SHORT COMMUNICATION

Human Endopeptidase 24.15 (THOP1) Is Localized on Chromosome 19p13.3 and Is Excluded from the Linkage Region for Late-Onset Alzheimer Disease

Matthew P. Torres,[1] Christa Prange, and Greg Lennon

Biology and Biotechnology Program, Lawrence Livermore National Laboratory, Livermore, California 94550

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The mapping position of human endopeptidase 24.15 (THOP1) has previously been reported to be within the linkage region for the late-onset Alzheimer disease AD2 locus on chromosome 19q13.3. After localizing THOP1 to the high-resolution cosmid contig map of human chromosome 19, we found that the previous report was incorrect. Results of the hybridization and FISH mapping of positive clones indicated localization of THOP1 to chromosome 19p13.3 and not 19q13.3. This localization is a correction of wrong chromosomal delegation and excludes THOP1 from the region that shows evidence of linkage to late-onset familial Alzheimer disease.

THOP1 is a well-known metalloproteinase distributed in animals and plants (11) and highly conserved between mammals such as pig, rat, and human. Previous work by Kest *et al.* (5) and Dahms and Mentlein (3) suggests that THOP1 is involved in the inactivation of somatostatin (a cyclic tetradecapeptide that inhibits release of growth factor, insulin, and gastrin) in the parietal cortex of human brain and in the degradation of several other neuropeptides with chain lengths between 8 and 30 amino acids. THOP1 is present and active throughout the brain but is densest in the nuclei of cerebellar Purkinje cells and in hippocampal dentate gyrus cells (4).

Prior to this report, the literature indicated that THOP1 is localized to chromosome 19q13.3 within the linkage region for late-onset Alzheimer disease. Based on this evidence, Meckelein *et al.* (8) suggested that THOP1 was a possible candidate for the amyloid β secretase responsible for the cleavage of amyloid β peptide from its precursor, the amyloid precursor protein, in the production of toxic amyloid in AD brain (9). This process is known to be a major initiator of the production of toxic amyloid and requires that the APP be membrane bound (10). [Amyloidogenic fragments, coupled with numerous neurofibrillary tangles in the hippocampus, amygdala, cerebral cortex, and other brain regions, are characteristic signs for the pathological diagnosis of Alzheimer disease (9)]. Chevallier *et al.* (1) have shown that THOP1 did not behave as an amyloid β secretase when studied for a specific role in amyloid β secretase activity in HK293 transfected cells. In addition, Marks *et al.* (7) have shown that a rat phosphoramidon-insensitive metalloproteinase similar to THOP1 was capable of degrading synthetic peptides containing β-secretase sites, suggesting that THOP1 may be involved in the processing of non-membrane-bound intermediate peptides involved in Alzheimer disease.

Meckelein's chromosomal localization for THOP1 was achieved by isolating an enzyme from human AD brain that was capable of generating amyloidogenic fragments, indicating that this enzyme had high homology to rat THOP1, and by FISH mapping of a rat THOP1 cDNA to human chromosomes. However, in an effort to map THOP1 to the high-resolution cosmid contig map of human chromosome 19, we discovered that THOP1 localizes to 19p13.3 and not 19q13.3.

We mapped THOP1 by hybridizing a partially sequenced cDNA clone to an arrayed, chromosome 19-specific cosmid library. ESTs (expressed sequence tags; partially or fully sequenced cDNA clones) for human THOP1 were discovered by using NCBI's BLAST program (http://www.ncbi.nlm.nih.gov) to query dbEST (the public database of expressed sequence tags) with the complete coding sequence for human THOP1 (GenBank Accession No. U29366). We opted to use IMAGE clone ID 663763 (GenBank Accession No. AA226759), which displayed a complete sequence homology with THOP1.

The clone used in this project was donated by the Integrated Analysis of Genes and their Expression (IMAGE) Consortium (6). IMAGE is a U.S. government funded collaborative effort to place the sequence, map, and expression data of EST arrays on the public domain as well as to create an all-inclusive array of all cDNA clones from each gene in the genomes under study. In this strategy, mRNAs are isolated from a variety of tissues in the organism of interest, converted

[1] To whom correspondence should be addressed. E-mail: mtorres@genelogic.com.

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to cDNAs, cloned into *Escherichia coli* plasmid vectors, isolated into separate wells of a 384-well plate, and then sequenced. The cDNA clones are stored in a library at Lawrence Livermore National Laboratory and are available free of royalties. The sequences and expression data have been placed on the internet (http://www-bio.llnl.gov/image/image.html).

Cosmid libraries specific to chromosome 19 were created from flow-sorted hamster–human somatic cell hybrids (2). Clones were spotted onto a nylon filter using a Hewlett-Packard ORCA robot (Hewlett-Packard, Palo Alto, CA). The ORCA is a 96-pin tool connected to a six-jointed arm that works atop a 1 × 2 m optical bench and is capable of producing up to 90 8 × 12 cm filters containing 3456 clones per filter in 8 h (2). Filters were prehybridized with 4× SSC, 2% SDS in 2 ml/filter at 65°C for 1 h prior to the addition of probe. To decrease the possibility of obtaining false-positive results, filters were blocked with a mixture of 50 μL salmon sperm DNA, 20 μL poly(U), 12 μL KS$^-$ (KS$^-$ is a purified mixture of fragmented Bluescript plasmid vector sequences, which are commonly used for cloning IMAGE ESTs), and 10 μL human COT DNA at 1 mg/ml (COT DNA is a purified mixture of short repetitive element sequences generated from human genomic DNA). The mixture was boiled for 5 min and then transferred to the prehybridization solution.

The probe was labeled by PCR using vector primers T3 and T7 and [^{32}P]dCTP incorporated into the reaction. Radiolabeled probe was then added to the cosmid filters. Hybridization took place at 65°C for 24 h. Following hybridization, filters were washed 2× in 2× SSC, 1% SDS for 15 min each, followed by a wash in 0.2× SSC, 1% SDS for 10 min. All washes were performed at 65°C. After being washed, filters were exposed to phosphor cassettes overnight and scanned with a STORM 860 (Molecular Dynamics). Filter images were analyzed with Image QuaNT software (Molecular Dynamics).

Verification of hybridization positives was accomplished by PCR using THOP1 sequence-specific primers (F2, TCCGTGCTCTGTGGTAAACGA, and R3, TGTAGGTGACCTCCACATCGG), generating a 179-bp fragment. Primers were designed from exon 1 (basepairs 104–316) of the complete coding sequence of THOP1 using the Baylor College of Medicine primer selection program (http://dot.imgen.bcm.tmc.edu:9331/seq-util/seq-util.html) to minimize the possibility of PCR failure caused by intronic sequence. Oligonucleotides were synthesized by Genset and optimized using human placental DNA. Once optimized, the primers were used in PCR of purified DNA from the hybridization-positive cosmid clones. All PCR experiments were accompanied by hamster genomic DNA as a negative control and human placental DNA as a positive control. Verified positives were localized to the restriction fragment cosmid contig map of chromosome 19. Results indicate that human THOP1 is located within the *Eco*RI restriction map localized to chromosome 19p13.3 between chromosome 19-specific markers D19S878 and D19S591 (the updated position has been marked on the LLNL physical map of chromosome 19 at http://bbrp.llnl.gov/genome/bin/../html/chrom_map.html). This mapping position is strengthened by two additional pieces of information: one, the same *Eco*RI contig map contains 12 independently FISH-mapped cosmid clones that localize to 19p13.3; and two, attempts to amplify other nearby cosmid clones within the contig were successful.

In conclusion, THOP1 is not located within the linkage region for the late-onset Alzheimer disease AD2 locus and is thus less likely to be a disease gene candidate for hereditary late-onset Alzheimer disease.

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