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The human lanosterol synthase gene maps to chromosome 21q22.3

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Abstract In order to contribute to the development of the transcriptional map of human chromosome 21 (HC21) we have used exon trapping to identify portions of HC21 genes. Using pools of random HC21-specific cosmids from the LL21NC02-Q library and cosmids from 21q22.3 we have identified five different coding regions with strong homology to the lanosterol synthase genes of rat and yeast. This enzyme catalyzes the cyclization of squalene-2,3-epoxide to lanosterol, which is the parental compound of all steroids in mammals. Using somatic cell hybrids and HC21 yeast artificial chromosomes (YACs) and cosmids, we mapped the human lanosterol synthase gene to 21q22.3 between markers D21S25 and 21qter. Cosmid Q7G8 from the LL21NC02-Q library and YAC 145D8 from the CEPH HC21 contig contain this human gene. We cloned a portion of the human lanosterol synthase cDNA (almost 85% of the coding region) from a brain cDNA library and determined its nucleotide sequence. The predicted human protein shows 83% identity to its rat and 40% to its yeast homolog. No obvious candidate human disease exists for lanosterol synthase deficiency and the role (if any) of triplication of this gene in the various phenotypes of trisomy 21 is unknown.

Introduction

Chromosome 21 (HC21) is the smallest human chromosome, its long arm contains about 1% of the human genome, and it is predicted to harbor between 600 and 1000 genes (Antonarakis 1993). The cloning and characterization of these genes is an essential step in the understanding of the pathophysiology of Down syndrome and the molecular etiology of monogenic disorders that map to this chromosome. Following the development of the physical and genetic maps of the chromosome (Chumakov et al. 1992; McInnis et al. 1993; Nizetic et al. 1994), a considerable international effort is now being made to create a transcriptional (genic) map of HC21 (Cheng et al. 1994; Xu et al. 1995; Peterson et al. 1994; Lucente et al. 1995; Chen et al. 1995). We have used exon trapping (Buckler et al. 1991; Church et al. 1994) to identify portions of HC21 genes; we report here the cloning of a partial cDNA sequence, from a cDNA library of infant brain of the human homolog of the (S)-2,3-oxidosqualene lanosterol synthase genes of rat and yeast (Corey et al. 1994; Roessner et al. 1993; Kusano et al. 1995) and its mapping to the most telomeric band of chromosome 21q22.3.

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Materials and methods

Exon trapping

Genomic DNA from the HC21-specific cosmid library LL21NCO2-Q (kindly provided by Dr. P. deJong; Soeda et al. 1995) was used in exon-trapping experiments. Exon trapping was carried out as previously described (Buckler et al. 1991; Church et al. 1994, and Gibco/BRL manual 18449–017) using two different input DNAs. In one experiment, pools of randomly picked HC21-specific cosmids from the LL21NCO2-Q library were used; and in another, specific cosmids from the terminal portion of HC21q between markers CBS and ITGB2 were used. *Pst*I-digested cosmid DNA was subcloned into vector pSPL3 and plasmid DNA was transfected into Cos7 mammalian cells using lipofectACE. Reverse transcribed polymerase chain reaction (RT-PCR) products from total Cos7-cell RNA were subcloned in the pAMP10 vector by UDG (Uracil DNA glycosylase) cloning (Gibco/BRL manual 18449–017) and sequenced by the dideoxynicleotide terminator fluorescent method using *Taq* polymerase on the ABI373A automated sequencer. Nucleic acid and amino acid homologies of the resulting sequences were obtained through BLASTX and BLASTN (Altschul et al. 1990) searches of nonredundant databases.

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Rat              186  GAVGIPSWGKFWLAVLNVYSWEGINTLFPEM  216
                      |||  |||||||||||||||  |||+|||||||
HMC14G01              GAVAIPSWGKFWLAVLNVYGWEGLNTLFPEM
                      ||+  |  |||  ||+  ||  |  |||+|   ||
S. cerevisiae    186  GAIGSPHWGKIWLSALNLYKWEGVNPAPPE  215

Rat              263  ELYVEDYASIDWPAQKNNVCPDDMYTPHSWLLHVVY  298
                      ||||||+|||||  ||+|||  ||++||||||||  |||
HMC14B03              ELYVEDFASIDWLAQRNNVAPDELYTPHSWLLRVVY
                      |+| + |  |++   || |   +|| |||  | +
S. cerevisiae    263  EIYTKPFDKINFSKNRNTVCGVDLYYPHSTTLNI  296
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Fig. 1 Database search results using BLASTX. Homology of HMC14g01 (Genbank X88398), and HMC14b03 (Genbank X88389) "trapped" sequences with the rat (Genbank D45252) and the *Saccharomyces cerevisiae* (Genbank P38604) lanosterol synthase polypeptide. Identical amino acids are shown by (I) and conservative changes are shown by (+)

Genomic mapping of the human lanosterol synthase gene

The PCR amplification product of a 102-bp trapped sequence, HMC14g01, that encodes the region homologous to nucleotides (nt) 623–714 of the rat lanosterol synthase gene (Genbank D45252) (Fig. 1) was used to map the human lanosterol synthase gene to HC21. Amplification was carried out using primers HMC14g01-F (5´-TGCTGTGGCCATCCCCTC-3´) and HMC14g01-R (5´-ACATCTCTGGGAACAGGGTA-3´) in a 30-cycle PCR in which template DNA was denatured for 5 min at 94°C followed by a "touchdown" program; 10 cycles: 94°C, 15 s, 60°C, 10 s (–1°C per cycle), 72°C, 15 s, followed by 20 similar cycles at an annealing temperature of 50°C. The template DNA for this PCR amplification included: yeast artificial chromosomes (YACs) 145D8, 94E4, 320B4, 200A9, 67D8 and 17C9 from the HC21 contig of Chumakov et al. (1992); 28 cosmids from the LL21NCO2-Q library that map between markers CBS to ITGB2; genomic DNA from a panel of rodent-human somatic cell hybrids with defined segments of HC21 (kindly provided by Dr. Patterson, Patterson et al. 1993). The 102-bp specific PCR-amplified product was subjected to electrophoresis on a 3% agarose gel (1:1 SeaKem ME:low-melting NuSieve GTG).

Cloning of a partial cDNA of the human lanosterol synthase gene

The 102-bp PCR product from trapped exon HMC14g01 was also used to screen 1×10^6 cDNA clones from a commercially obtained cDNA library of human fetal brain (Clontech HL3003a). Screening was carried out by probing the library with a [^{32}P]ATP-labeled insert of cloned HMC14g01 DNA, which contained an exon of the human lanosterol synthase gene. Positive clones were sequenced in both orientations using standard oligonucleotide-walking protocols for the ABI373A automated sequencer.

Northern blot analysis

A 1.8-kb long human cDNA clone (LS13) that contains the entire sequence reported in Fig. 4 was used to probe a Northern blot containing poly(A)$^+$ RNA from eight human tissues (Clontech filter 7760–1). Northern blot analysis was performed according to standard protocols with high-stringency washing.

Results and discussion

We have used randomly picked HC21-specific cosmids (from the LL21NCO2-Q library) in an exon-trapping experiment to capture partial gene sequences from HC21 and have identified more than 550 different potential exons (Chen et al. 1995). A total of five clones were identified that showed homology to the lanosterol synthase genes of rat and yeast. Clone HMC14g01 (Genbank X88398,102 nt) was homologous to rat lanosterol synthase (Genbank D45252) from amino acid 186–216 ($P = 1.3 \times 10^{-13}$) (Fig. 1). The additional four clones HMC14b03 (Genbank X88389,113 nt), also shown in Fig. 1, HMC14b02 (Genbank X88386,162 nt), HMC42b10 (Genbank X88067,83 nt) and HMC28g07 (Genbank X88191,146 nt) were homologous to amino acids 263–298 ($P = 2.6 \times 10^{-16}$), 374–423 ($P = 1.3 \times 10^{-18}$), 665–690 ($P = 1.5 \times 10^{-10}$) and 691–732 ($P = 1.6 \times 10^{-18}$) of the rat lanosterol synthase, respectively. When using cosmids in exon trapping from the region of HC21 between markers CBS to ITGB2 in 21q22.3 we identified two clones (MDL13c02 and MDL12g04), the sequences of which were both included in the above-mentioned HMC14b02 clone. This suggested that the human homolog of rat lanosterol synthase is potentially localized on 21q22.3.

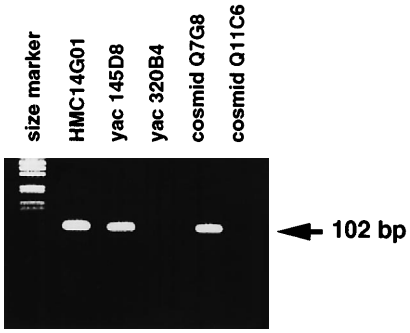

Fig. 2 Mapping of the human lanosterol synthase gene. Polymerase chain reaction (PCR) amplification with primers HMC14g01-F and HMC14g01-R from selected yeast artificial chromosomes (YACs) and cosmids on HC21. Plasmid HMC14g01 is the originally trapped sequence; YACs 145D8 and 320B4 are from the HC21 contig of Chumakov et al. (1992); cosmids Q7G8 and Q11C6 are from the LL21NCO2-Q CH21-specific library

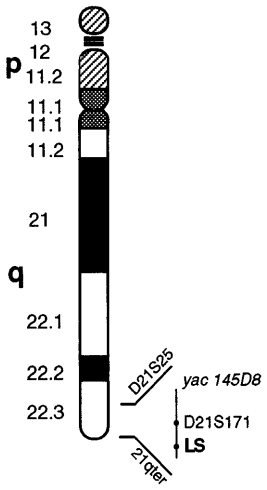

Fig. 3 Schematic representation of the mapping position of the human lanosterol synthase gene. Mapping with somatic cell hybrids localized this gene between marker D21S25 and 21qter. The YAC from Chumakov et al. (1992), positive for the lanosterol synthase gene, is indicated. The same YAC is positive for marker D21S171; however, the order of D21S171 and the lanosterol synthase gene relative to the telomere is not known

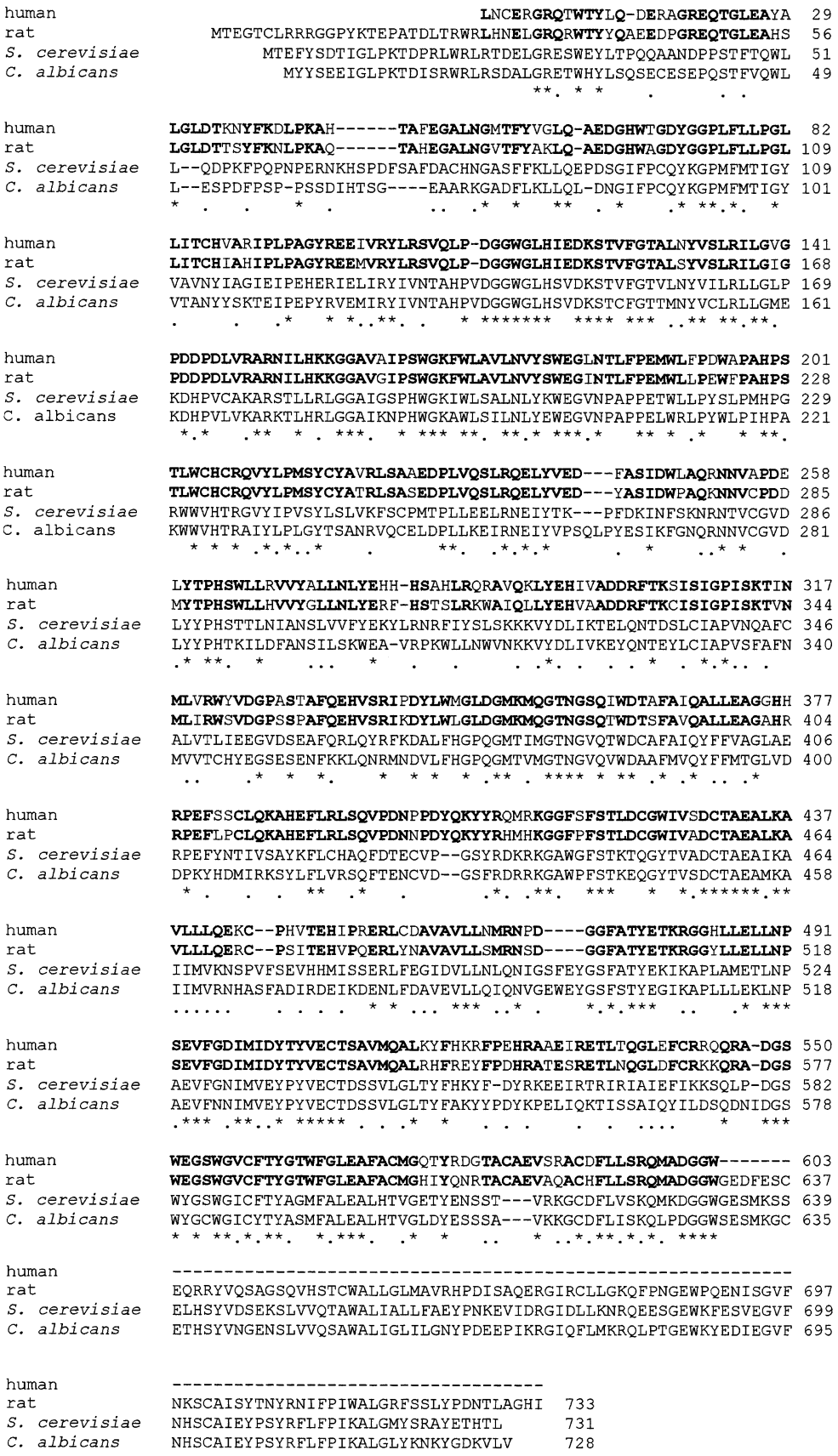

Fig. 4 Multiple alignment of sequences of the lanosterol synthase peptides generated with the CLUSTAL V program. The accession numbers of the sequences are: human (Genbank X87809), rat (Genbank D45252), *S. cerevisiae* (Genbank P38604) and *Candida albicans* (Genbank Q04782). Identical amino acids in the human and rat sequences are in *bold letters*. Amino acids identical in all four sequences are indicated by a (*), while conservative amino acids are indicated by a (.) below the alignment. The one-letter code of amino acids is used. The human sequence is not full length (see text)

In order to determine the precise localization of these exons, PCR amplification of the specific 102-nt insert of clone HMC14g01 was performed using DNA from rodent-human somatic cell hybrids that contain the entire or specific segments of HC21 (Patterson et al. 1993). Somatic cell hybrids amplifying HMC14g01 were R451-29C-5, WA17, 2Fur1, JC6A, ACEM2-10d, 8q-, GA9-3, R50-3, 1881C-13b and 9528C-1 whereas hybrids 6918–8a1, Raj5, 9542C-5a, MRC2G and R2-10 W did not show amplification. These data localized the human lanosterol synthase gene to the terminal region of 21q22.3 between marker D21S25 and the 21q telomere. Similar PCR amplification using total yeast DNA from HC21 containing YACs from the Chumakov et al. (1992) contig mapped the HMC14g01 exon to YAC 145D8. This YAC has previously been localized to 21q22.3 and also contains marker D21S171. Similarly, cosmid Q7G8 from the LL21NCO2-Q library located between markers CBS and ITGB2 was positive for HMC14g01 (Fig. 2). These results are in agreement with the data from the somatic cell hybrids and confirm the localization of a human homolog of the rat lanosterol synthase gene to the distal part of 21q near marker D21S171 (Fig. 3).

Clone HMC14g01 was used to probe a cDNA library of human fetal brain and several positive clones were obtained. The predicted amino acid sequence of the longest clone, LS13, (which is not full length since it lacks part of the coding and the 5´- and 3´-untranslated regions) is shown in Fig. 4. There is significant nucleotide homology between the human and rat lanosterol synthase (82% identity). An alignment of the predicted polypeptide sequences of lanosterol synthase of human (Genbank X87809), rat (Genbank D45252), *Saccharomyces cerevisiae* (Genbank U04841) and *Candida albicans* (Genbank L04305) is shown in Fig. 4. There is 83% identity between the human and the rat peptide sequences; 40% and 39% identity between the human and *S. cerevisiae*and *C. albicans* peptide sequences, respectively, was observed.

Northern blot analysis using the cDNA clone LS13 as a probe revealed a prominant 4.4-kb mRNA in all tissues (Fig. 5).

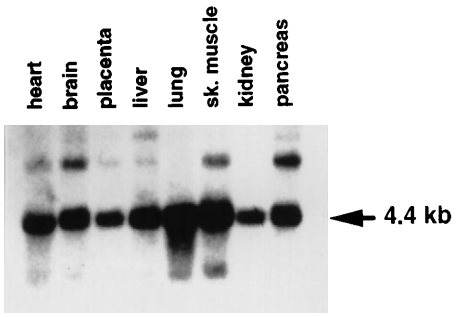

Fig. 5 Northern blot analysis of human mRNA (Clontech filter 7760–1) hybridized to LS13, a 1.8-kb cDNA probe for lanosterol synthase. An intensely hybridizing 4.4-kb mRNA species is observed in all tissues. Additional bands may represent either alternatively spliced mRNAs or mRNA species from genes with homology to the lanosterol synthase probe

Lanosterol synthase or (S)-2,3-oxidosqualene lanosterol cyclase is an enzyme involved in the synthesis of lanosterol, which is the initial sterol precursor to cholesterol, steroid hormones and vitamin D (Corey et al. 1966, 1994). It catalyzes the cyclization of (S)-squalene-2,3-epoxide into lanosterol; this catalysis is complex as this enzyme is involved in the creation of the four-ring parental steroid in a one-step reaction (Corey et al. 1994).

The human lanosterol synthase gene maps in a region of 21q22.3 that is distal to the so-called Down syndrome critical region (Delabar et al. 1993; Korenberg et al. 1994; McCormick et al. 1989). Triplication of this gene is therefore unlikely to contribute to the phenotype of Down syndrome. Complete absence of lanosterol synthase or amino acid substitutions that abolish its activity may not be compatible with life in humans. However, partial deficiency of the enzyme may be associated with a phenotype with low levels of all steroid compounds. No candidate disorders have been mapped to the 21q22.3 region to date, and no known phenotypes with very low levels or absent steroid compounds have been described. Further experiments such as the production of a mouse with targeted disruption or mutagenesis of its lanosterol synthase gene are necessary to correlate a lanosterol synthase defect with a specific phenotype.

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