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# Construction of YAC Contigs at Human Chromosome 11q22.3–q23.1 Region Covering the *Ataxia Telangiectasia* Locus

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**Abstract**

*Ataxia telangiectasia* (*AT*) is an autosomal recessive disease of unknown etiology associated with cerebellar ataxia, telangiectasia, immune dysfunction, higher cancer risk, genomic instability and hypersensitivity to ionizing radiation. The major *AT* loci, *AT-A* and *AT-C*, are shown to be closely linked at chromosome 11q22–q23. The most recent genetic linkage mapping and linkage disequilibrium analysis have localized the major *AT* loci to a sequence of approximately 850 kb between the markers *D11S1819* and *D11S1818*. The isolation of yeast artificial chromosomes spanning the *AT* region is an essential step to identify the gene or genes responsible for the mutation(s). We isolated a total of 20 YAC clones from three independent YAC libraries, using sequence tagged sites mapped in the *AT* region as primers for PCR-based YAC screening. The PCR assay for the presence or absence of 16 different DNA markers allowed us to construct and to order four YAC contigs at the *AT* region. One of the contigs which consists of the 10 YAC clones, covers about 2 Mb of DNA at the boundary between Giemsa-positive band 11q22.3 and Giemsa-negative band 11q23.1 and includes the entire region of the major *AT* locus between *D11S1819* and *D11S1818*. Thus, the YAC contigs will facilitate the positional cloning approach for searching transcribed sequences from the defined genomic region.

**Key words:** YAC contigs; human chromosome 11q22.3–23.1; *ataxia telangiectasia*

## 1. Introduction

*Ataxia telangiectasia* (*AT*) is a human autosomal recessive disorder characterized by progressive cerebellar ataxia, oculo-cutaneous telangiectasia, immunodeficiency, and a high incidence of cancer.[1] The AT cells exhibit various abnormalities consistent with a defect involving DNA metabolism and/or maintenance of genomic integrity: i.e., elevated frequencies of chromosomal aberrations and intrachromosomal recombination, aberrant immune gene rearrangements, hypersensitivity to the killing effects of ionizing radiation and radiomimetic chemicals, radio-resistant DNA synthesis, and defective damage-responsible cell-cycle checkpoints.[2–4] The AT genotypes have been classified into four complementation groups, designated AT-A, AT-C, AT-D, and AT-E, on the basis of heterodikaryon complementation assay for radioresistant DNA synthesis.[5]

Linkage studies have demonstrated that the genetic defect responsible for AT-A,[6,7] AT-C,[8] and AT-E[9] localize to chromosome region 11q22–q23. An international mapping consortium has analyzed linkage data of 111 AT-families from the UK, Israel, Italy, Turkey and USA, and mapped a major *AT* locus to an 8 cM sex-averaged interval between the marker *STMY* and *D11S132/NCAM* of chromosome 11q22–q23.[10] More recent linkage analyses narrowed the *AT-A/AT-C* locus to an interval of 4.1 cM between the loci *D11S611* and *D11S535/D11S384*[9] or of 3.4 cM flanked by *GRIA4* on the centromeric side and *D11S535* on the telomeric side.[11,12] The most recent haplotype analysis by the international consortium has indicated that the major *AT* locus is further localized to an ~850 kb interval flanked by *D11S1819* and *D11S1818*.[13]

Despite considerable efforts to isolate of the *AT* gene(s) by functional complementation using its marked sensitivity to ionizing radiation as a selective phenotype, functional cloning of the gene has been unsuccessful. This has been due to some technical difficulties such as low transfection frequencies, instability of the integrated DNA and difficulties in recovering transfected DNA sequences from

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the cells showing functional complementation.[14–16] In the case of AT-D, the functional cloning of a candidate gene, designated ATDC, has been reported.[17] However, the cDNA of the ATDC was localized to 11q23, just distal to the marker *THY1*. The precise relationship of this candidate gene to *AT* phenotype has not as yet been clearly established.[18]

An alternative strategy for gene cloning, the so-called "positional cloning" approach,[19,20] must be a more efficient way to identify the *AT* gene. More than 50 polymorphic markers have been localized to the 11q22–q23 region by chromosome mapping,[21,22] linkage mapping,[9,10,23] and physical mapping.[11,12,24,44] These mapping information will provide landmarks to facilitate the construction of yeast artificial chromosome (YAC) and cosmid contigs spanning the *AT* locus at 11q22–23.

In an attempt to develop a long-range YAC contig covering the major *AT* locus, we have first constructed an 11q22.3/11q23.1 (G-band/R-band) junction-specific DNA library by means of micro-dissection and microcloning techniques,[25] and sequence tagged sites (STSs) were designed for PCR-mediated screening of YAC library.[26] By using the STSs from the microclones and other DNA markers mapped in the AT region, we have identified 20 YAC clones from three different YAC libraries.[27–29] We describe here the generation of an STS-content map of YAC clones encompassing the entire region of the major *AT* locus.

## 2. Materials and Methods

### *2.1. YAC library screening*

The YAC libraries used in the present study were constructed at Washington University, St. Louis[27] and at the Centre d'Etudes du polymorphisme Humain (CEPH), Paris.[28,29] CEPH Mega-YAC library was purchased from Research Genetics (Huntsville, AL). PCR amplification was performed from 12.5–100 ng of template DNA in a reaction volume of 5–20 $\mu$l including 10 mM Tris-HCl (pH 8.8), 2.5 mM $MgCl_2$, 150 $\mu$M each dNTP, 2 $\mu$M each primer, and 0.2 unit of Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT). The PCR conditions were denaturation at 95°C for 2 min followed by 30–35 cycles of 30 sec at 95°C, annealing at the predicted temperature for each primer set, and extension 1 min at 72°C.

The STSs for microclones (pMD11C) and oligonucleotide pairs used for PCR have been described.[26] Other primer sequences used for YAC screening and PCR assay for the physical mapping of the YACs were as previously published for *STMY*,[30] *D11S385* (CJ52.75), *D11S611* (J12.1C2), *D11S2020* (Y11B11R), *GRIA4*, *D11S2022* (CJ52.3), *D11S2025* (Y11B29L), *ACAT*, *D11S384* (CJ52.193), and *D11S535* (J12.8),[11] and three microsatellite markers, *D11S1818* (A2), *D11S1819* (A4), *D11S1343* (AFM26).[24]

### *2.2. YAC characterization*

For sizing of YAC clones, high molecular weight genomic DNA was prepared from yeast cells and embedded in agarose plugs.[31] Yeast chromosomes were separated by pulsed-field gel electrophoresis (PFGE) through 1% agarose gels in 0.5XTBE buffer (1XTBE=90 mM Tris borate, pH 8.3/2 mM EDTA) at 200V and 14°C, using a Bio-Rad CHEF-Mapper apparatus (Bio-Rad Laboratories, Richmo, CA). For *Alu*-PCR finger-printing assay, LA (long and accurate)-PCR (Takara, Japan) of YAC DNA was carried out with a PDJ34-1 primer, 5′TGAGCCGAGATCGCGCCACTGCACTCCAGCCT-GGG[32] to detect the *Alu* consensus sequence.[33] LA-PCR reactions were performed in a 20-$\mu$l volume, containing 25–50 ng total YAC yeast DNA, 0.2 $\mu$M PDJ34-1 primer, 2.5 mM $MgCl_2$, 400 $\mu$M each of dNTPs, and 1.5 units of ExTaq DNA polymerase (Takara) according to the protocol recommended by Takara. Temperature and time profile were 30 cycles of 95°C for 20 sec and 68°C for 15 min. Aliquots of LA-PCR product were examined on 0.8% agarose gels.

### *2.3. Fluorescence in situ hybridization (FISH)*

The LA-*Alu*-PCR products were ethanol precipitated, 1 $\mu$g of DNA was labeled by nick-translation with biotin-11-dUTP, and applied to a direct mapping system which is based on FISH combined with replicated prometaphase R-bands.[34] A suppression hybridization procedure with human Cot1 DNA (GIBCO-BRL Gaithersburg, MD) was used for elimination of repetitive sequences in the PCR products.[35] The concentration of the probe DNA was 250 ng/20 $\mu$l of hybridization mixture containing 5–10 $\mu$g human Cot1 DNA as a competitor per chromosome spread. The procedures of hybridization, rinsing, detection with avidin-FITC, counter-staining with propidium iodide and microphotography were described previously.[22,35]

### *2.4. STS-content mapping of YAC clones*

The long-range physical and genetic maps across the 11q22–q23 regions have been reported.[11,24,36,44] By using the STS markers and microsatellite markers in these maps, the YAC clones were physically mapped based on the presence or absence of their PCR products.

## 3. Results and Discussion

### *3.1. Characterization of YAC clones*

Two YAC libraries, the CEPH[28] and the Washington University D[27] were screened by PCR in YAC DNA pools with primers for *STMY*, pMD11C-C-115, pMD11C-C-104, pMD11C-C-7, pMD11C-C-107, and *D11S384*. The CEPH "Mega-YAC" library[29] was also screened by PCR

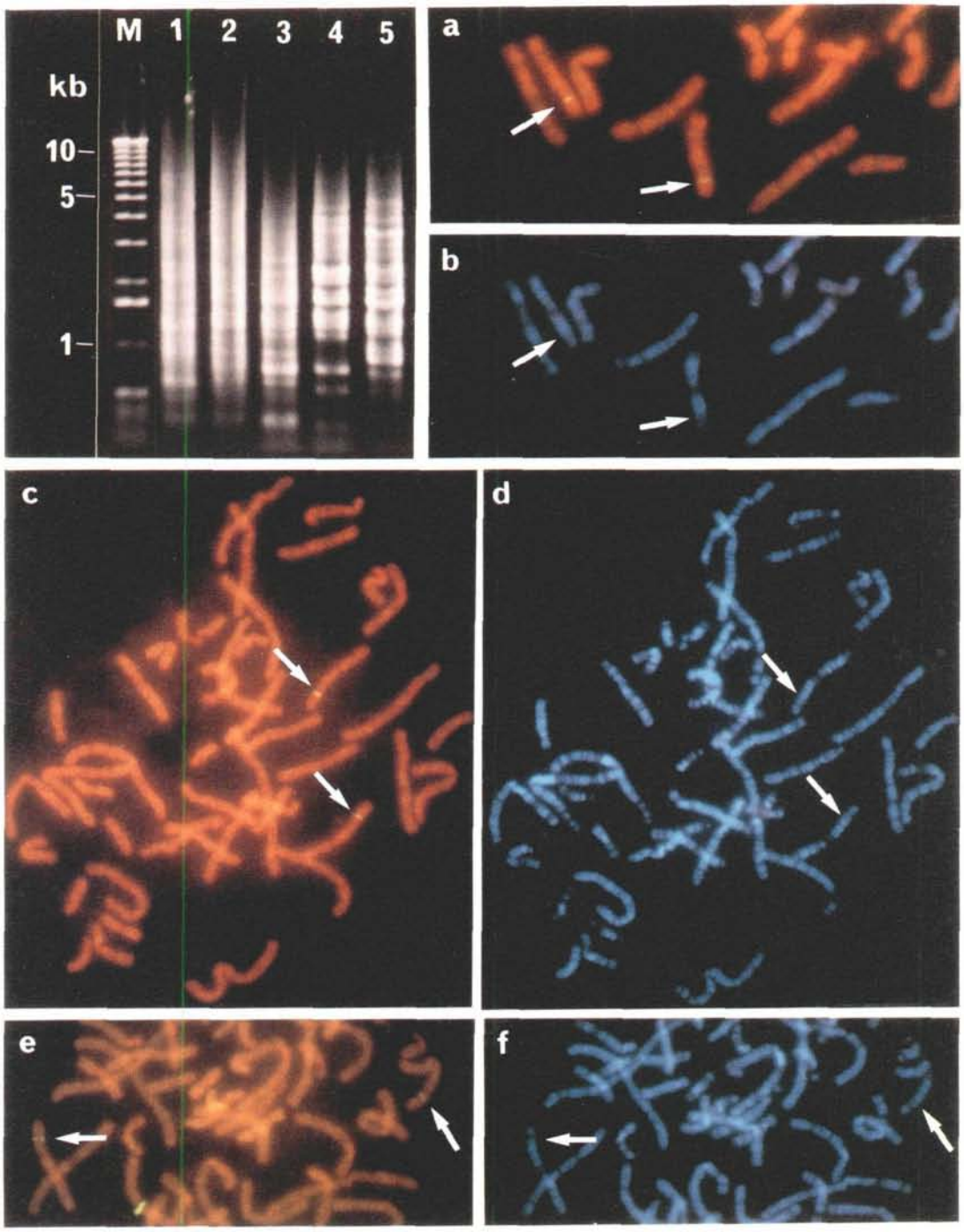


**Figure 1**. Chromosomal location of YAC clones at the junction of 11q22.3 and 11q23.1 determined by FISH with biotinylated LA-*Alu*-PCR products. Top-left: LA-*Alu*-PCR products derived from YAC clones 961D6 (lane 1), 890C12 (lane 2), 910D12 (lane 3) and 984E7 (lane 5), using a PDJ34-1 primer. a, c, and e show FISH result of YAC clones 984E7, 910D12 and 961D6, respectively. b, d, and f show their G-band patterns. Arrows indicate the location of signal at the 11q22.3–11q23.1 boundary.

**Table 1.** YAC clones subjected to PCR analyses.

| DNA marker | YAC clone | Source | Size (kb) | Ref. |
|---|---|---|---|---|
| pMD11C-7 | DA2411D11 | Washington D | 360 | 26 |
| | DD005F5 | Washington D | 530 | 26 |
| | DB019D4 | Washington D | 200 | 26 |
| pMD11C-104 | DB018D8 | Washington D | 800 | 26 |
| | 341D11 | CEPH | 180 | 26 |
| pMD11C-107 | DA1904B1 | Washington D | 520 | 26 |
| | DA2207F9 | Washington D | 500/300 | 26 |
| pMD11C-115 | 432H2 | CEPH | 190 | 26 |
| | 474E7 | CEPH | 330 | 26 |
| STMY | 381H4 | CEPH | n.d. | - |
| | 470D10 | CEPH | n.d. | - |
| CJ193 | DA2418H3 | Washington D | n.d. | - |
| (D11S384) | DA2409G1 | Washington D | n.d. | - |
| | DA2112B2 | Washington D | 400 | - |
| | 890C12 | CEPH | 670 | - |
| | 961D6 | CEPH | 1500 | - |
| J12.8 | 961D6 | CEPH | 1500 | - |
| (D11S535) | 984E7 | CEPH | 1200 | - |
| AFM296 | 876G4 | CEPH | 1150 | - |
| (D11S1343) | 910D12 | CEPH | 1150 | - |
| | 950C12 | CEPH | 1700 | - |

n.d., not determined.

with primers for *D11S384*, *D11S535* and *D11S1343*. In total, 20 YAC clones were isolated and characterized. The sizes of YAC clones were determined by pulsed-field gel electrophoresis of yeast chromosomes. The results of the size analysis are shown in Table 1. The serious major problem of using YAC clones in a large-scale genome mapping is the presence of artifactual "chimeric" YACs in virtually all the currently available YAC libraries. Chimeric YAC clones consist of two or more noncontiguous segments of genomic DNA co-cloned in the same vector and may arise from co-ligation during the cloning or from recombination events during transformation.[37] Because of the relatively high frequency of chimerism (approximately 40–60%),[38] we first determined the chromosomal origin of each YAC clone by FISH. The accuracy and usefulness of YAC mapping by FISH have been documented previously. Particularly, the INSPCR (interspersed repetitive sequence polymerase chain reaction) with human-specific *Alu* and *L1* primers made it possible to characterize and map human YACs by FISH with its PCR products, without isolating them from the host chromosomes.[39,40] The conditions for LA (long and accurate)-*Alu*-PCR were optimized to yield longer PCR products (more than 2–5 kb) of inter-*Alu* DNA sequences without any amplification of the yeast genome. Figure 1 shows examples of ethidium bromide-stained LA-*Alu*-PCR products from the 5 YAC clones. The fingerprint pattern of the inter-*Alu* PCR product of each clone was unique.

To determine the chromosomal location and chimerism of the YACs, the LA-*Alu*-PCR products from each YAC were biotinylated and hybridized to prometaphase chromosome spreads from a normal XY individual. The *in situ* suppression hybridization was performed to eliminate possible non-specific signals from repetitive sequences present in the PCR products. Figure 1 shows representative analyses of three YAC clones identified from the Mega-YAC library (CEPH), exhibiting unique twin spots at or near the junction of q22.3 and q23.1 of both homologues of chromosome 11. As shown in Fig. 1, in our optimized suppression conditions, virtually all spurious signals from hybridization of repetitive elements to other nonspecific sites could be eliminated. In the most of the cases, the percentage of labeled chromosomes was more than 80% and was therefore equivalent with the efficiencies obtainable with cosmid clones.[22,34] Although a large number of chimeric YAC clones in the libraries was reported,[38] no chimera signal was detected by FISH using the *Alu*-PCR products of each YAC clone as probes. In all cases except for DA2207F9, over 90% of the metaphase spread examined for each YAC showed two twin-spots at the 11q22.3–q23.1 region of chromosome 11, without any detectable signals on other chromosomes. In the case of a YAC, DA2207F9, the yeast cells contained two YACs with different insert size (see Table 1), and appeared to be a mixture because FISH analysis indicated that they are localized at the 11q22.3–q23.1 and at 2p13 regions (data not shown).

Our data indicate that the FISH by using LA-*Alu*-PCR products as probes are a rapid method for chromosomal location of YACs. However, it is difficult to estimate the sensitivity of this method for detection of chimeric YACs because the chance of detection depends upon the *Alu*-richness of the genomic DNA segments, and the PCR efficiency of diverse *Alu* sequences in a given YAC clone. Furthermore, possible internal deletion or rearrangement in a YAC clone can not be detected by this method. Some YACs may be reassessed upon further analyses, including the physical mapping described below.

### *3.2. STS-content mapping of YACs*

Figure 2 shows a long-range physical map across the 11q22–q23 chromosomal region covering the major *AT* locus. This was based on the map reported by Gatti et al.,[13] and modified slightly according to the data from Uhrhammer et al.,[44] Ambrose et al.,[11] Vanagaite et al.[36] and Rotman et al.[24] A total of 16 markers were incorporated in the map spanning an approximately 8-Mb interval between or near the markers *STMY* and *D11S927*.

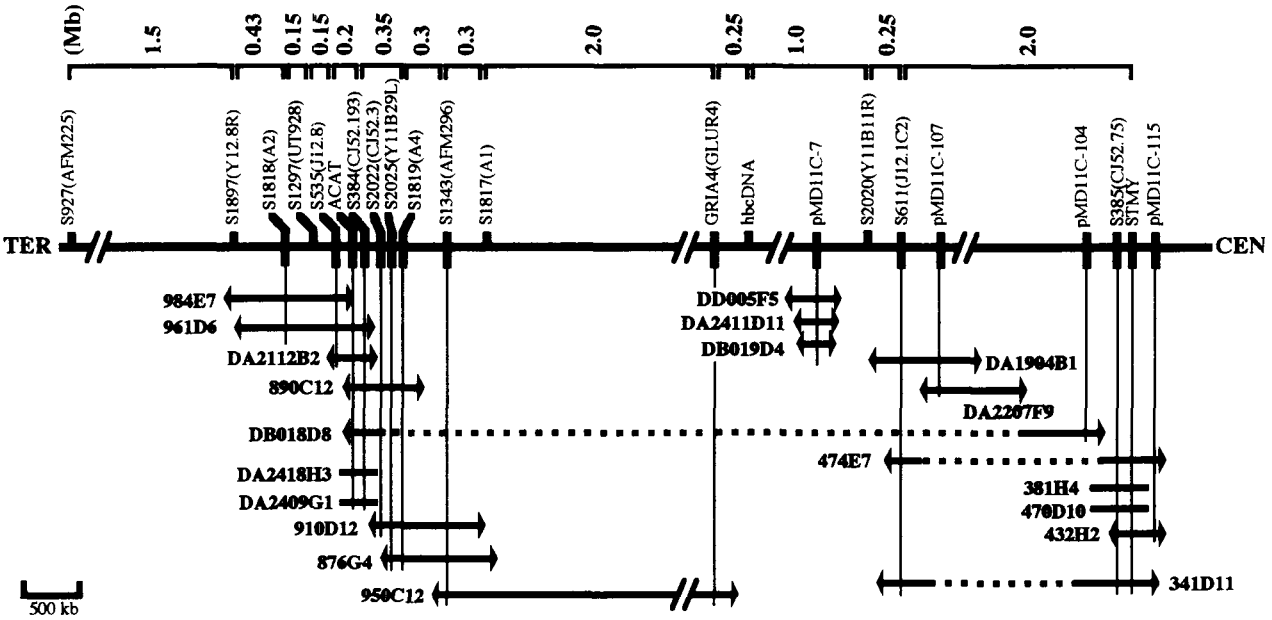


**Figure 2.** A YAC-based STS-content map of the *AT* major region. The order and distance between markers are based on the maps reported by Gatti et al.,[13] Ambrose et al.[11] Vanagaite et al.,[36] and Rotman et al.[24] Size and origin of the YACs are summarized in Table 1. The size of the YAC clones without arrow-ends was not determined. STSs used for analysis of the YACs are indicated by vertical thick bar crossing the horizontal line. The presence of an STS sequence in a YAC is demonstrated by thin line. The YACs represented by dashed line demonstrate that these have discontinuous segments. // indicates that physical length in the maps reported previously are not confirmed by the present YAC contigs.

The distances between the markers ranged from 150 kb to 2000 kb. Thus, we used the STS content mapping[41] to construct a DNA contig of the YAC clones we isolated. The 20 YACs were examined for the presence or absence of the markers in the map by PCR. Examples of such analyses are shown in Fig. 3 in which four YACs identified by the marker *D11S384* and another three YACs isolated by the marker *D11S1343* were examined for the presence or absence of five different markers. Inspection for the presence or absence of each marker in each YAC clone facilitated the ordering of the YACs into contigs and also for reassessing the ordering of these marker loci in the physical map reported. A summary of the results of this analysis is illustrated in Fig. 2. The 20 YAC clones were classified into four ordered groups. A ~2 Mb YAC contig assigned in telomeric side of the map was formed at an interval between *D11S1897* and *D11S1817*. This contig consists of 10 YAC clones and the entire region is covered by four representative YAC clones; i.e., 984E7 (1200 kb), 961D6 (1500 kb), 890C12 (670 kb), and 910D12 (1150 kb). Our physical ordering of the YACs and their size estimates appears to be consistent with the genetic ordering of the markers and physical distances between them reported by Ambrose et al.,[11] Rotman et al.,[24] and Gatti et al.[13] Recently, the genetic linkage mapping and linkage disequilibrium analyses in an international consortium have indicated that the major locus for *AT-A* and *AT-C* is confined within an ~850 kb interval flanked by the markers *D11S1819* and *D11S1818*.[13] Thus, the ~2 Mb YAC contig should contain the entire locus for the major AT gene(s). The ACAT gene is located in the middle of the 850-kb region (Fig. 2) and is closely (~50 kb) linked to the marker *D11S384*.[11] We have previously mapped the ACAT gene, using high-resolution R-band FISH, to the junction between the Giemsa-positive band 11q22.3 and Giemsa-negative band 11q23.1.[42] The Giemsa-negative bands, R- and T-band, of human chromosomes are shown to be rich in both *Alu* family of human repetitive sequences and CpG islands and contain a majority of housekeeping genes: These genes are early replicating and are in the GC-rich isochores.[43] The 11q23.1 band corresponds to the T-band in which the majority of housekeeping genes may be clustered. Consequently, it seems probable that the AT gene, a housekeeping gene functioning in DNA metabolism, is located close to the markers of ACAT and *D11S384* in the T-band at 11q23.1 rather than at a more proximal position in the G-band of 11q22.3.

The physical map of markers in the 11q22–q23 region reported by Gatti et al.[13] includes the data from PFGE analyses of Uhrhammer et al.[44] and Ambrose et al.[11] Uhrhammer et al.[44] constructed a physical map span-

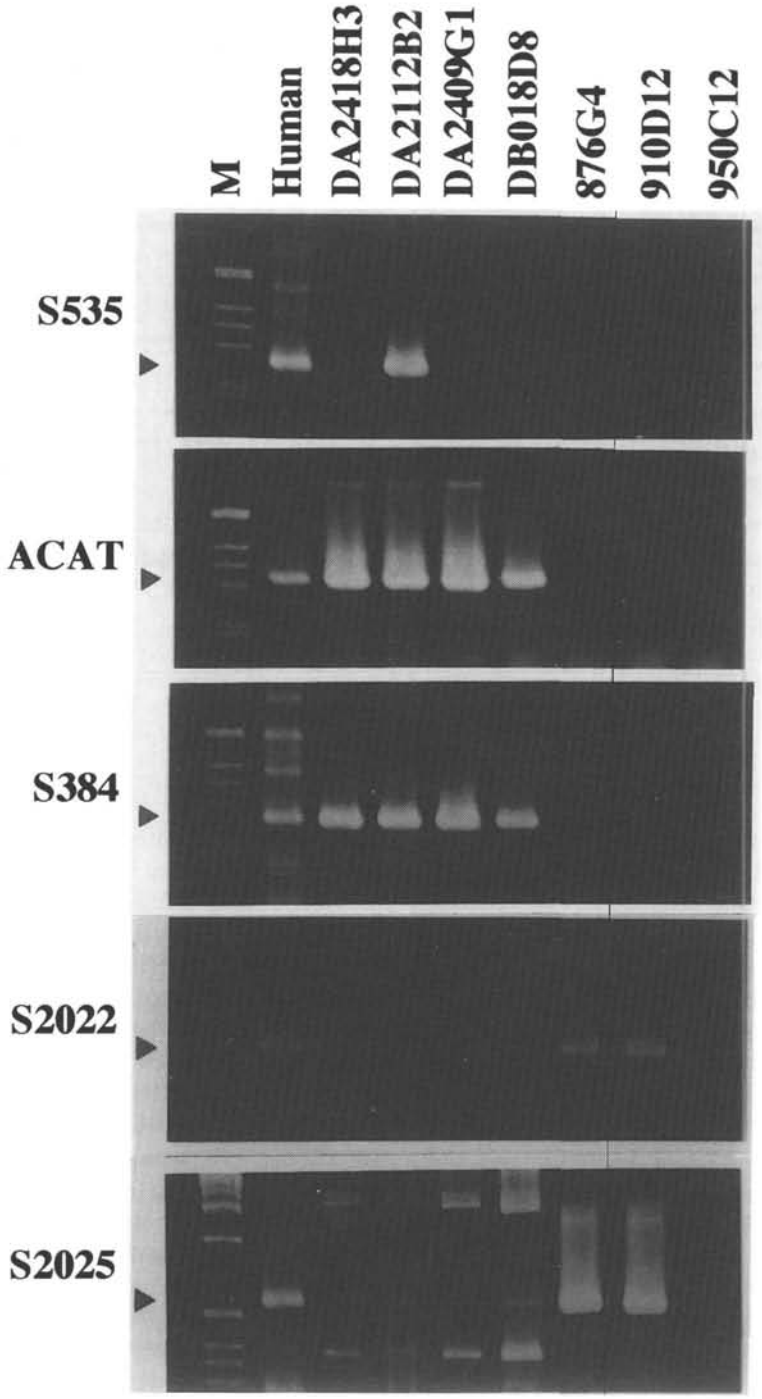


**Figure 3.** An example of PCR assay for the presence or absence of markers mapped at the 11q22–23 region. YAC clones isolated with a marker *D11S384* (DA2418H3, DA2112B2, DA2409G1 and DB018D8) or *D11S1343* (876G4, 910D12 and 950C12) were further analyzed by PCR with STS markers indicated on the left side of each panel. The prefix "*D11*" is omitted from the marker designations. M indicates size marker (1 kb ladder, BRL) for agarose gel electrophoresis. An arrowhead represents the expected size of the PCR product for each STS.

ning the distal part of the *AT* region, using *D11S384* and *D11S424* as anchor loci to relate the PFGE map to published genetic linkage map. They examined a total of 22 markers, including 17 cosmid clones that had been mapped to the 11q22–q23 region using somatic cell hybrids[21] and by FISH.[22] Despite the relatively large number of cosmids examined. the PFGE map was not extended in the centromeric direction beyond the locus *D11S384*. This may reflect a non-random distribution of cosmid mapping by FISH which has a tendency to localize to Giemsa-negative R-bands,[22] possibly due to a screening bias caused by the use of *Alu* sequences to identify cosmid clones with human inserts.

Ambrose et al.[11] constructed a more complete, contiguous physical map which stretches from *D11S535* to *D11S385* at the centromeric end of the *AT* region. However, as shown in Fig. 2, it is evident that there are still long genomic regions (1–2 Mb) in which only a few markers have been identified. Eight YAC clones identified by the STSs from the microclones, pMD11C-7, -104, -107, and -115, were localized to the ~3 Mb interval between markers *GRIA4* and *STMY*. Unfortunately, three YAC clones, DD005F5, DA2411D11, and DB019D4, identified by pMD11C-7 did not contain any of the markers examined in this region. However, a two-color FISH experiment indicated that they are localized close to the telomeric site of two YACs, 384H2 and 470D10, which were isolated by the marker *STMY* and positive for the presence of *D11S385* (data not shown). Thus, we tentatively mapped them to an interval flanked by hbcDNA and *D11S2020*. Judging from the physical distances between the markers reported by Uhrhammer et al.[44] and Ambrose et al.[11] and our estimates of YAC sizes, it seems likely that three YAC clones, 341D11, DB018D8 and 474E7, have an internal deletion. A YAC clone, *DB018D8*, was identified independently by two markers of *D11S384* and pMD11C-104 in the PCR-mediated screening of the St. Luis library. The DA018D8 YAC was about 800 kb in size and positive for the presence of two other markers, *ACAT* and *D11S2025*. This YAC appeared to contain a complex internal deletion. In view of the relatively low marker density at the centromeric portion of the *AT* region, the integration of our STS markers from the microclones into the physical map will facilitate the extension of the YAC contig, by isolating the mega-YACs.

Rotman et al.[24] have recently reported a long-range YAC contig which contained a minimum of 10 YAC clones, spanning at least 4.5 Mb in the 11q22–q23 region. They screened 3 YAC libraries, the ICRF human 4X and 4Y library, a chromosome 11-specific YAC library, and Washington University's libraries and applied chromosome walking methods to assemble YAC clones into order. They estimated the physical distance between *D11S1817* and *D11S927* to be approximately 3.5 Mb. The 2 Mb YAC contig described in the present study is included in the 3.5 Mb YAC contig reported by Rotman et al.[24]

We present here a YAC contig, which is represented by a minimal number of 4 contiguous and overlapping YACs, spanning about 2 Mb of genomic interval of the *AT* region at the boundary between 11q22.3 (G-band) and 11q23.1 (R-band). This contig includes the 850-kb genomic region between *D11S1819* and *D11S1818* in which the major locus of *AT-A* and *AT-C* is confined.[13] Although the physical maps at the *AT* region need to be refined more and the major *AT* locus is to be further narrowed down, one can initiate the positional cloning approaches to identify the AT gene(s), by searching candidate transcripts from the YAC clones described here. These transcripts can be tested functionally by using hypersensitivity to ionizing radiation as a selective phenotype in complementation assay.

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