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Identification of positional candidates for neurological disorders on chromosome 13q14→q22

H.G. Nothwang, J. Wirth, B. Brandl, T. Haaf, K.B. Nielsen, N. Tommerup, and H.H. Ropers

Abstract. In the course of a research project aimed at the molecular characterization of balanced chromosome rearrangements associated with mental retardation (MR), several YACs spanning MR-associated chromosomal rearrangements in the 13q14→q22 region were identified. To facilitate the search for relevant candidate genes, we have analyzed a total of 102 EST clones from this region. Sequence comparisons revealed that these 102 clones represent up to 72 distinct transcripts. When no physical mapping data were available, a minimal YAC contig was screened for each unique transcript by the polymerase chain reaction (PCR) or hybridization. Fifty-eight independent ESTs could be localized to YAC clones between the markers D13S1248 and D13S1201. Several ESTs are located on YAC clones detecting chromosomal rearrangements in MR patients. One EST was mapped within the critical region for Rieger syndrome type 2, and three transcripts were identified in the relevant candidate genes, including the cadherin-related tumor suppressor gene from Drosophila, the yeast mitotic control protein DIS3, and the human α-2-macroglobulin receptor associated protein.

Mental retardation (MR) is a common and distressing disorder that affects about 1–2% of the human population. Despite recent advances in understanding the molecular basis of many inherited disorders, progress in unraveling the molecular genetics of mental retardation has been slow, partially because of its etiological heterogeneity. Cytogenetically detectable chromosomal anomalies are found in up to 28% of cases with MR (Curry et al., 1997). The molecular genetic analysis of these chromosomal rearrangements will provide an important key for identifying genes that play a role in brain development and function. Recently, an international network of cytogenetic laboratories, the Mendelian Cytogenetics Network (MNC), was established in order to collect and analyze disease-associated balanced chromosome rearrangements (DBCRs) (Tommerup, 1993). A survey of the MNC database revealed 12 breakpoints in chromosome 13q14→q22 in MR patients (Tommerup, 1993). A more detailed molecular cytogenetic analysis of DBCRs from this region has led to the identification of YAC probes encompassing the regions of rearrangement (van der Maarel et al., 1996; Wirth et al., submitted for publication).

In addition, 13q14→q22 harbors two other known loci associated with neurological disorders: the variant form of late infantile neuronal ceroid lipofuscinosis (CNL5) and a second locus for Rieger syndrome (Klockars et al., 1996; Phillips et al., 1996). Neuronal ceroid lipofuscinosis is a progressive encephalopathy characterized by psychomotor deterioration, visual failure, seizures and ceroid- and lipofuscin-like cytosomes in both neural and extraneural tissues. Rieger syndrome is an autosomal dominant disorder with an abnormal development of the anterior segment of the eye, which is derived from the...
Table 1. Assignment of 57 ESTs to a minimal YAC contig between markers D13S1248 and D13S1201 on chromosome 13q14→q22

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All YAC clones were taken from the Whitehead contigs WC13.1, WC13.2 and WC13.3; (a) = mapping data taken from the Whitehead physical map.
embryonic neural crest. Furthermore, microdeletions in 13q14 were reported in several patients with MR, indicating a further locus for MR in this region (Cowell et al., 1989). Finally, a new tumor suppressor locus (DBM) was assigned to 13q14→q22 (Brown et al., 1993).

Because of the frequent involvement of this region in human genetic disease, we set out to construct an EST map in the marker interval D13S1248 to D13S1201 on chromosome 13q14→q22 and placed 58 transcripts onto YAC clones.

Materials and methods

YAC contigs

YAC clones and information on positive STS hits were taken from Whitehead YAC contigs (http://www-genome.wi.mit.edu) (Hudson et al., 1995). YAC DNA and dot blots were prepared using standard methods (Sambrook et al., 1989).

Sequence analysis and EST screening

STS sequences were taken from the Genome Data Base (GDB) (http://gdbwww.gdb.org) and analyzed by BlastN (http://www.ncbi.nlm.nih.gov) for similarity with expressed sequences. To group ESTs, the program UniGene was used (http://www.ncbi.nlm.nih.gov).

ESTs were localized by the polymerase chain reaction (PCR), using the respective primer pairs from GDB. In this work, 10 µl of a 50-µl PCR reaction was analyzed in a 2% agarose gel containing ethidium bromide. When hybridization was performed, the respective EST clone was amplified by PCR using the universal primers T3, T7, and M13 forward and reverse, depending on the vector. Aliquots (5–10 ng) of the purified PCR product was radioactively labeled using Klenow enzyme and hybridized to YAC dot blots containing ~ 200 ng of total YAC DNA from each clone. Hybridization of the probes in the presence of excess human competitor DNA was done essentially as described elsewhere (van der Maarel et al., 1996). Autoradiographs were taken for 12 h at −70°C using two intensifying screens.

Fluorescence in situ hybridization (FISH)

CEPH Mega YAC clones were isolated by pulsed field gel electrophoresis (PFGE) and amplified by degenerate oligonucleotide-primed PCR (DOP-PCR) according to standard procedures (Telenius et al., 1992). Chromosome in situ hybridization of biotinylated and digoxigenated YAC probes to patient chromosomes was carried out as described previously (Kingsley et al., 1997). Images were taken with a Zeiss epifluorescence microscope, equipped with a thermoelectronically cooled charge-coupled device (CCD) camera (Photometrics), which was controlled by an Apple Macintosh computer. Oncor imaging software was used to capture grayscale images and to superimpose the images into a color image.

Clinical reports

Detailed clinical reports of the DBCRs will be given elsewhere (Wirth et al., ms. submitted for publication). Briefly, female patient K92-4290B has a t(X;4)(4;13)inv(2) and exhibits mental retardation and epilepsy. Female patient K96-26671B has a t(3;13)(q21,q22.2) and was diagnosed with moderate mental retardation, hypotonia, and strabismus. Female patient PMI
has a t(X;13)(q13.3;q13.2) and suffers from mental retardation, scoliosis, and spotty hypopigmentation of the skin. Finally, male patient 377-771 has a cytogenetically visible interstitial deletion on 13q, viz., del(13)(q13.3q13.21), associated with mild epicanthus and facial anomalies, including a broad root of the nose, a large forehead, and synophrys. Psychological testing at age 12 mo showed the mental development of an 8-mo-old infant (Tranchjang et al., 1988).

Results and discussion

Numerous YAC clones from the 13q14→q22 interval were identified by screening the Whitehead Institute data base (Hudson et al., 1995). Thirty-eight YAC clones were selected that encompass the 13q14→q22 region between markers D13S1248 and D13S1201, except for five gaps of 1–2 cm each. These gaps are located between YAC clones 875 A and 846 A11, 917 E11 and 882B4, 801 B11 and 806 F6, 947 B9 and 799 F3, and 799 F3 and 842 G6. Together, these contigs cover a region of 28 cm.

From the Human Gene Map, 87 ESTs were selected that had been mapped within or close to this interval (http://www.ncbi.nlm.nih.gov/SCIENCE96/) (Schuler et al., 1996). Another eight EST sequences have been described previously by Still et al. (1996) and in a recent chromosome 13 workshop report (Warburton et al., 1996). All nonpolymorphic markers in this region were analyzed by BlastN for similarity with expressed sequences. Seven STSs generated by the Whitehead Institute were found to be highly similar or identical to EST sequences with \( P \) values >1.2e-35: D13S1447, D13S1158, mapping of these clones to the YAC contig revealed that all ESTs are localized proximal to the reported interval.

In a first computer-based screen, BlastN and UniGene analyses were performed with all EST sequences in order to detect redundancy (Schuler et al., 1996). This analysis resulted in the identification of 72 independent sequences, representing a total of 102 ESTs. This number, however, is likely to be an overestimate and will decrease upon further sequence information, as indicated by our own analysis. In the course of this project, new sequence information led to the merging of two previously independent transcripts to one sequence. Furthermore, several ESTs are localized in clusters and thus may be derived from the same gene (see below).

Eleven ESTs had already been localized to YAC contigs by the Whitehead Institute, each of them mapping to several overlapping YAC clones (Table 1). For all other apparently unique genes, single primer pairs were employed to screen the YAC contig by PCR or else EST clones were amplified and used as hybridization probes. To compare both methods, seven ESTs were mapped both by PCR and hybridization, and identical results were obtained. Thus, a total of 84 ESTs, representing 58 so far independent clones, could be localized to YAC clones (Table 1).

Most EST clones were mapped to one or two YAC clones. ESTs D13S831E, WI-17550, and D13S668E were positive with three overlapping YAC clones. A large cluster of ESTs was mapped to YAC clone 775 C8, which contains 13 EST clones (Table 1). Although this YAC clone is more than 1 Mb in length, it is plausible to assume that some ESTs from this cluster are derived from the same gene. For example, for the human serum albumin gene, 455 ESTs were reported (Wolfsberg and Landsman, 1997). Overall, our physical mapping data confirm the reported intervals to which the EST clones had been mapped previously, but there were also marked differences. For example, EST clones WI-12824, SHGC-3226, SGC31580, and WI-11923 are to code for the human \( \alpha \)-2-macroglobulin receptor-associated protein precursor (\( \alpha \)-2-macroglobulin receptor-associated protein precursor) (Schizosaccharomyces pombe). 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Fig. 1. FISH of YAC clones to metaphase spreads of the patient 377-77H with a deletion on 13q13.1→q21.1. YAC 951 A3 (arrowhead) show signals on both the deleted chromosome (arrowhead) and its normal homolog. YAC 818 B12 (long arrow) hybridized only to the normal chromosome 13, indicating a deletion on the derivative 13.

including 843 E9, 800 D6, 818 B12, 984 G6, 800 D6, 911 F6, 937 C7, 955 G7, 921 F11, and 922 A8, produced no hybridization signal on the deleted chromosome (Fig. 1). This patient is therefore most likely hemizygous for 31 ESTs located between YAC clones 853 G8 and 925 E11 (Table 1). Furthermore, this molecular cytogenetically characterized deletion, together with the phenotype, might help to delineate other disease loci in this region. Three other ESTs (D13S813E, W1-17550, and D13S668E) were mapped to YAC clone 852 G2. This YAC contains the locus for the variant form of late infantile neuronal ceroid lipofuscinosis (CNL5) (Klockars et al., 1996). Interestingly, it also spans the chromosome 13 breakpoint in a mentally retarded female with a balanced X;13 translocation (van der Maarel et al., 1996). An X-chromosomal gene, DXS6673E, was isolated that is disrupted by the translocation in the 5'-untranslated region. However, expression studies revealed that DXS6673E is still expressed from the derivative chromosome 13 (van der Maarel et al., 1996). This indicates that another important gene is disrupted on the der(13) or that a promoter swapping, as reported for pleiomorphic adenoma of the salivary glands, leads to a different expression of DXS6673E (Kas et al., 1997).

Several ESTs are portions of strong positional candidate genes for several genetic disorders that have been assigned to this region by linkage analyses. ESTs D13S1158, D13S181E, and D13S1161 are located on YAC clone 846 A11 containing marker D13S291. This polymorphic marker was shown to be most tightly linked to nocturnal enuresis type 1 (ENUR1), with a lod score of Z = 3.55 at θ = 0.07 in a genome-wide exclusion analysis (Eiberg et al., 1992). One EST, D13S1447, was identified within the Rieger syndrome type 2 region between markers D13S1253 and D13S1297 (Phillips et al., 1996). Finally, ESTs assigned to YAC clones 830 C8 and 851 F1 are potential candidates for the new tumor suppressor gene DBM, since they map close to but mainly telomeric of the retinoblastoma 1 (RBI) gene. RBI was shown to reside in the vicinity but at least 530 kb centromeric of the DBM locus, whose inactivation contributes to the initiation or progression of low-grade B-cell malignancy (Brown et al., 1993). ESTs near the retinoblastoma locus on YAC clone 830 C8 are potential candidate genes for MR sometimes associated with retinoblastoma (Cowell et al., 1989).

In summary, the approach described in this study represents a fast and efficient strategy for screening a chromosomal region of interest for transcripts. The mapping of most ESTs to a single YAC might lead to wrong assignments due to the reported chimerism of YAC clones. However, this is highly unlikely, since all ESTs examined here have already been independently mapped by other techniques, including radiation hybrid or somatic cell hybrid analysis, to chromosome 13q. The precise localization of 84 ESTs to YAC clones on chromosome 13q14→q22 led to the identification of positional candidate genes for several disorders, including MR. Fine mapping to PAC and cosmid clones and mutation analysis in affected patients will clarify their involvement in the respective disorder. Furthermore, all other ESTs presented here will be of high value for further positional cloning projects in this region. The molecular cytogenetic characterization of microdeletions may aid in integrating further chromosome maps and clinical disease entities.

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References


