A 3-Mb Sequence-Ready Contig Map Encompassing the Multiple Disease Gene Cluster on Chromosome 11q13.1-q13.3

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Abstract

Despite the presence of several human disease genes on chromosome 11q13, few of them have been molecularly cloned. Here, we report the construction of a contig map encompassing 11q13.1-q13.3 using bacteriophage P1 (P1), bacterial artificial chromosome (BAC), and P1-derived artificial chromosome (PAC). The contig map comprises 32 P1 clones, 27 BAC clones, 6 PAC clones, and 1 YAC clone and spans a 3-Mb region from D11S480 to D11S913. The map encompasses all the candidate loci of Bardet-Biedle syndrome type I (BBS1) and spinocerebellar ataxia type 5 (SCA5), one-third of the distal region for hereditary paraganglioma 2 (PGL2), and one-third of the central region for insulin-dependent diabetes mellitus 4 (IDDM4). In the process of map construction, 61 new sequence-tagged site (STS) markers were developed from the Not I linking clones and the termini of clone inserts. We have also mapped 30 ESTs on this map. This contig map will facilitate the isolation of polymorphic markers for a more refined analysis of the disease gene region and identification of candidate genes by direct cDNA selection, as well as prediction of gene function from sequence information of these bacterial clones.

Key words: chromosome 11; contig; SCA5; BBS1

1. Introduction

Human chromosome 11q13 is one of the most intensively studied regions of the human genome because it is particularly rich in a number of disease-associated genes. Certain of these genes, such as PYGM for MacArdle disease1 or, more distally, MYOTA (11q13.5) for Usher type 1B syndrome,2 have already been identified and cloned. Recently, a large-scale collaborative effort led to the discovery of MEN1N, a gene responsible for multiple endocrine neoplasia type-1 (MEN1).3 Other genes that have been assigned to subregions on 11q13 include those for Best’s disease,4 atopy,5 osteoporosis-pseudoglioma syndrome,6 insulin-dependent diabetes mellitus 4.7,8 spinocerebellar ataxia type 5,9 hereditary paraganglioma 2,10 and Bardet-Biedl syndrome type 1.11,12 Among these, the loci for Bardet-Biedl syndrome type 1 (BBS1), spinocerebellar ataxia 5 (SCA5), insulin-dependent diabetes mellitus 4 (IDDM4) and hereditary paraganglioma 2 (PGL2) have been shown to overlap at 11q13.1-q13.3.

The development of an accurate physical map and the assembly of contiguous cloned genomic reagents are necessary for the identification of candidate genes using a positional cloning approach. A number of maps of the 11q13 chromosome have been published, including genetic maps13-18 or physical maps.19-26 More recently, high-resolution physical maps of subregions of 11q13 were reported.27-29

We previously constructed a complete Not I restriction map covering the entire long arm of chromosome 11 using linking-clone mapping. This map provides the most accurate ordering and distance estimation to date.30 In the course of developing the map, it was observed that CEPH mega YAC library31 screening of the 11q13 region gave only a few YACs; almost all of the other YACs isolated showed severe deletions or rearrangements, and many were not suitable for further analysis.30 Here, we present a 3-Mb contig map of 11q13.1-13.3 constructed by using the Not I restriction map as a scaffold and Escherichia
coli-based large insert cloning systems (P1, BAC, and PAC). The contig map constructed using E. coli-based large-insert cloning systems should be useful for the generation of genomic sequences and transcription maps of this important region. Since our map covers the whole or at least some parts of the regions implicated in BBS1, SCAl5, IDDM4, and PGL2, it should contribute to the positional cloning of these disease-related genes.

2. Material and Methods

2.1. Libraries

Five libraries were screened to construct the contigs: the CEPH mega YAC library,31 the P1 library, the Keio University BAC library, the BAC library from the Research Genetics, and the PAC library. The P1 library was constructed at Du Pont, Inc.32 and corresponds to 3.5 equivalents of the human genome. The Keio University BAC library was developed by Asakawa et al.33 with a modification of the method by Shizuya et al.,34 and DNA pools corresponding to about 3.5 genome equivalents were used for screening. The PAC library (prepared in pCYPAC2 or pPAC4) which consists of 500,000 clones, corresponds to 20 equivalents of genome (from Rosewell Park Cancer Institute, Joannou et al.35).

2.2. Development of STS primers

P1, BAC and PAC DNAs with yields of 5–15 µg were prepared from 100-ml overnight cultures using an automated plasmid isolation apparatus (PI-100; Kurabo, Japan), followed by PEG precipitation, phenol chloroform extraction, and ethanol precipitation. Each SP6 and T7 end of a P1 clone was sequenced directly using primers, PSup-2 and PTup-2, respectively. The sequences of the primers (5’ to 3’) are as follows: PSup-2, described above; PTup-2, GGGGCTGGCAGATT TAGGTTGACTA and PTup-2, CGGGCGCTATAACGAGACTA. For BAC end-sequencing, universal and reversal primers (5’ to 3’) were used; universal primer, CGACGTTCGTTAAAACGACGGCCAGT and reversal primer, TTTTACACAGGAACAGCTATGAC. The partial sequences of Not I linking clones were obtained by end-sequencing of a subclone using the following primers (5’ to 3’); T7, TAATACGACTCAG TTAGGG and T3, ATTAACCCTCACTAAGGGGA or universal and reversal primers. End fragments from PACs were isolated using a modification of the ligation-mediated PCR as previously described.37 Approximately 0.5 µg of PAC DNA was digested with Acc II, Pvu II, Rsa I, or Sca I and blunt-end ligated to a double-stranded linker in a 25-µl reaction. The ligated mixture was diluted four times, and 2 µl of the dilution was amplified using the linker-specific primer and a pCYPAC2- or pPAC4-specific primer (5’ to 3’) which were as follows: PASup-1, ATCTCTTCCGAATTTGAC TAGTGGTT; PSup-2, described above; PTup-1, TC GAGCTGATTTAGGACTA and PTup-2, described above. The amplified fragments were sequenced to develop STSs and were used as probes for Southern blot analysis. Out of the 61 newly generated STS markers, 45 were designed from the insert end sequences of P1, BAC, and PAC clones and 14 were developed from the sequences of Not I linking clones using the PRIMER program (Stephen E. Lincoln et al., Whitehead Institute, MIT). Primer SPRK-ctd was derived from the sequence of the src-homology 3 domain-containing proline-rich kinase (SPRK) gene.38 Primer GP17 was developed from the sequence of the human muscle glycogen phosphorylase (PYGM) gene.39 Newly generated STSs were tested for localization on chromosome 11q using five somatic hybrid cell lines (J1-ke, J1-44, J1-7, P3-27A, and R229-3A).40

The additional 12 STS markers used for screening were as follows: CN2402A (GDB:6054130), CN3168A (GDB:6054131), cC111-291B (GDB:6054133), cC111-534B/D11S711 (GDB:6054134), LN32-32T (GDB:6054135), CN3071B (GDB:6054136), P22E7S (GDB:6054137), P125F11S (GDB:6054138), Y77E2-NB9B (GDB:6054139), P31G3S (GDB:6054140), CN1002A (GDB:6054141), P31G3T (GDB:6054142).30 Other markers were obtained from the Genome Data Base (GDB), GenBank, and the scientific literature.41

2.3. Screening and clone analysis

An STS-based PCR screening of DNA pools was done to determine positive clones within each library. PCR was performed with a GeneAmp 9600 thermal cycler (Perkin-Elmer) in a reaction volume of 10 µl containing 10 mM Tris-HCl (pH 8.0), 1.5 mM MgCl2, 50 mM KCl, 200 nM each primer, and 1 unit of Taq DNA polymerase (Boehringer). DNA was denatured at 94°C for 3 min and subjected to 35 cycles of amplification where each cycle consisted of denaturation at 94°C for 30 s, annealing at temperatures specific for each primer for 1 min (Table 1), and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min.

Structural analysis of all PCR-positive clones was carried out by PFG electrophoresis (CHEF Mapper system: Bio-Rad) after digestion with Not I, Not I–Sal I, or Eag I (New England Biolabs). The digested DNA was electrophoresed through a 1% agarose gel at 200 V for 10 h with an initial pulse time of 0.3 s and a final pulse time of 6.0 s, and blotted onto Hybond N (Amersham) membrane according to the manufacturer’s recommendations. Southern blots were hybridized with each radioactively labeled PCR product for screening under stringent conditions. STSs and RNA probes derived from SP6 and T7 promoters of isolated P1 clones were used to confirm
Table 1. STSs used for contig construction and for mapping into the contig.

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3. Results

3.1. Library screening and contig construction

Figure 1 shows the region containing the cluster of disease-associated genes investigated in this study. Previously, it has proven difficult to obtain coverage of the approximately 3-Mb region flanked by D11S480 and D11S913 in a CEPH mega YAC library and, thus, the region has been considered to contain areas of uninformative DNA refractory to YAC cloning. Therefore, we began to survey Du Pont’s total human Pl library to generate a contig map using PCR-based screening (Fig. 2). Nineteen P1 clones were initially selected by using 11 STSs developed from Not I linking clones, one STS (GP17) derived from the PYGM gene and two existing STS markers (WI-4003, FAU). The generation of STSs from some of the ends of the P1 clone inserts was hampered due to high GC contents, and the presence of high levels of Alu and of LINE sequences. In those cases, a BAC library constructed by Asakawa et al. which possesses larger inserts than the P1 library (110 kb versus 75-95 kb) was employed as an additional library to isolate new clones, assuming that clones with different terminal sequences might allow to develop new STSs. This actually allowed the development of 14 new STSs from the termini of 10 BAC clones isolated using 6 STSs derived from Not I linking clones and 3 STS markers (AFMa085yc9/D11S4205, WI-9890 and COX8). In total, 33 P1 and BAC clones were obtained in the initial screening. Successive walking from the ends of these P1 and BAC clone inserts allowed us to build six contigs. These contigs were located between D11S4205 and CN3168 (contig 1), among PLCB3 (contig 2), between the overlap between clones.
Figure 1. The Not I restriction map and the loci of PGL2, IDDM4, BBS1, and SCA5 on the 11q13 region. The map represents a part of Not I restriction map of chromosome 11q (Hosoda et al. 1997). The vertical bold lines on the right of the map indicate the loci of respective disease genes (see text). The arrow shows the region where we constructed the contig map.
Figure 2. The 3-Mb contig map of the 11q13.1-q13.3 region. The top horizontal line represents the \textit{Not} I restriction map. Vertical bars on the restriction map show \textit{Not} I sites and dotted bars show a partially restricted site on the genome. Numerical values adjacent to the map represent the physical distance in kb between \textit{Not} I sites. \textit{Not} I linking clones are indicated above the line and other mapped markers are marked below the line. The locations of 66 clones aligned on the basis of their loci content are indicated by thin lines. Walking paths are shown by yellow lines. Overlaps confirmed by the use of clone-end sequences are also shown by dotted thin lines. The sizes of individual PIs, BACs, PACs and YACs are illustrated according to scale. The loci of 30 ESTs placed on the contig map are illustrated at the bottom. The bold arrow above the map represents a recombination hot spot (see text). One gap is labeled with a red arrow. Others are shown on the left-side down panel.
3.2. Analysis of the isolated clones and contig

The STSs developed from the end sequences of P113H6 and P22CS did not map on 11q13, indicating that these 1PI clones were chimeras (Fig. 2). P113H6 was isolated by the cCI11-367A marker which is proximal to PYGM. YAC clones DA1908F2 (350 kb) and 199A7 (200 kb) were isolated from two YAC libraries, which were isolated by Toda et al. 44 Chimerism was also observed for the YAC clone DA1908F2. The map contained one gap in the region around D11S711 where P22CS was found to map. These observations suggest that DNA in the region from D11S480 to D11S460, and especially around PYGM and D11S711, might contain sequences that are hard to clone even with the low-copy-number vectors. Not I restriction sites were identified for all the clones along with the developed contigs. The Not I restriction patterns of all isolated clones coincided well with the previously constructed Not I restriction map, indicating that the genomic structure is faithfully retained within individual clones. Our map represents a twofold average coverage of the region comprised by the contigs and the depth of coverage varied from no clone in one gap to 4 clones in some regions (CN3168 and CN3179).

3.3. Mapping of ESTs

Seventy ESTs have been mapped to the region between D11S1357 and D11S913 (see Fig. 1) by radiation hybrid mapping. 30 Thirty of them could be integrated within the present contig map (Fig. 2) and 40 ESTs were localized on a YAC contig spanning the region from 11q11 to the centromeric end of q13.1 (data not shown), which was constructed using genetically anchored YACs from the CEPH mega YAC library with the help of the Not I restriction map (Hosoda et al., unpublished data). As shown in Fig. 2, this contig map established the unequivocal order of ESTs previously mapped to the interval between D11S480 and D11S460. This information should provide a valuable tool for cDNA screening.

4. Discussion

Chromosome 11q13 is a region characterized by the presence of a particular high density of disease genes previously mapped by genetic linkage analysis (Fig. 1). 30 However, with the exception of MEN1, the molecular cloning of these genes remains to be accomplished, despite the resources that have been made available by the rapid progress of the human genome project. We recently reported the construction of a complete Not I restriction map spanning the entire long arm of chromosome 11 and showed that the Not I sites are highly clustered in the q13 region with an average span of 160 kb between them. 30 We have also found that CEPH mega YAC clones, which were isolated from eight sites within
the 6-Mb region lying between PGA5 and D11S1917 (see Fig. 1), possessed severe deletion or rearrangement, demonstrating the unsuitability of YAC-based mapping for this region. Based on these results, we attempted the construction of a contig map spanning the 3 Mb region between D11S480 and D11S913 in q13 using E. coli-based large-insert cloning systems and the assembled Not I linking clones as scaffolds. Our success in the construction of the present contig map for this region underlines the importance of physical maps which serve as a guide in this type of analysis. In addition, this study supports the use of large insert bacterial clones as an alternative to YACs for the contig construction of large genomic intervals.

The gap in the contig map (illusrated by an arrow in Fig. 2) is located in the 200-kb region between Not I sites CN3191 and D11S711, and has a maximal size of 70 kb. The gap size was estimated from the size of the Not I fragments of the P1 clones P110A10 and P77F10 which protrude from Not I sites CN3191 and D11S711, respectively. As b144L4 is located at the distal end of the centromeric contig and overlaps with P77F10, determination of its insert size and the extent of overlap should allow us to narrow the gap. In spite of extensive screening using various E. coli vector-based libraries including a P1 library, Du Pont's total human library (3.5 genome equivalents), two BAC libraries (Keio University and Research Genetics total human libraries containing 3.5 and 4 genome equivalents, respectively) and a PAC library (20 genome equivalents), our efforts to fill this gap were unsuccessful. This failure might be attributable to the existence of sequences with high GC contents, high gene density, or to the presence of unusual sequences affecting the growth of host cells.

Two groups have recently reported high-resolution maps that overlaps ours. Wood et al. constructed a 1.5-Mb physical map from PYGM to D11S460. Comparison of our map with their physical map reveals a difference in the order of some of the markers. Our map indicates the order, cen-D11S457-D11S427-PYGM-pter (see Fig. 1), while Wood et al. reported the order, cen-PYGM-D11S427-D11S457-pter. This difference could be due to the fact that these markers are clustered within a very restricted region, and that ordering of markers within such small regions is difficult to resolve by FISH analysis or mapping with somatic cell hybrids. Courseaux et al. reported a physical map of the region between D11S471 and D11S460 (see Fig. 1). Our Not I restriction map is in good agreement with their mapping information. However, there exist some discrepancies with respect to the estimation of the distances. For example, intermarker distances between COX8 and D11S457 and between D11S427 and PYGM are much longer in their physical map than in ours (600 kb versus 340 kb and 400 kb versus 80 kb, respectively). Conversely, the intermarker distance between PYGM and FAU is shorter in their map than in ours (180 kb versus 400 kb). This difference may be due to the scarcity of markers in their experiments.

By comparing our Not I restriction map with an integrated YAC-RH-genetic map constructed by the MIT/Whitehead genome center, we found that previously mapped STS markers are very scarce in the q13 region. As this region is expected to contain a particularly high density of genes, we predicted that the introduction of EST markers as landmarks would make up for the deficiency in marker density. The recent version of the Whitehead map allocates a number of EST markers to the q13 region, although their precise ordering remains to be defined, probably because of the limited resolution of RH mapping. The EST mapping shown in Fig. 2 demonstrates that 30 out of the 70 EST markers located between D11S1357 and D11S913 in their map are within the 3 Mb region with an average spacing of 120 kb and without strong bias in their distributions. This information suggests that if YAC clones are unavailable, contig construction could be achieved by combining the extensive use of EST markers as scaffolds with the construction of physical maps such as the Not I restriction map.

We have revealed the presence of seven recombination hot spots on the long arm of chromosome 11 by comparing Not I restriction map with the Genetic map. One of them is located between D11S4205 and D11S1883; this site is found within the contig map presented in this report and is covered by the clones KB306G8, KB191G5, KB292F6, and KB387H11. Sequence analysis might provide some insight into why the region displays such a high recombination frequency compared to the surrounding region.

This study provides reagents and mapping information that can be used to identify candidate genes assigned to 11q13.1-q13.3. In addition, our map provides a set of minimal overlapping bacterial clones that can be used as templates for determining the nucleotide sequence of this region of chromosome 11.

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