Contig Map of the Parkinson's Disease Region on 4q21-q23

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Abstract

We have constructed a yeast artificial chromosome contig (YAC) map of human chromosome 4q21-
q23 across the Parkinson's disease region by combining molecular and fluorescence in situ hybridization
techniques. This map contains 55 YACs and 51 molecular markers, including 23 polymorphic markers.
We have also isolated one PI and 33 bacterial artificial chromosomes located within this contig. Plasmid
libraries were generated from 11 of these BAC and PI clones, and 614 random plasmid clones were sequenced
for a total of about 200 kb. This contig allowed us to precisely determine the location of 18 transcripts
within the D4S2460-D4S2986 interval, including the alpha-synuclein gene found to be mutated in some
families with Parkinson's disease.

Key words: Parkinson's disease; chromosome 4; contig; map; synuclein

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects 1% to 2% of the pop-
ulation over age 50. The major clinical features of PD include resting tremor, bradykinesia and muscular
rigidity.1,2 Linkage analysis in a large Italian kindred has demonstrated for the first time the existence of a
gene responsible for PD on human chromosome 4q21-q23.3 The location of this gene was first established be-
tween polymorphic markers D4S2361 and D4S421,3 and later narrowed down to a ~ 6 cM region between poly-
morphic markers D4S2371 and D4S2986 (unpublished data). In an effort to identify this PD gene by posi-
tional cloning, we constructed a high-resolution physical map of the region between markers D4S2371
and D4S2986 which we also extended on the centromeric side to include marker D4S2460. The map was con-
structed using the Centre d’Etude du Polymorphism Humain (CEPH)/Génethon,4 MIT/Whitehead,5 and Stanford
(http://shgc.stanford.edu/Mapping/phys_map/index.html) databases. High throughput screening of yeast
and bacterial artificial chromosome libraries (YACs and BACs) and one PI library was performed, as well as fiber
and metaphase fluorescence in situ hybridization (FISH) analyses. These data were used to construct a map that
includes the location and order of polymorphic markers, transcripts, and YAC and BAC clones. In addition,
construction of 11 plasmid libraries and random sequenc-
ing of clones were performed to offer additional data on the gene content within this chromosomal region. This
contig map provides useful mapping information in the 4q21-q23 chromosomal region.

2. Materials and Methods

2.1. Oligonucleotides

Specific oligonucleotides were designed: For the ADH5
gene ADH5-5F (5’-CTTTTATAAGGCATTGCTGC-3’)
and ADH5-5R (5’-CAAGAGAATCACTGGGTTTC-3’)
amplify a 498-bp fragment that includes exon 5 (acces-
sion # M81116), and ADH5-8F (5’-TGTGATTTCTTT-
tagCATGC-3’) and ADH5-9R (5’-TACCTGAGATTTAGCTGTGAAGCTCTACGAG-3’) amplify a 415-bp fragment that in-
cludes exon 8 and part of exon 9 (accession # M81118).
MPK-F (5’-TTGTTGCAGGTGACTAGCCG-3’) and
MPK-R (5’-TTAGCTGAGATTTAGCTGTGAAGCTCTACGAG-3’) amplify a 149-bp fragment of the MAP kinase gene (acces-
sion # U07620). All oligonucleotides and Map Pairs
were purchased from Research Genetics (Huntsville, AL.
USA).
2.2. Libraries

YAC clones from the CEPH library were grown in 25 ml culture in AHC medium (Bio 101, Vista, CA, USA) at 30 °C for 2–3 days. One milliliter of culture was transferred to a deep 96-well plate (Beckman Instruments) and aliquoted into MicroAmp Optical 96-well reaction plates (Perkin Elmer, Foster City, CA, USA) using a Hydra 96 (Robbins Scientific). Twenty-microliter PCR reactions were carried out on 2 μl of liquid culture, in a 9600 thermocycler (Perkin Elmer) under standard conditions in the presence of 0.1 mM cresol and 12% sucrose. Amplification products were migrated in 3% Ultra Pure Agarose (Gibco BRL) and visualized by ethidium bromide staining. Fifty- and 100-bp ladders (Gibco BRL) were used as molecular weight standards. BAC and PI libraries purchased from Research Genetics and Genome Systems (St. Louis, MO, USA) were screened as described earlier.

BAC and YAC DNAs were prepared using Qiagen kit (Qiagen, Santa Clarita, CA, USA), or the Peregene kit (Geutra Systems, Research Triangle, NC, USA) respectively.

2.3. Sizing of clone inserts

BAC and PI clone DNA was digested with Not I and Sfi I, as recommended by the manufacturer (Boehringer Mannheim, Indianapolis, IN, USA). Digestion products were subjected to a clamped homogeneous electric field (CHEF) gel electrophoresis, carried out in 1% agarose gel, 0.5 x TBE, at 6 volts/cm for 16 h at 14 °C with a 50- to 90-s ramp, using a CHEF-DRIII system (BioRad, Hercules, CA, USA). Size standards used were low and mid range pulsed-field gel (PFG) markers and lambda PFG marker (New England BioLabs, Beverly, MA, USA). DNA was visualized by SYBER Green I staining (FMC BioProducts, Rockland, ME, USA).

2.4. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) experiments were performed as previously described with the following modifications. Colcemid was added after 72 h of mitogen stimulation, and bromodeoxy uridine (BrdU) was added to cultures at 72 h. On each slide, 1 mg of labeled BAC or YAC DNA was applied. Non-unique and nonspecific DNA hybridizations were blocked by pre-annealing the probes with a ten fold of human Cot-1 DNA. The signals were detected with either Cy5-Avidin (Biological Detection Systems Inc., Pittsburgh, PA, USA) or anti-digoxigenin conjugated to fluorescein isothiocyanate (FITC) (Oncor, Gaithersburg, MD, USA). Slides were counterstained with 4',6-Diamine-2'-phenylindole dihydrochloride (DAPI), 250 ng/ml (Boehringer Mannheim) with antifade solution. For fiber-FISH experiments, one million cells of Epstein-Barr virus (EBV)-transformed lymphoblast lines were washed twice in phosphate-buffered saline (PBS, pH 7.4, Quality Biological, Inc., Gaithersburg, MD, USA). Four drops were placed on each slide. After air drying, the slides were immersed in lysis buffer (2 M NaCl, 25 mM Tris-HCl, 1% Triton X) for 20 min at room temperature. The buffer was then removed and the slides placed in a vertical position for 2 min to allow the chromatin fibers to extend with the flow of the liquid. The slides were then fixed in a 3 : 1 mixture of methanol : acetic acid (v/v) for 15 min and a FISH protocol was performed as described above.

2.5. Analysis of random sequences

Shotgun plasmid library construction from BAC inserts, arraying, and single-pass sequencing of random clones were carried out under commercial contract at Sequenom (Houston, TX, USA). The sequences were analyzed for potential microsatellite repeating elements. Sequences were compared to GenBank database sequences (nr and dbest) using BLAST. Sequences have been submitted to the GSS division of GenBank (Table 3).

3. Results and Discussion

3.1. Contig map

As a first step toward constructing a contig map of the PD critical region, we searched the following databases to identify YAC clones and molecular markers known to map near the critical 4q21-q23 interval; MIT/Whitehead: http://www.genome.wi.mit.edu/cgi-bin/contig/phys_map, Stanford: http://shgc.stanford.edu/Mapping/phys_map/index.html, CEPH/Généthon: http://www.cephb.fr/ceph-genethon-map.html. A set of 95 YAC clones was selected and typed by polymerase chain reaction (PCR) for 136 markers. The markers were then ordered so as to minimize the number of clones with a non-contiguous set of markers. A contig map formed of 55 YAC clones (Fig. 1) was generated, that contained 18 transcripts and 33 anonymous markers, including 23 polymorphic markers.

Of these, a minimum set of 12 YACs formed a contig which covered the entire critical PD region from marker D4S2460 to marker D4S2986 (Fig. 1). YAC addresses were also identified for 34 additional 4q21-q23 markers, including 25 ESTs, which could not unambiguously be placed within this minimum contig (Table 1).

3.2. BAC and PI clones

Our strategy was to develop sequence based polymorphisms near the existing polymorphic markers and known genes. Towards that goal, BAC libraries were screened for 28 sequence-tagged sites (STSs) of the YAC contig (Table 2). A PI library was also screened for marker D4S1647, since no BAC or YAC library clone could be identified with this marker. A total of 33 BAC and one
### Table 2. BAC and PAC clones isolated with chromosome 4q21-q23 markers.

| Mark | Accession | Insert size (Kpb) | Parental clones | GenBank accession |
|------|-----------|------------------|----------------|------------------|
| D4S34 | 710839 | 2051 | RS(2) | AF010830 -> AF010931 |
| D4S25 | 710702 | 2394 | RS(2) | AF010710 -> AF010811 |
| D4S23 | 710616 | 3351 | RS(2) | AF011127 -> AF011221 |
| D4S21 | 710657 | 3352 | RS(2) | AF011127 -> AF011221 |
| D4S20 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S19 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S18 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S17 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S16 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S15 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S14 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S13 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S12 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S11 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S10 | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S9  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S8  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S7  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S6  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S5  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S4  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S3  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S2  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S1  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |
| D4S0  | 710702 | 3551 | RS(2) | AF011127 -> AF011221 |

54F1 is a PAC clone, all the other clones are BACs. Accession & refer to GenBank ID # or GDB ID # (*). RS and GS refer to Research Genomics and Genome Systems, respectively. Plasmid libraries were constructed for clones shown in bold. Insert sizes are given in kilobase pairs; ND: not determined. The number of restriction sites present within the clone insert is indicated.

### 3.3. Fluorescence in situ hybridization

Metaphase and fiber-FISH analysis of YAC, BAC and PAC clones was carried out from marker D4S2460 to marker D4S2986. Metaphase FISH analysis allowed us to orient the map with respect to the centromere and telomere (Fig. 1). YACs were also evaluated by FISH analysis for chimerism; only one of the 12 YAC clones from the minimum contig (Fig. 1) was apparently chimeric. Clone 966A6 hybridized to chromosome 4q22 as well as to 6p22 (data not shown). YAC clones 888B9, and 783H11 which were previously found by PCR to be chimeric with chromosome 5 (Whitehead Institute/MIT Center for Genome Research) appeared to hybridize only with chromosome 4q22 (data not shown).

Furthermore, fiber-FISH experiments were carried out in order to determine the order of clones previously determined by the STS-EST contig-map. BAC clones 293H15 and 360G16 and the MAP kinase and the ADH5 genes outside the D4S2460-D4S2986 interval, on the centromeric and telomeric side, respectively (data not shown).

Finally, by combining estimated distances between BAC clones obtained by fiber-FISH, and sizes of YAC and BAC clones, we evaluated the D4S2460-D4S2986 interval to span about 6 Mb.

#### 3.4. Random sequences

Plasmid libraries were generated for 10 BACs and for 1 PAC from 7 of these 11 libraries, a total of 614 random clones covering a total of about 200 kb were sequenced. Sequences were compared to GenBank databases (nr and ddbst) using BLAST.8,10 Sequences were submitted to GenBank (Table 3) and search results are available at http://www.ncbi.nih.gov/DR/projects.html.

### Table 3. Clones from the PD region on chromosome 4q21-q23.

| Clone | Number of sequences analyzed | GenBank accession numbers |
|-------|-------------------------------|---------------------------|
| 102J7 | 102                          | AF010608 -> AF010709      |
| 225H6 | 102                          | AF010710 -> AF010811      |
| 335P21| 18                           | AF010812 -> AF010829      |
| 355N11| 102                          | AF010830 -> AF010931      |
| 358M14| 94                           | AF010932 -> AF011025      |
| 54F1  | 101                          | AF011026 -> AF011126      |
| 94E7  | 95                           | AF011127 -> AF011221      |
Figure 1. Map of the human chromosome 4q21-q23 between markers D4S2460 and D4S2986. **Top:** Metaphase FISH analysis showing the location of YAC 922g11 on the human chromosome 4. **Bottom:** YAC contig. Only unambiguous results on YAC clones with at least 2 contiguous markers are shown. Markers are designated by their D4S number. YACs are represented by horizontal lines with black or white circles for markers present or absent, respectively. The YAC addresses are also indicated. Polymorphic markers are highlighted in yellow. The location of the alpha-synuclein gene is indicated by marker D4S3159 (in red). Brackets show blocks of markers which were not ordered. *indicates transcripts. The minimum contig of 12 YAC clones is shown above the thin horizontal line.
3.5. Conclusions

We have constructed a high-resolution 6-Mb physical map of the human chromosome 4q21-q23 region between markers D4S2460 and D4S2986. This contig map contains 55 YACs and 51 molecular markers, resulting in an average density of about one marker per 120 kb. Out of 18 transcripts that we mapped within this PD critical region, only one (D4S3159) corresponded to a known gene: alpha-synuclein or non-A4 component of amyloid precursor.11 This gene was found to carry a mutation in four families with PD.12

YAC addresses were determined for 34 additional STSs, and we have re-defined the location of 42 transcripts including the alpha-synuclein gene. Thirty-three BAC and one P1 clone were isolated using 28 different markers. Plasmid libraries were generated from 11 of these clones, and about 200 kb of raw sequence was obtained. This contig map provides physical mapping tools as well as DNA sequence information for gene discovery in the 4q21-q23 region.

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