $(CA)_{15}$ suggested that cosmid p194B contained a single $(CA)_n$ repeat. Screening with radiolabeled $(CA)_{15}$ at high stringency identified a 1-kb *Sau*3A subclone of cosmid 194B that contained an uninterrupted $(CA)_{22}$ repeat. Using the DNA sequence flanking this repeat (GenBank Accession No. U12896), we designed PCR primers to type this marker locus: p194B/F, 5′ AGGGCAATGAGGACATGAAC 3′, and P194B/R, 5′ ATGTGGCTGGTCCACCTG 3′. PCR reactions were performed in a volume of 10 μl containing 25 ng genomic DNA, 5 pmol each primer, 1.5 m*M* $MgCl_2$, 200 μ*M* dNTPs, 50 m*M* KCl, 10 m*M* Tris–Cl, pH 8.3, and 0.25 units of *Taq* polymerase (AmpliTaq). Three-temperature "touchdown" PCR amplification was performed in a Perkin–Elmer/Cetus System 9600 thermocycler using an initial annealing temperature of 70°C, which was decreased by 1°C in each of the first 10 cycles and then maintained at 60°C for 20 more cycles. Each cycle consisted of a 30-s 94°C denaturing step, a 15-s annealing step, and a 30-s extension step at 72°C. A final 5-min 72°C extension was used. PCR products were resolved on denaturing polyacrylamide gels, capillary blotted onto Hybond-$N^+$ membrane (Amersham), and detected by probing with a 5′ $^{32}P$-labeled $(CA)_{15}$ probe. Sizes of allelic fragments were determined relative to an M13mp18 DNA sequence ladder. Allele sizes and frequencies in 72 unrelated CEPH parents are shown in Table 1. The observed heterozygosity was 86%, and the PIC was 0.83. Genotypes of four CEPH parents are 133101:4,14, 133102:16,16, 133301:2,6, 133302:2,14.

Initially, localization of D11S2071 to chromosome 11 was verified by showing that the STS was present in several rodent–human somatic cell hybrids that contained this chro-

**TABLE 1**

**Allele Sizes and Frequencies in 72 Unrelated CEPH Parents**

| Allele | Size (nt) | Frequency |
|---|---|---|
| A1 | 202 | 0.013 |
| A2 | 200 | 0.21 |
| A3 | 198 | 0.06 |
| A4 | 196 | 0.19 |
| A5 | 194 | 0.07 |
| A6 | 192 | 0.17 |
| A7 | 190 | 0.05 |
| A8 | 188 | 0.00[a] |
| A9 | 186 | 0.007 |
| A10 | 184 | 0.00[a] |
| A11 | 182 | 0.013 |
| A12 | 180 | 0.007 |
| A13 | 178 | 0.00[a] |
| A14 | 176 | 0.05 |
| A15 | 174 | 0.007 |
| A16 | 172 | 0.13 |
| A17 | 170 | 0.007 |
| A18 | 168 | 0.007 |

[a] Alleles A8, A10, and A13 were not observed in the population studied, but may be observed as the sample size increases.

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