Genomic structure, expression pattern, and chromosomal localization of the human calsenilin gene: no association between an exonic polymorphism and Alzheimer’s disease

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Abstract

Calsenilin is a recently-identified member of the neuronal calcium sensor family. Like other members of this family, it is found in the brain and binds calcium. Calsenilin was discovered by virtue of its interaction with both presenilin-1 and -2, proteins that are involved in the etiology of Alzheimer’s disease. Because calsenilin may play a role in Alzheimer’s disease and other disease with alterations in calcium homeostasis, we characterized the human gene. The gene, which we localized to chromosome 2, extends over a region of at least 74 kb and includes nine exons. Interestingly, the ninth exon of calsenilin contains a highly polymorphic CA repeat, adjacent to the stop codon. In a study of Alzheimer patients and their unaffected siblings, there was no evidence of association of AD with any calsenilin allele. This CA repeat will be useful for linkage and linkage disequilibrium studies to determine whether calsenilin variants contribute to risk in other diseases. © 2000 Published by Elsevier Science Ireland Ltd.

Keywords: Alzheimer’s disease; Calsenilin gene; Presenilin-1 and -2

Alzheimer’s disease (AD) is a progressive, neurodegenerative disease involving the accumulation of amyloid plaques in the brain [3,14]. Amyloid plaques are comprised of aggregates of a 40–43 amino acid long peptide, A\textsubscript{β}. A\textsubscript{β} is derived by the action of two proteases, \( \beta \)- and \( \gamma \)-secreasate, on a large transmembrane protein, the Alzheimer amyloid protein precursor (APP). A \( \beta \)-secreasate has recently been identified [15]. Two homologous proteins, presenilin-1 and presenilin-2 are somehow intimately involved with \( \gamma \)-secreasate activity and have been proposed as either \( \gamma \)-secreasates themselves, or as being necessary cofactors for \( \gamma \)-secreasate [1,6,10]. These two proteins were identified by positional cloning as underlying certain forms of early-onset, autosomal dominant, familial Alzheimer’s disease. To date, more than 50 mutations in presenilin-1, as well as two mutations in presenilin-2, have been identified that lead to Alzheimer’s disease. Disruption of the murine presenilin-1 gene leads to a nearly total loss of \( \gamma \)-secreasate activity in neuronal culture, supporting a critical role for presenilin-1 in the proteolysis of APP and the formation of A\textsubscript{β} [1,6]. Interestingly, presenilins are also involved in Notch signaling [1,6] and in the unfolded-protein response (UPR) [8,12].

Studies have indicated that the COOH-terminal of presenilin-1 and -2 play important roles in programmed cell death and in A\textsubscript{β} formation. We had carried out yeast two-hybrid studies to identify proteins that interact with the COOH-terminus of the presenilins [5]. We identified a neuron-enriched protein of the neuronal calcium family as a potent interactor with both presenilin-1 and -2. Because this protein was able to bind both calcium and presenilins, we denoted it as calsenilin. The function of neuronal calcium
sensors is not known, although it is known that these proteins have very high affinities for calcium and may modulate signal transduction cascades. In our studies with calsenilin, we observed that it can regulate the levels of presenilin fragments [5], and can regulate the modulatory effects of the presenilins on IP₃-receptor mediated calcium release from the endoplasmic reticulum [11].

To determine the structure of calsenilin, we first identified and sequenced full-length cDNA clones from human libraries. In addition, to ensure the presence of the complete sequence, we carried out 5' rapid amplification of cDNA ends (RACE) (GenBank No. AF120102). This cDNA sequence was aligned to the genomic sequence of two portions of an incompletely sequenced BAC (GenBank No. AC009238). The results showed that the human calsenilin gene spanned a region of at least 74 kb and is organized into nine exons (Fig. 1; Table 1).

Expression of calsenilin in human tissue was determined by immunoblotting. All members of the neuronal calcium sensor family share sufficient homology such that there may be cross-reaction of polyclonal antibodies across family members. We therefore developed a highly specific monoclonal antibody that recognized only a single band on immunoblots. This band had a molecular weight of 30 kDa which corresponds to the size of recombinant calsenilin expressed in cultured cells and is ~4 kDa larger than that of other neuronal calcium sensors because of the presence of a unique amino terminal in calsenilin. We can therefore be certain that the band recognized is in fact calsenilin. In extracts of human muscle, lung, heart, liver, kidney, and

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Table 1

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Fig. 1. Genomic structure of calsenilin. Exons (black bars) are numbered 1–9, and the position of the ATG and TAG are shown. The untranslated region of exon 9 is indicated by the unfilled segment of the bar. The CA repeat is in exon 9, immediately downstream of the TAG.
brain, calsenilin was only observed in brain extracts (Fig. 2). These results were consistent with a previous analysis of calsenilin mRNA [5]. The highly selective expression of calsenilin in the brain suggests that the calsenilin promoter may control brain-specific expression. Examination of the sequence upstream of the start of transcription failed to identify a TATA box, although there were some consensus sites for transcription factor (Sp1) binding.

Analysis of the cDNA and genomic sequence identified a dinucleotide (CA) repeat in the 3′UTR of the calsenilin cDNA, coded for by exon 9. This CA repeat was polymorphic as determined by sequencing from distinct libraries and by comparison with expression sequence tags in the GenBank database, (e.g. AA349365). Comparing the sequence with previously characterized CA repeats demonstrated that this CA repeat had been characterized in the Genethon database (ID no. AFMa125yg1). This repeat showed high heterozygosity in the Centre d’Etude du Polymorphome Humain (CEPH) families (maximal heterozygosity of 0.74) and was included as a polymorphism (D2S2159) for the development of a genetic map of the human genome. This marker was mapped to chromosome 2.

Because of the potential relevance of calsenilin to AD, we genotyped the calsenilin polymorphism (D2S2159) in a cohort of 280 sibships and other mostly small families with AD collected under the auspices of the NIMH Genetics Initiative. The ascertainment and collection of these families are described elsewhere [2,4]. In the 115 of these families containing a sibship discordant for AD, we tested for linkage between AD and D2S2159 using the multi-allelic sibship disequilibrium test (SDT; [7] also see http://www.biostat.harvard.edu/~fbat/default.html), a family-based test of association that does not require parental data, and found no evidence of association with this marker (Table 2). In addition, parametric and non-parametric linkage analysis in the full sample of 280 families, performed under various models, also failed to show any evidence for linkage (data not shown). Recent full genome scans for late onset Alzheimer’s disease did not demonstrate linkage to this region [9,13].

Although the exact function of neuronal calcium sensors is unknown, it is known that members of this family are sensitive to very low concentrations of calcium, and respond by undergoing a profound conformational change that in certain cases leads to the translocation of the protein to a different subcellular compartment. This translocation then leads to the regulation of function of proteins that interact with the neuronal calcium sensor. The important role of the presenilins in γ-secretase cleavage of APP, in Notch signaling, and in the UPR support a role for this protein in several important cellular functions. The interaction between calsenilin and presenilin appears to modulate some of the functions of the presenilins. Further characterization of calsenilin and the calsenilin gene in normal and disease states may elucidate a role for this protein in these conditions.

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