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SHORT REPORT

Saturating density of STSs (1/6 kb) in a 1.1 Mb region on 3q28-q29: a valuable resource for cloning of disease genes

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We have fine mapped 29 ESTs of Genemap'99 to YACs and radiation hybrids covering 8 cM of the chromosomal region of 3q28-q29. Focusing on the genetic interval of ~1 Mb between markers *D3S3669* and *D3S3562* we established a sequence-ready PAC contig which covers the *OPA1* locus containing the gene causing autosomal dominant optic atrophy (ADOA; OMIM*165500). The fidelity of the contig was increased by the generation of 181 PAC end sequences, 84 of which resulted in PCR-able STSs. Sequence content evaluation of the PAC ends by BLAST analysis identified two novel ESTs localising to the *OPA1* crucial interval. *European Journal of Human Genetics* (2001) **9,** 307–310.

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Introduction

With the raw sequence data of the human genome accumulating rapidly in public databases, the direct screening of candidate genes is becoming the most straightforward approach in identifying disease genes. The exact location of ESTs and genes within a particular linkage interval for a disease is, therefore, a very valuable tool in order to reduce the list of putative candidates for mutation screening. Genemap'99[1] provides rough mapping information for ESTs to fairly large genetic intervals each usually spanning several cM. In order to complete a gene map of 3q28-q29 we covered the region with YACs and finemapped ESTs and genes listed in Genemap'99. Subsequently, we constructed a high resolution PAC contig encompassing the *OPA1* locus.[2]

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Methods

Clone libraries, RH cell lines and primers

The CEPH Mega YAC library,[3] the ICI/ICRF YAC library[4,5] and the RPCI PAC library[6] are available from the RZPD (resource center/primary database in Berlin, Germany at http://www.rzpd.de). The radiation hybrid cell lines #15 and #75 were kindly donated by Dr Mariano Rocchi (Bari, Italy).[7] DNA of the cell lines was isolated using the Nucleon Kit (Scotlab). STS and EST primer sequences were obtained from 'The Genome Database' (GDB) at http://www.gdb.org/ and 'Genemap'99' at http://www.ncbi.nlm.nih.gov/genemap/.

EST analysis

Twenty-five-microlitre PCR reactions using 5 μl of a 1 : 1000 dilution (10^6 pfu/ml) of a foetal retina cDNA library (Stratagene), a foveal retina cDNA library[8] and a cerebellum cDNA library (Clontech) as templates were run with EST specific primers.

Construction of PAC contig

STSs and inter*Alu* PCR products were labelled using the 'Prime it' kit (Stratagene). Excessive radioactive nucleotides were eliminated by column-centrifugation (200H, Pharma-

cia). STS hybridisation was carried out over night at 42°C in formamide buffer. Inter*Alu* PCRs were performed using A3,[9] pDJ34,[10] and 451[11] primers and products were hybridised overnight at 65°C in CHURCH buffer.[12] After washing, filters were exposed for 2–4 h at −80°C using enhancement screens.

Generation of PAC ends

PAC DNA was isolated by standard alkaline lysis followed by phenol/chloroform extraction. PAC-ends were generated by DOP-vector PCR[13] and subsequently sequenced using Dye-terminator chemistry (ABI). Sequencing products were column-purified (Princeton Separation) and analysed on an ABI373 (Perkin Elmer, Amersham).

Results

YAC-EST mapping

35 contiguous YAC clones[14] covering the chromosomal area of 3q28-q29 were identified by screening pools of genomic YAC libraries with STSs depicted in Figure 1. 53 ESTs taken from the genetic interval flanked by the markers *D3S1601* and *D3S1265* spanning 8 cM according to Genemap'99 were tested on 11 selected YAC clones. Two radiation hybrid (RH) cell lines were used as positive controls to compensate for possible deletions in the YAC inserts. Twenty-nine ESTs were localised both on our YAC clones and radiation hybrids (Figure 1). SGC33690, SGC36945, WI-11613, SGC33919, and WI-16815 showed positive signals only on the RH DNA. Since all ESTs were assigned to the genetic interval between markers *D3S3642* and *D3S1265* on Genemap'99, their most likely localisation is between stSG4566 and *D3S1265*. In contrast to Genemap'99, 5 ESTs mapped outside the genetic interval defined by *D3S1601* and *D3S1265*. This can be explained by the fact that a pair of flanking markers defining the boundaries of a given genetic interval in Genmap'99 are identified as the most centromeric or telomeric markers on a particular RH fragment. Hence, ESTs can map further up- or down-stream towards the actual physical breakpoint of the RH fragment. We identified six ESTs to localise within the *OPA1* critical interval. *HRY* (MIM*139605), *DLG1* (MIM*601014), SDH Fp subunit pseudogene (represented by stSG4566), 83 kDal carboxypeptidase *CPN2* (represented by SHGC-12402), and *CLC2* (OMIM*600570; data not shown) were excluded from the *OPA1* candidate region.

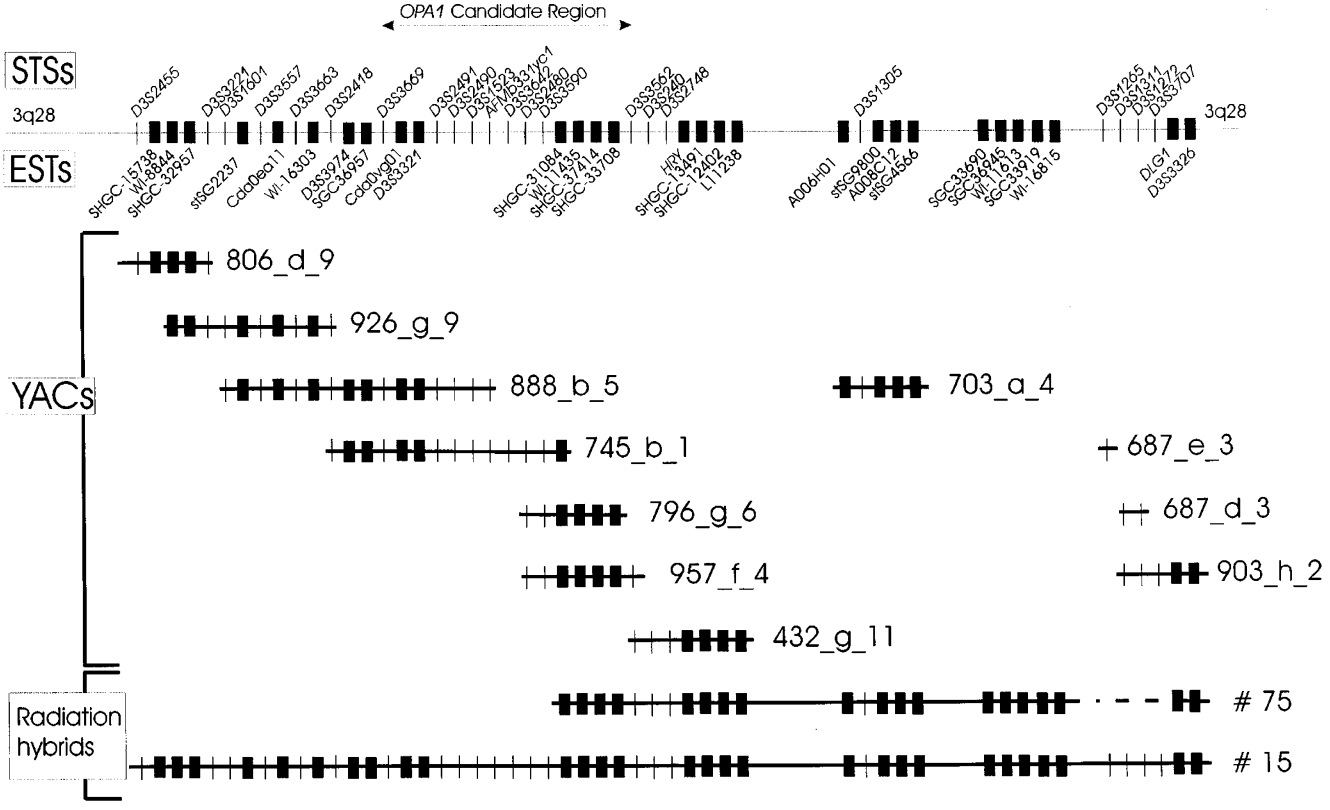

Figure 1 EST-YAC map of chromosome 3q28-q29. Twenty-two STSs (short vertical bars) and 29 ESTs (black boxes) were physically mapped to 11 selected YAC clones (horizontal bars) and two radiation hybrid cell lines (vertical bars; #15 and #75). The dashed line marks a deletion in RH #75.

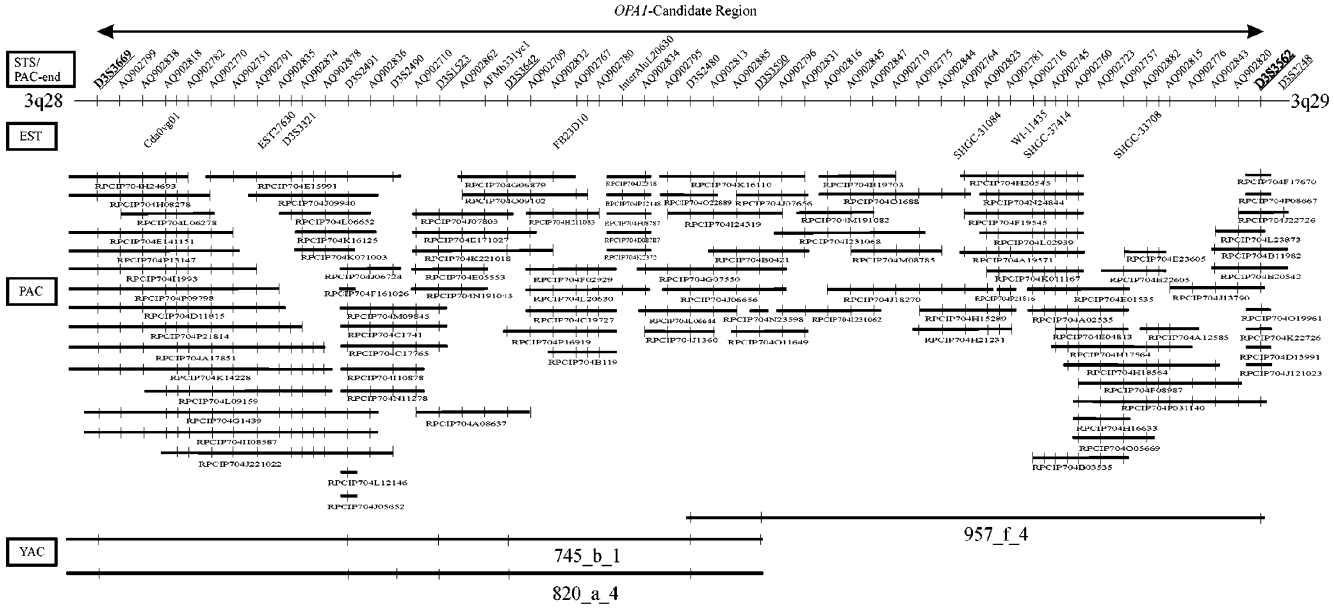

Figure 2 PAC/YAC contig covering the *OPA1* region. Assuming an even distribution, 60 STSs are listed across the top of the figure consisting of six polymorphic STSs (underlined), 42 PAC ends (Genbank Accn), eight 4 single-copy STSs. Inter*Alu*L20630 probe was mapped by hybridisation of the PAC filters. The *OPA1* candidate region between flanking markers *D3S3669* and *D3S3562* is marked. Ninety-two PACs and three YACs are shown represented by horizontal bars; positive PCR signals for the STSs are marked as short vertical bars.

On PCR amplification using three different cDNA libraries, EST Cda0vg01, SGC36957 and WI-11435 were detected exclusively in the foetal retina library, whereas SHGC-31084 was transcribed only in the fovea. SHGC-37414 was present in all tested libraries. Genomic contamination was excluded based on a number of genetic markers tested on the cDNA libraries. Therefore, the ESTs characterised are most likely to represent functional gene transcripts.

Construction of a PAC contig covering the OPA1 candidate region

Screening of the RPCI PAC library allowed us to construct a PAC contig covering the entire *OPA1* candidate region (Figure 2). Radioactively labelled, pooled STSs (Figure 1) were used as 'anchor'-probes in the initial round of screening. Forty-three PAC clones were identified and confirmed positive in a PCR rescreening applying the STS primers. Pooled inter-*Alu* PCR products of the YACs 957_f_4, 745_b_1 and 820_a_4 identified additional 72 PAC clones. Iterative rounds of hybridisation applying PAC ends as probes helped in closing the remaining gaps by chromosome walking. We used Inter*Alu*-fingerprint PCR to independently validate the order of PACs. From the 115 PAC clones, we generated 182 PAC end-sequences using the DOP-vector PCR method. Those were scanned for repetitive DNA elements, which were discovered in more than 60% of cases. However, 84 novel PCR-able STSs within the *OPA1* region were established and localised on the physical map. Taking into consideration all 178 (182 −4 identical matches) PAC ends, eight genetic markers and eight ESTs mapping within the 1.1 Mb interval, the STS density on average was raised to 1 STS every 6 kb throughout the contig.

Computational analysis of PAC ends

BLASTN analysis of PAC ends revealed sequence matches to known genes and markers. J221022-T7 (AQ902818) and K011167-SP6 (AQ902822) confirmed the location of two Unigene clusters, Cda0vg01 (Hs.12251) and SHGC-37414 (Hs.147946), with 97% (104 bp) and 96% (105 bp) identity, respectively. SHGC-37414 represents a fragment of the human mRNA KIAA0567. J07803-T7 (AQ902808) overlapped with microsatellite *D3S1523* over 376 bp with 95% identity and O09102-T7 (AZ536558) is 94% identical to the human foetal brain cDNA clone FB23D10 (T03086), which was not previously known to map to this region. The PAC ends P09798-T7 (AQ902871) and L06652-SP6 (AQ902835) scored an identity of 98% over a length of 320 bp and of 92% over 167 bp, respectively, with EST27630 (AA324733).

Discussion

We report the establishment of an EST map for 3q28-q29 based on YACs highlighting six candidate ESTs for *OPA1*. We believe that the construction of the sequence-ready PAC contig with a fivefold coverage of the *OPA1* candidate region represents an excellent resource for gene identification

approaches including strategies such as large-scale sequencing, as well as EST-mapping and elucidation of genomic structures of candidate genes by bioinformatical tools. Based on the physical mapping data presented here, KIAA0567 was recently identified as the *OPA1* gene.[15]

Acknowledgments

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