Mutations in the open reading frame of the β-site APP cleaving enzyme (BACE) locus are not a common cause of Alzheimer’s disease

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Abstract Amyloid β-peptide (Aβ) plays a central role in the pathogenesis of Alzheimer’s disease (AD). The gene encoding the β-site APP cleaving enzyme (BACE), one of two enzymes that sequentially cleave the β-amloid precursor protein to generate Aβ, has recently been cloned. We tested the hypothesis that BACE might be genetically associated with AD by linkage analysis (56 pedigrees), by direct nucleotide sequencing of the entire open reading frame (20 subjects with familial AD, and 10 subjects with sporadic AD) and by allelic association analysis (155 AD cases and 173 non-demented controls). Our results revealed no evidence for either genetic linkage or allelic association between BACE and AD, and no coding sequence mutations were detected in the open reading frame of the BACE gene. These data suggest that while BACE protein plays an important role in the pathogenesis of AD, and may be a robust therapeutic target, it is unlikely to be a major AD susceptibility locus.

Keywords β-site · APP cleaving enzyme · Alzheimer’s disease · Linkage analysis

Introduction

One of the main neuropathological hallmarks of Alzheimer’s disease (AD) is the accumulation of senile plaques in the brain, a principal component of which is the amyloid β-peptide (Aβ). Amyloid β-peptide is generated via sequential proteolytic cleavage of the β-amloid precursor protein (βAPP) by β- and γ-secretase. The β-secretase cleavage is mediated by the β-site APP cleaving enzyme (BACE), a type 1 transmembrane aspartyl protease encoded on chromosome 11q23.3 [1, 2, 3, 4, 5]. Although the etiology of AD is complex, genetic factors do play a prominent role. Four different genes associated with inherited susceptibility to AD have been identified to date: presenilin-1 (PS 1) [6], presenilin-2 (PS 2) [7], βAPP [8] and APOE [9]. However, because these genes account for only one-half of the genetic variance of AD, other AD susceptibility loci are thought to exist. The BACE gene is a reasonable candidate for a familial AD (FAD) susceptibility gene because Aβ pep-
tide, and the processes involved in generating Aβ (i.e., β- and γ-secretase cleavage), play a central role in the genesis of AD.

To test the hypothesis that BACE might be the site of mutations associated with AD, we used a direct sequencing approach, a case-control association study, and a segregation analysis of three polymorphic genetic markers flanking the BACE locus.

Materials and methods

Subjects

Fifty-six pedigrees multiply affected by AD were available. Alzheimer’s disease was diagnosed by NINDS-ADRA criteria and confirmed by autopsy in 38 families. The majority (50) of the families had late-onset AD cases (mean age of onset, >65 years) and six families had early-onset AD cases (mean age of onset, <65 years). Samples were obtained from at least three living AD-affected family members distributed in at least two continuous generations. We also investigated BACE in 173 sporadic cases and in 155 unrelated controls who were cognitively normal (typically spouses). Both cases and controls were Caucasian, and were collected through Memory Disorder Clinics at the University of Miami and the University of Toronto. The mean age of AD onset of the patients was 76.1±7.8 years and the mean age at examination of the controls was 69.6±12.5 years. As with the FAD cases, the diagnosis of AD was made using NINDS-ADRA criteria.

Genotyping

Mutations in PS 1, PS 2 or βAPP were previously excluded in our pedigrees by direct sequencing and the APOE genotype was ascertained as previously described [6, 7, 8, 9]. The primer sequences and polymerase chain reaction (PCR) conditions for the three anonymous polymorphic markers were obtained from the Genome Database (http://www.ncbi.nlm.nih.gov/genemap). These markers have been mapped in order according to the Marshfield Map (available at http://www.marshmed.org/genetics): D11S1347- (6.0 cM)-D11S4127- (0.5 cM)-D11S1348. The BACE gene has been physically mapped between D11S1347 and D11S939 (Gene Map '99 available at http://www.ncbi.nlm.nih.gov/genemap).

Linkage analysis was performed using non-parametric and parametric approaches implemented in the computer software package GENEHUNTER [10]. A parametric analysis was carried out by assigning a constant low penetrance (0.02) to all unaffected relatives. The maximum NPL score marker (NPL statistic=0.28; P=0.9) was observed at the location of D11S1347. Stratification of the dataset into groups of families positive or negative for APOE ε4 showed no evidence for linkage in any subset. Because the results were negative for almost all families, the hypothesis of linkage in the presence of heterogeneity can be rejected.

The power of linkage methods to detect genes of small effect is limited. Consequently, we next searched the entire open reading frame of the BACE gene in the affected members of 20 families (one per family) in which no obligate recombinants were detected between AD and the three microsatellite markers. In addition, 10 autopsy-proven cases of sporadic AD (obtained from the Canadian Brain Tissue Bank) were sequenced to explore the possibility that a polymorphism within the BACE gene might be a risk factor for sporadic AD.

The only sequence variation detected in the open reading frame of the BACE gene in 60 different chromosomes was a common silent substitution of G to C in codon 262 at nucleotide 1239. To test whether this polymorphism might show allelic association with AD, we performed a case-control association study in a sample of 173 well-characterized sporadic AD cases and 155 normal subjects drawn from the same ethnic group. Inspection of the allele and genotype distributions showed no association at the allelic (P>0.3) or genotypic (P>0.4) level (Table 1).

Results

Maximum likelihood calculations excluded linkage in our FAD pedigrees to the entire 7 cM interval containing the BACE gene (Fig. 1). The individual family scores were negative or close to zero for 52 of the 56 families. The four remaining families had LOD scores of 0.5–0.6. Non-parametric linkage (NPL) analyses also failed to detect significant evidence for allele sharing in affected relatives. The maximum NPL score marker (NPL statistic=0.28; P=0.9) was observed at the location of D11S1347. Stratification of the dataset into groups of families positive or negative for APOE ε4 showed no evidence for linkage in any subset. Because the results were negative for almost all families, the hypothesis of linkage in the presence of heterogeneity can be rejected.

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