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Research Report

CAT 53: A protein phosphatase 1 nuclear targeting subunit encoded in the MHC Class I region strongly expressed in regions of the brain involved in memory, learning, and Alzheimer's disease

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Abstract

We identified CAT 53 by cDNA hybridization selection as an expressed sequence tag (EST), located in the vicinity of HLA-C and designated as CAT (for HLA-C associated transcript) 53. CAT 53 encodes a protein described by others and commonly known as phosphatase 1 nuclear targeting subunit (PNUTS). PNUTS is a potent inhibitor of nuclear serine/threonine protein phosphatase 1 (PP1). We present the genomic organization of CAT 53, localize specific sites of mRNA transcription in thin sections of mouse brain by in-situ hybridization, and perform a structural analysis of the peptide domains. We also characterize the protein expression pattern for PNUTS by Western blotting and immunohistochemistry with PNUTS antibody in Alzheimer's disease (AD) brains and age-matched control brains. In-situ hybridization and immunohistochemistry analysis of human and mouse brain show high CAT 53 expression in the olfactory cortex, piriform cortex, and hippocampus. Very high expression of CAT 53 was found mainly in the hippocampus, frontal, and entorhinal cortex of control brains and in the neurofibrillary tangles of AD brain. In the hippocampus, CAT 53 is expressed in CA1 and CA3 cell layers and in the dentate gyrus. The hippocampus is known to play a fundamental role in learning and episodic memories and has been implicated in a number of neurological and psychiatric disorders, including AD, epilepsy, and schizophrenia. Our findings suggest that PNUTS, encoded by CAT 53 on 6p21.3, may have a role in the progression of AD.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Alzheimer's—miscellaneous

Keywords: CAT 53; Alzheimer's disease; Phosphatase 1 nuclear targeting subunit; MHC

1. Introduction

The major histocompatibility complex (MHC) is an extended cluster of 230 genes distributed over 4 million bases (Mb) on human chromosome 6p21.3 [8]. The large number of related genes encoded in this region is remarkable for their diverse functions and varied roles in immunity. During the last two decades, several groups including our own have identified new genes in the MHC cluster and characterized their role in immunity and disease associations. The MHC is of major biomedical interest because of its contribution to transplant rejection and susceptibility to a variety of autoimmune diseases such as

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insulin-dependent diabetes mellitus, multiple sclerosis (MS), systemic lupus erythematosus, and rheumatoid arthritis [19,20,43]. In addition, the MHC contains the gene for at least one non-immune mediated disorder, hemochromatosis (HFE), an iron overload syndrome associated with the HLA-A3 class I allele [37].

The MHC has also been associated with several neurobehavioral phenotypes, but specific causative or susceptibility genes have yet to be identified. These include narcolepsy associated with certain HLA class II alleles [26], early onset Alzheimer's disease (AD) with HLA-A2 [34,44], and Parkinson's disease with HLA-DR [29,47]. Reading disability, or dyslexia, has been associated with MHC alleles by several independent studies [9,13,18].

To identify uncharacterized genes in the extended MHC class I region, gain clues to the evolution of this highly conserved region, and identify possible candidate genes for dyslexia, AD, and MS, we created several transcription maps of the MHC [1,2,21]. In this paper, we describe one such uncharacterized transcript and gene originally identified by cDNA hybridization selection as an expressed sequence tag (EST) [45,46], located in the vicinity of HLA-C and designated as CAT (for HLA-C associated transcript) 53. Fine mapping using PCR and regional YAC clones localized it 70 Kb centromeric of HLA-E (Fig. 1) [21], but the subsequent description of a full-length cDNA (FB19) did not suggest a particular function [45]. Other investigators identified the putative translation product of CAT 53, eponymously known as p99 and PNUTS. p99, a nuclear modulator of protein phosphatase I, was purified from HeLa cell nuclei [27]. Phosphatase I nuclear targeting subunit (PNUTS) was independently identified by a yeast two-hybrid system [3]. Antibody staining showed that PNUTS was enriched in dendritic spines of neuronal cells in the brain where it has been implicated in ionic conductance and long-term synaptic plasticity. However, its putative role in disorders that map within or close to the MHC remains largely unstudied.

AD is the most common cause of dementia in the elderly population. It is characterized by the presence of two pathological hallmarks, extracellular β-amyloidal deposits in senile plaques and intracellular neurofibrillary tangles (NFT). NFT are composed of bundles of paired helical filaments (PHF), whose major protein subunit is abnormally hyperphosphorylated Tau [15,28]. In AD brains, Tau contains three to four times more phosphates than Tau in normal brains [25]. Hyperphosphorylation, both in-vitro and in-vivo, has been shown to decrease the affinity of Tau for microtubules leading to disruption of the neuronal cytoskeleton and axonal transport [17,23,30]. Phosphorylation also causes Tau resistance to proteolytic degradation leading to its gradual accumulation in the cell. Co-localization of CAT 53 and an AD locus within the MHC suggests a possible connection.

Below, we present the genomic organization of CAT 53, characterize the unique expression pattern in AD and age-matched human control brain and mouse brain, localize specific sites of mRNA transcription in thin sections of mouse brain by in-situ hybridization, and perform a structural analysis of the peptide domains. To extend these results, we performed Western blotting and immunohistochemistry studies on human brain tissue sections. These new data on CAT 53 and PNUTS further increase the likelihood that the gene could be involved in the pathophysiology of AD, dyslexia, or schizophrenia.

2. Materials and methods

2.1. cDNA library screening

Human cDNA fetal brain and mouse cDNA brain libraries were purchased from Clontech (Palo Alto, CA). Library screenings were performed by plating ~5 × 10^6 plaque-forming units with insert sizes in the range of 500–1500 nucleotides. The CAT 53 EST [46] was radiolabeled by random priming as previously described [16]. All

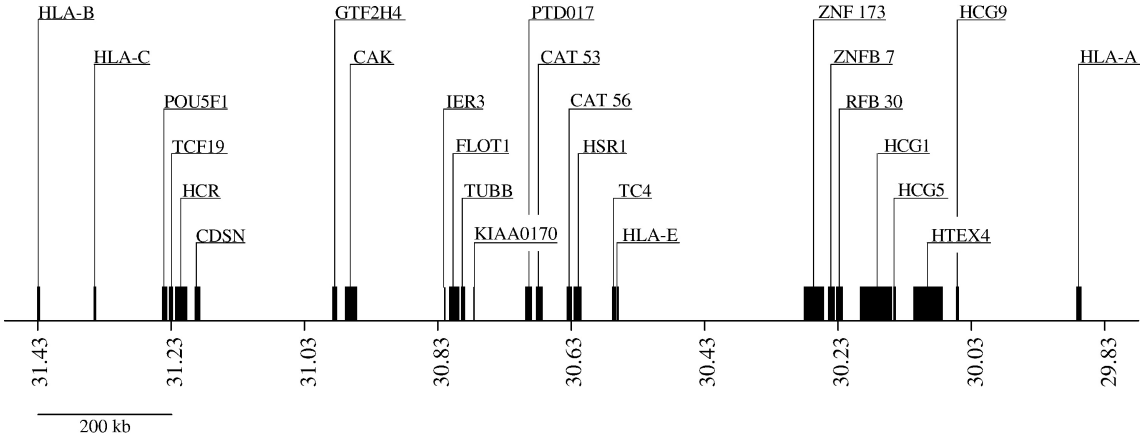

Fig. 1. Genes encoded in the MHC class I region. Regional map of the MHC class I region spanning 1.6 million bases and 26 genes. Numbers beneath the median are distances from pter in millions of bases. Cen is to the left. CAT 53 is in the center. Gene sizes and locations are from the Ensembl database (3/31/04, www.ensembl.org).