Gene 488 (2011) 1–12

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

The Alzheimer's amyloid β-peptide (Aβ) binds a specific DNA Aβ-interacting domain (AβID) in the *APP*, *BACE1*, and *APOE* promoters in a sequence-specific manner: Characterizing a new regulatory motif

Bryan Maloney [a], Debomoy K. Lahiri [a,b,*]

[a] Laboratory of Molecular Neurogenetics, Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[b] Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202, USA

ARTICLE INFO

Article history:
Accepted 6 June 2011
Available online 15 June 2011

Received by Astrid M. Engel

Keywords:
Alzheimer's disease
Amyloid beta
DNA–protein interaction
Gene regulation
Transcription factor

ABSTRACT

Deposition of extracellular plaques, primarily consisting of amyloid β peptide (Aβ), in the brain is the confirmatory diagnostic of Alzheimer's disease (AD); however, the physiological and pathological role of Aβ is not fully understood. Herein, we demonstrate novel Aβ activity as a putative transcription factor upon AD-associated genes. We used oligomers from 5′-flanking regions of the apolipoprotein E (*APOE*), Aβ-precursor protein (*APP*) and β-amyloid site cleaving enzyme-1 (*BACE1*) genes for electrophoretic mobility shift assay (EMSA) with different fragments of the Aβ peptide. Our results suggest that Aβ bound to an Aβ-interacting domain (AβID) with a consensus of "KGGRKTGGGG". This peptide–DNA interaction was sequence specific, and mutation of the first "G" of the decamer's terminal "GGGG" eliminated peptide–DNA interaction. Furthermore, the cytotoxic Aβ25–35 fragment had greatest DNA affinity. Such specificity of binding suggests that the AβID is worth of further investigation as a site wherein the Aβ peptide may act as a transcription factor.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly (Hebert et al., 2003) and is associated with heterogeneous risks including genetic, epigenetic, dietary, and lifestyle factors (Lahiri and Maloney, 2010b). It is characterized by neuronal loss, intraneuronal tangles of hyperphosphorylated microtubule-associated τ protein (MAPT), and extracellular deposition of β-amyloid peptide plaque. This amyloid plaque is composed primarily of the amyloid β peptide (Aβ), 39–42 amino acids in length (Lahiri et al., 2003; De Strooper, 2010). Aβ is a cleavage product of the Aβ precursor protein (APP) by the β- and γ-secretases. Cleavage of APP by β-secretase or β-site APP cleaving enzyme 1 (BACE1) releases the large extracellular domain of APP (Hussain et al., 1999; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999), and subsequent cleavage by γ-secretase releases Aβ and the APP intracellular domain fragment (AICD) (Kimberly et al., 2001). Aβ can accumulate in neurons without plaque formation in both human AD cases and transgenic AD models (Gouras et al., 2000; Shie et al., 2003). The non-pathological functions of Aβ in addition to its activity in aging-related disorders, such as AD, are multifaceted (Lahiri and Maloney, 2010a), including but not necessarily limited to kinase activation (Bogoyevitch et al., 2004; Tabaton et al., 2010), protection against metal-induced oxidative damage (Zou et al., 2002; Baruch-Suchodolsky and Fischer, 2009), regulation of cholesterol transport (Yao and Papadopoulos, 2002; Igbavboa et al., 2009), and formation of ion channels (Jang et al., 2010). However, the pathological role of Aβ is not fully understood.

The greatest risk factor for AD is age (Thies and Bleiler, 2011). DNA damage and changes in gene regulation have been reported in the aging human brain (Lu et al., 2004). Specifically, aging of the human cortex is characterized by a distinct transcriptional signature that includes reduced expression of genes that mediate synaptic plasticity and correlates with age-dependent DNA damage to the promoters of these genes. Recent work also suggests an important transcriptional role of several genes and their products in AD, including APP, AICD (from APP), BACE1, and presenilin complex (Zhang et al., 2007; Checler et al., 2010). We report results herein that Aβ interacts directly with specific DNA sequences from regulatory region(s) of AD-associated genes.

The *APP* gene is subject to complex regulation. Its promoter includes distal (Lahiri et al., 1999) and proximal (Ge et al., 2004) regulatory elements. The *APP* promoter also includes tissue–specific elements (Lahiri et al., 2000; Ge et al., 2004). Coding sequence

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E gene; AICD, APP intracellular domain fragment; APP, Aβ-precursor protein; *APP*, Aβ-precursor protein gene; *ASCL1*, achaete–scute complex homolog 1 gene; ATF, AICD/KAT5/APBB1 transcription factory complex; Aβ, amyloid beta-peptide; *BACE1*, β-amyloid cleaving enzyme 1/β-secretase gene; EMSA, electrophoretic mobility shift assay; FAD, familial AD; HSE, heat shock element; *OLIG2*, oligodendrocyte lineage transcription factor 2 gene; p53, tumor protein 53; SNP, single-nucleotide polymorphism; *SLC38A1*, solute carrier family 38 member 1 gene; *TP53*, tumor protein 53 gene.

* Corresponding author at: 791 Union Drive, Indianapolis, IN 46202, USA. Tel.: +1 317 274 2706; fax: +1 317 274 1365.
E-mail address: dlahiri@iupui.edu (D.K. Lahiri).

0378-1119/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2011.06.004

mutations in the *APP* gene are linked to autosomally–dominant AD (Selkoe and Schenk, 2003). However, these are a minority of AD cases. Links have been determined between the more common sporadic AD and single–nucleotide polymorphisms (SNPs) in the *APP* promoter (Lahiri et al., 2005; Guyant-Marechal et al., 2007; Lv et al., 2008). Multiple transcription factors interact with the *APP* promoter, including activator protein (AP)1, specificity protein (SP)1, and upstream stimulatory factor (USF) (La Fauci et al., 1989; Lukiw et al., 1994; Kovacs et al., 1995), among others. SP1 activation of the APP gene may be subject to perturbation by environmental metal exposures (Zawia et al., 1998). In addition to regulating *APP*, AP1, SP1, and other shared transcription factors regulate other AD–associated genes such as *BACE1* (Christensen et al., 2004; Sun et al., 2005; Dosunmu et al., 2009). In this context, our discovery of this sequence–specific Aβ/DNA interaction opens an avenue for exploration of APP gene self–regulation and participation of Aβ in co–regulation of other AD–associated genes.

Extracellular Aβ is transported into the cell under oxidative and heat stress (Bailey et al., 2011; Ohyagi et al., 2005; Ohyagi and Tabira, 2006; Ohyagi et al., 2007), and Aβ nuclear localization occurs under the regulation of the Aβ-related death-inducing protein (AβDIP). Aβ can also induce an increase in levels of the apoptosis-associated tumor protein 53 (p53, gene name *TP53*) (Ohyagi et al., 2005), the transcription factor achaete–scute complex homolog 1 (*ASCL1*) (Uchida et al., 2007) and transcription of the *BACE1* (Tabaton et al., 2010) gene, while inducing reduction in levels of oligodendrocyte lineage transcription factor 2 (*OLIG2*) gene expression (Uchida et al., 2007). In the case of p53, this induction was from direct action of Aβ upon the *TP53* promoter, Aβ binding centered around a known heat shock element (HSE), "GGATTGGGGT" (Ohyagi et al., 2005).

We identified similar decamers in the 5′-flanking sequences of the *APOE*, *APP*, and *BACE1* genes, all of which are implicated in the pathogenesis of AD (Lahiri et al., 2002). These particular genes were chosen since polymorphisms in the genomic sequences *APOE*, *APP*, and *BACE1* have been associated with AD (Lv et al., 2008; Maloney et al., 2010; Wang and Jia, 2010). We generated thirteen 20-mer pairs, each containing a decamer that had at least 80% homology to the *TP53* decamer. We investigated decamers' interaction with different Aβ peptides. Six of the oligomer pairs showed interaction with Aβ, including the *TP53* oligomer. The "positive" sequences were used to generate a consensus, "KGGRKTGGGG", and a similarity matrix with the Target Explorer utility (Sosinsky et al., 2003). Negative controls consisting of an HSE within the *APP* 5′-flanking region (La Fauci et al., 1989) and an oligomer pair derived from *APP* promoter, both of which had ≤40% homology with the *TP53* decamer, did not interact with Aβ.

Of particular interest, an oligomer pair containing a single-nucleotide polymorphism (SNP) in one of the "positive" *APP* oligomer pairs significantly reduced binding with both Aβ1–40 and Aβ1–42 peptides, although binding was not qualitatively reduced with the Aβ25–35 peptide. The G→A substitution is a functional *APP* SNP that we have previously associating with increased AD risk (Lahiri et al., 2005). We also investigated the regions of the Aβ peptide that would bind the DNA decamer and determined that maximum DNA binding was obtained with the cytotoxic Aβ25–35 peptide. Taken together, we have demonstrated DNA sequence-specific interaction with the Aβ peptide. In one important instance, this specificity was to a SNP in that APP gene that has been implicated in AD risk. This suggests functional investigation of this interaction as a potential regulatory pathway for control of Aβ and possibly in development of AD.

2. Materials and methods

2.1. Chemicals/reagents

Unless otherwise specified, reagents were purchased from Sigma (St. Louis, MO) and were of "molecular biology" or "analytic" quality. Enzymes were purchased from Roche (Indianapolis, IN). Cell culture reagents were purchased from Invitrogen (Carlsbad, CA).

2.2. Aβ peptides and their fragments

Peptides Aβ1–42, 1–40, 1–28, 25–35, 29–40, 31–35, 42–1, 40–1, and 35–25 were purchased as trifluoroacetic acid salts from Bachem (Torrance, CA) and resuspended at stock concentrations of 1 mg/ml in different solvents per manufacturer's recommendations. Consultation with manufacturer indicated that peptides dissolved under these conditions would be dimers or larger aggregates.

2.3. Synthesis of different oligomers representing putative AβIDs in the regulatory regions of APOE, APP, BACE1, and TP53

The 5′-flanking regions of 4 genes (*APOE*, *APP*, *BACE1*, and *TP53*) were selected for investigation. *TP53* was chosen to as a "positive control" for Aβ–DNA interaction (Ohyagi et al., 2005). *APOE*, *APP*, and *BACE1* were selected as genes with a strong contribution to AD etiology. The *TP53* HSE decamer, 5′-GGATTGGGGT-3′ (Ohyagi et al., 2005) was used to identify potential Aβ-binding decamers within the *APOE* (Paik et al., 1985; Du et al., 2005), *APP* (Lahiri and Robakis, 1991; Hattori et al., 1997), and *BACE1* (Christensen et al., 2004; Sambamurti et al., 2004) 5′-flanking regions and introns upstream of the "ATG" start codon. A minimum 80% homology to the *TP53* sequence was required for selection. Of the decamers located on these four sequences, twelve were selected (Fig. 1). Twenty-mer pairs (Table 1) of decamers plus flanking DNA were synthesized (Invitrogen), annealed, and radiolabeled with ^{32}γP-ATP via polynucleotide kinase (Roche).

2.4. Electrophoretic mobility shift assay (EMSA) with Aβ1–42 peptide and DNA oligomers

A quantity of 1 μg of Aβ1–42 peptide was incubated in EMSA buffer 4 (ActiveMotif, Carlsbad, CA), supplemented with 25% glycerol, 0.04% Triton X-100, for 20 min at room temperature (25 °C). Oligomer pairs (50,000 CPM, approximately 75 pg), corresponding to sites at *TP53* HSE (*TP53* −1); *APOE* +171, +284, +660/+665 (two overlapping sites); *APP* −1862, −2871, −3364, −3833G; and *BACE1* +36, −119, −1766, −1939 were added, and incubation proceeded for an additional 30 min. Reactions were analyzed on native 5% polyacrylamide tris–glycine-EDTA (TGE) gels. Gels were dried and DNA–protein interactions were visualized by autoradiography. EMSA was repeated independently with Aβ1–42 and Aβ1–40, using the *APOE APP*, and *BACE1* oligomers pairs from the previous experiment, plus the following additions: An oligomer pair based on *APP* −3833G with a G→A substitution, which corresponded to a naturally occurring single-nucleotide G↔A polymorphism at the sixth base of the decamer beginning at −3833, i.e., −3829 in the *APP* promoter sequence (Lahiri et al., 2005); a pair crossing a second *APP* promoter polymorphism at −1023 (Lahiri et al., 2005), with 40% maximum homology to the *TPE* decamer; and a pair corresponding to an HSE within the *APP* promoter, with 40% maximum homology to the *TP53* decamer. Films were scanned, and signals were densitometrically quantified with ImageJ software package (Girish and Vijayalakshmi, 2004). To improve consistency of the data, the results for Aβ1–40 and Aβ1–42 EMSA were combined. Data within each film was standardized to that film's mean and standard deviation with the equation $\frac{x-m}{s}$, where x is densitometric reading, m is mean densitometric readings within that gel, and s is sample standard deviation of that gel's readings. Data was analyzed by Waller–Duncan multiple range test to determine a standardized densitometric cut-off between "non-binding" and "binding" levels of DNA–peptide interaction.

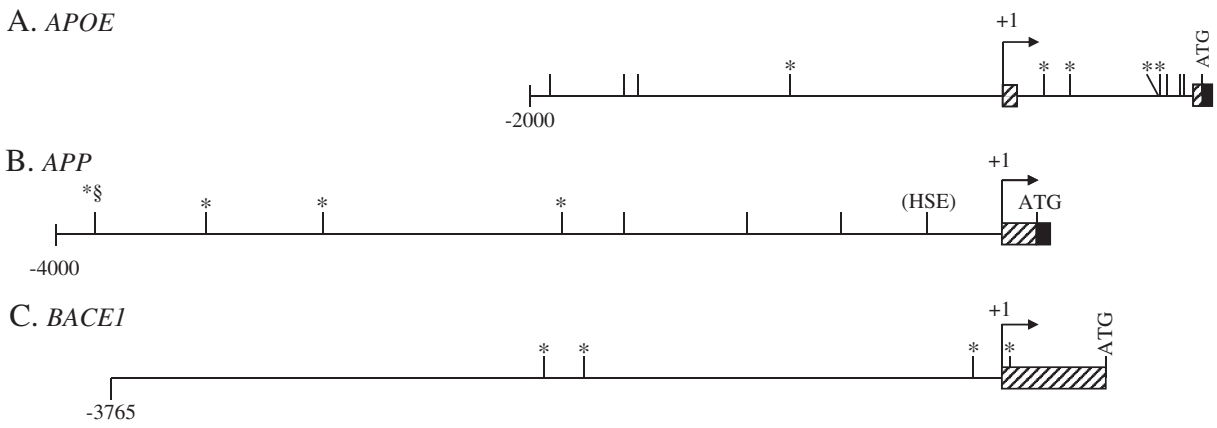


Fig. 1. Presence of Aβ-binding motifs (AβID) with 80% homology to p53 HSE sequence on *APOE*, *APP*, and *BACE1* 5′-flanking sequences. The 5′-flanking sequences of the *APOE*, *APP*, and *BACE1* genes were searched for decamers with at least 80% homology to the "GGATTGGGGT" Aβ-binding HSE site of the *TP53* promoter (Ohyagi et al., 2005). Sites marked with "*" were chosen for further study in this report. A. A region of the *APOE* gene from 2 kb upstream of the +1 TSS to the end of the first coding exon, which contains the first intron, (Paik et al., 1985; Du et al., 2005) was searched. B. The *APP* 5′-flanking region from 4 kb upstream of the +1 TSS to the end of the first coding exon (Lahiri and Robakis, 1991; Hattori et al., 1997) was searched. C. The *BACE1* 5′ flanking region from 3.8 kb upstream of the +1 TSS to the "ATG" start codon (Christensen et al., 2004; Sambamurti et al., 2004) was searched.

2.5. Competition EMSA with Aβ1–42 and Aβ1–40 peptides and DNA oligomers

Specificity of interaction between Aβ peptides and DNA sequences was tested by homocompetition EMSA, using those oligomer pairs that had previously shown interaction with Aβ. These were incubated as described, with the addition of 140× molar excess unlabeled oligomer pairs.

2.6. Concentration-dependency of Aβ peptide–DNA interactions

Reactions were performed with the oligomer pairs *APP* −3833G, *APOE* +660/+665, and *BACE1* −119. The *APP* −3833G pair was reacted with Aβ1–40 at 10, 100, 250, 500, and 1000 ng of peptide; with Aβ1–42 at 0.5, 1, 2, and 4 μg of peptide, with Aβ42–1 and 1–28 at 2, 4, and 10 μg of peptide; and with Aβ25–35 at 10, 100, 250, and 500 ng of peptide. The *APOE* +660/+665 and *BACE1* −119 oligomers were reacted with Aβ1–40, 1–42, 42–1, 1–28, and 25–35 at 500 ng and 1 μg of each peptide.

2.7. Determination of the protein-binding DNA consensus within oligomer sequences

Sequences of five experimental potential AβID-containing 20-mers that had greater than "non-binding" interaction by EMSA (see Section 3.2) plus the positive control *TP53* derived 20-mer were aligned by Target Explorer (Sosinsky et al., 2003) without pre-defining the length of the conserved motif. Target Explorer determined the motif to be a decamer. Target Explorer calculates weights for individual bases at specific motif positions using prior probabilities (priors) for each base, but available alternatives on the Target Explorer server were limited to *Drosophila* whole genome priors or estimation based on nucleotide frequencies within the submitted sequences. Neither was a desirable alternative, since appropriate priors would, in theory, be better derived from promoter sequences instead of an entire genome. Therefore, human promoter sequences were downloaded from the Eukaryotic Promoter Database (Schmid et al., 2006), version based on EMBL release 104. This database consists exclusively of experimentally confirmed PolII binding sites associated with a confirmed RNA transcript. Sequences that were not complete between −4000/+1000 were excluded, leaving 1840 sequences. Priors for each base were then calculated at each position for this database.

To determine the weight of each nucleotide (G, A, T, C) at each position, we used a position weight matrix equation: $\ln\left[\frac{(n_{i,j}+p_i)/(N+1)}{p_i}\right]$, where ln is the natural logarithm, $n_{i,j}$ = the number of times nucleotide i appears at position j in the alignment, p_i = prior probability of nucleotide i at a specific position relative to

Table 1
Oligomers generated for electrophoretic mobility shift (EMSA) assays.

Oligo	Sequence	Oligo	Sequence
p53 −1F	5′-TGATGGGATTGGGGTTTTCC-3′	BACE −119F	5′-GGGCTGGAGAGGGGTCTGGG-3′
p53 −1R	5′-GGAAAACCCCAATCCCATCA-3′	BACE −119R	5′-CCCAGACCCCTCTCCAGCCC-3′
APOE +171F	5′-GGTCGGGCTTGGGGAGAGGA-3′	BACE −1766F	5′-CTTATTGATTAGGGTTTTCT-3′
APOE +171R	5′-TCCTCTCCCCAAGCCCGACC-3′	BACE −1766R	5′-AGAAAACCCTAATCAATAAG-3′
APOE +284F	5′-CAGCTGGACTGGGATGTAAG-3′	BACE −1939F	5′-AAATTGGATTTGGTTTTTTT-3′
APOE +284R	5′-CTTACATCCCAGTCCAGCTG-3′	BACE −1939R	5′-AAAAAAACCAAATCCAATTT-3′
APOE +660/+665F	5′-AGGGAATGGGTTGGGGGCGG-3′	BACE +36F	5′-GAGCTGGATTATGGTGGCCT-3′
APOE +660/+665R	5′-CCGCCCCCAACCCATTCCCT-3′	BACE +36R	5′-AGGCCACCATAATCCAGCTC-3′
APOE −899F	5′–CACAGGTATTGTGGTTTCCA-3′	*APP* −3833GF[a]	5′-TGGGGGTGGGGGTACATAAT-3′
APOE −899R	5′-TGGAAACCACAATACCTGTG-3′	*APP* −3833GR[a]	5′-ATTATGTACCCCCACCCCCA-3′
APP −1862F	5′-AGTAGAGATGGGGGTTTCAC-3′	*APP* −3833AF[a]	5′-TGGGGGTGAGGGTACATAAT-3′
APP −1862R	5′-GTGAAACCCCCATCTCTACT-3′	*APP* −3833AR[a]	5′-ATTATGTACCCTCACCCCCA-3′
APP −2871F	5′-AACTAGGATGGGGATGCTGT-3′	*APP* HSE F[b]	5′-GCTCTCGACTTTTCTAGAGC-3′
APP −2871R	5′-ACAGCATCCCCATCCTAGTT-3′	*APP* HSE R[b]	5′-GCTCTAGAAAAGTCGAGAGC-3′
APP −3364F	5′-AAATAGAAATGGGGTATCTG-3′	*APP* −1023F[a,b]	5′-GGGGATACATCTGGGCAGTT-3′
APP−3364R	5′-CAGATACCCCATTTCTATTT-3′	*APP* −1023R[a,b]	5′-AAGTGCCCAGATGTATCCCC-3′

[a] These oligomers were previously used in characterization of two *APP* − promoter polymorphisms implicated in late-onset familial AD (Lahiri et al., 2005). The SNP site that occurs at −3829 in the *APP* promoter sequence, represented by the −3833G and A oligomers, is underlined.
[b] These oligomers were selected as negative controls due to low (≤50% homology) to the *TP53* HSE decamer.

the +1 TSS, and N = number of sequences in the alignment. Prior probabilities for individual bases are not constant along the range of human promoter sequences. Therefore, instead of deriving a static weight matrix to screen sequences of genes of interest, we used a "dynamic" calculation, where the Target Explorer equation was applied to each position in turn from −4000 to +1000, and these weights were then compared in ten-base blocks with sequences of interest, according to the position of each possible decamer within the sequences. While this is a computationally intensive method, it inherently corrects for position-dependent effects of background nucleotide frequency.

Individual bases within DNA sequences were compared to calculated weights for each base at specific positions, and the results for each decamer of bases totaled. If this total was less than the total observed for the lowest-scoring decamer in the matrix training set at that specific position between −4000/+1000, the decamer was rejected. In addition to weights based on the oligomer alignment, a sequence logo was calculated from the six aligned sequences (Schneider and Stephens, 1990).

The dynamic weight method was used to predict potential AβID sites on the *APOE* (Paik et al., 1985; Du et al., 2005), *APP* (Hattori et al., 1997), *BACE1* (Sambamurti et al., 2004), *BACE2* (Maloney et al., 2006), along with *ASCL1*; insulin-degrading enzyme (*IDE*), microtubule associated protein τ (*MAPT*), *OLIG2*, *SLC38A1*, and *TP53* (Sayers et al., 2011) 5′-flanking regions.

2.8. EMSA of selected oligomers with different Aβ peptides

Selected oligomers that bound to Aβ1–42, specifically *APOE* +171, *APOE* +660/+665, *APP* −3833G, *APP* −3833A, and *BACE1* −119, were used to screen binding capacity of Aβ peptides 1–42, 1–40, 1–28, 20–29, 25–35, 29–40, and 31–35 (Bachem) for both qualitative binding/non-binding and concentration-dependency. Each peptide was resuspended per manufacturer's instructions and 1 μg of each peptide was used in EMSA *vs.* the oligomer pairs.

2.9. Data analysis

EMSA films were scanned at 300 dpi, 14 bit-depth grayscale and densitometrically measured with ImageJ. All statistical analysis was carried out using SAS 9.1.3 (SAS Institute, Cary, NC).

3. Results

3.1. Determination of potential Aβ-interacting sequences in the APOE, APP, and BACE1 5′-flanking regions

The *APOE*, *APP*, and *BACE* 5′-flanking regions were compared to a "GGATTGGGGT" decamer from the *TP53* HSE sequence. Sites with at least 80% homology were accepted as potential AβID (Fig. 1). Within the *APOE* 5′-flanking region, 11 decamers were located. The *APP* sequence had 7 decamers, while the *BACE1* sequence had 4. One site in the *APP* 5′-flanking region included an *APP* promoter SNP we have previously linked to risk of AD (Lahiri et al., 2005).

3.2. The Aβ peptide binds DNA in a sequence-specific manner, and the reverse peptide lacks binding

To determine specificity of a potential AβID motif based upon the *TP53* decamer, Aβ1–42 and Aβ1–40 peptides were incubated with radiolabeled double-stranded oligomers corresponding to putative AβIDs in the *APP* (−3833G, −3364, −2871, −1682), *APOE* (−899, +171, +284, +660/+665), *BACE1* (−1939, −1766, −119, +36) and *TP53* (−1) 5′-flanking regions plus two additional oligomer pairs from the *APP* 5′-flanking region, specifically *APP*HSE and *APP* −1023 as controls for presumably non-specific "background" levels of DNA–peptide interaction. Mixtures were analyzed on 5% polyacrylamide-TGE gel. The gels were dried and subjected to autoradiography (Fig. 2). DNA–protein interactions were visualized as radioactive bands on the gel. Interactions were similar between Aβ1–42 and Aβ1–40. The Aβ42–1 peptide had no apparent DNA–protein interaction, while the Aβ40–1 peptide interacted with the *APP* −3833G DNA oligomer pair. However, the signal was less intense than the forward peptide (Fig. 2B). The −3833G *APP* oligomer pair is of particular interest as the sixth base of its AβID is a previously characterized *APP* promoter SNP at −3829 (Lahiri et al., 2005). The majority population sequence at this SNP is a "C" in the sense strand (corresponding to a "G" in the AβID if read in the same orientation as the *TP53* −1 decamer). The minority SNP variant would correspond to an "A" in the decamer. Of particular note, this G→A substitution significantly reduced apparent Aβ binding. DNA–protein interactions in these and further gels migrated very slowly after entering through the well. Our consultation with the peptide manufacturer indicated that their peptides would most likely resuspend as oligomers, explaining very slow electrophoresis. Pilot tests done with peptides that the manufacturer (rPeptide, Bogart, GA) deemed more likely to resuspend as monomers produced no interaction. This is touched upon further in Section 4.

Analysis of standardized densitometric scans (Fig. 3) was based on our pre-designation of the *APP*HSE and *APP* −1023 oligomer pairs as "non-specific binding" controls from their low homology with the prototype *TP53* decamer. They were used to reflect a "background signal" of non-specific DNA–protein interaction between Aβ and a given oligomer pair. The normalized EMSA signals were compared by Waller–Duncan multiple range test. Those oligomer pairs that were grouped with either of the *APP*HSE and *APP* −1023 controls by the statistical test (either of group "D" or "E" in Fig. 3) were also classified as "non-specifically binding", i.e., indistinguishable from "background". Five oligomers were excluded from the "non-specifically binding" statistical group and were, therefore, considered to have DNA sequence specific interaction with Aβ (Table 2). Two of these were from *APOE* (−171 and −660/665), two from *APP* (−3833G and −2871), and one from *BACE1* (−119). These oligomer sequences were used to determine the DNA sequence motif for the AβID. To further test the specificity of the aforementioned EMSA results, reactions were repeated in the presence of 140× molar excess of unlabeled homologous oligomer. In all cases, self-competition visibly reduced Aβ–DNA interaction (Fig. 4).

3.3. The Aβ-binding DNA motif/AβID is a specific degenerate decamer

When those oligomers that showed positive DNA–peptide interaction (Table 1), along with the *TP53* oligomer, were aligned by Target Explorer (Table 2), a consensus decamer of "KGGRKTGGGG" was determined. This decamer was "offset" 1 base upstream of the decamer originally chosen from the *TP53* sequence, and 1 or 2 bases upstream of the predicted decamers. A sequence logo was generated (Fig. 5). Four "G" bases at the 3′ end of the decamer did not vary. Altering the "G" indicated by "*" in the figure to an "A" (*APP* −3833G *vs.* −3833A) significantly diminished Aβ binding capacity. Dynamic weight analysis predicted that a decamer with a sequence of "GGGGTTGGGG" would have the maximum possible score, although this specific decamer did not appear in sequences of interest searched or in the database used to establish priors.

3.4. DNA binding of Aβ is strongest with the cytotoxic Aβ25–35 peptide

To determine the specific DNA-binding site within the Aβ peptide, various peptide fragments of Aβ, specifically 1–42, 1–40, 1–28, 20–29, 25–35, 29–40, 31–35, 42–1, 40–1, and 35–25, were reacted with the oligomers for the AβIDs at *APOE* +171 (Fig. 6A, lanes 1–10), *APOE* +660 (Fig. 6A, lanes 11–20), *APP* −3833G (Fig. 8B, lanes 1–10), and *APP* −3833A (Fig. 6B, lanes 11–20). Of the peptides tested, the 1–28,

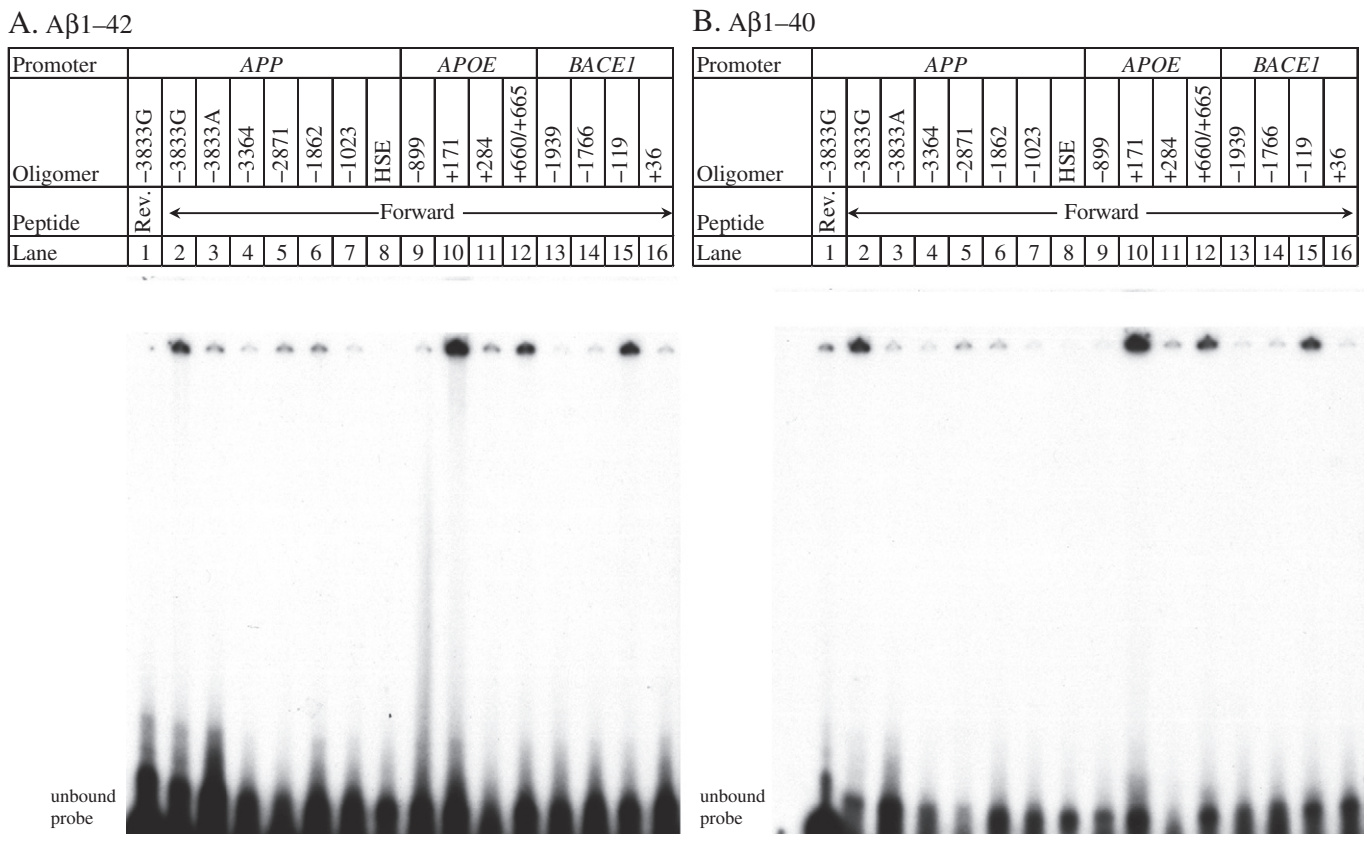


Fig. 2. Electrophoretic mobility shift assay (EMSA)/gel shift assay of Aβ1–42 *vs.* different putative binding decamer-containing oligomers. Figure shows representative EMSA of the A. Aβ1–42 and the B, Aβ1–40 peptides. The reverse Aβ40–1 and 42–1 peptides were independently incubated with radiolabeled oligomer containing the putative AβID at −3833 (lane 1). Aβ1–42 or 1–40 (lanes 2–16) were incubated with radiolabeled oligomers that contained the putative AβIDs in the *APP* (lanes 2–8) 5′-flanking region at positions −3833G (lane 2), −3833A with a "G" to "A" substitution at −3829 (lane 3), −3364 (lane 4), −2871 (lane 5) and −1862 (lane 6). In addition, an oligomer corresponding to the FAD mutation site in *APP* at −1023 (lane 7) and a distinct HSE-binding site within *APP* (lane 8) were incubated and run; in the *APOE* (lanes 9–12) 5′-flanking region at positions −899 (lane 9), +171 (lane 10), +284 (lane 11), +660/+665 (lane 12); and in the *BACE1* (lanes 13–16) 5′-flanking region at positions −1939 (lane 13), −1766 (lane 14), −119 (lane 15), and +36 (lane 16). The gel was dried and exposed to X-ray film for autoradiography. DNA–peptide interactions appeared as dark signal near the top of the gel, unbound oligomer ran to the bottom of the gel.

20–29, 31–35, 35–25, and 42–1 peptides had no apparent DNA-binding capacity. Of the remaining peptides, binding capacity was, from highest to lowest, 25–35>29–40>1–40>1–42>40–1 (Figs. 6 and 7). The particularly cytotoxic (Millucci et al., 2010) Aβ25–35 peptide had the greatest DNA-binding capacity with any of the DNA oligomer pairs studied.

In addition, the polymorphic *APP* −3833A oligomer pair was allowed to interact with the same series of Aβ peptides. This pair did not have apparent binding with either Aβ1–42 or 1–40, although the high binding affinity of the 25–35 peptide did result in apparently strong interaction (Fig. 6B). Apparent differences between different peptide migration rates were minimal. This was probably because our particular preparations of Aβ were likely to be aggregates instead of monomers, according to the manufacturer.

3.5. Binding of Aβ1–42 and 1–40 occurred in a concentration-dependent manner

To determine if Aβ–DNA interaction occurred in a concentration-dependent manner typical of transcription factors, we selected one "positive" oligomer pair each from *APOE*, *APP*, and *BACE* to bind with the Aβ1–42, 1–40, 42–1, 1–28, and 25–35 peptides (Fig. 8A). The 1–40 and 1–42 peptides both showed qualitative concentration-dependent increases in interaction with the target DNA oligomer pairs. The 1–40 peptide (10 ng–1 μg with *APP* −3383G) showed a greater apparent interaction at any given amount of peptide when compared to the 1–42 peptide (500 ng–4 μg with *APP* −3383G). The interaction of the 25–35 peptide with the −3833G oligomer pair was less dependent upon peptide concentration than for the 1–40 and 1–42 peptides. The reverse peptide (and negative control) 42–1, and forward 1–28 peptide showed no DNA–protein interaction. Two different concentrations of each of these peptides was interacted with the *APOE* +660/+665 and *BACE1* −119 oligomer pairs, with similar results (Fig. 8B).

3.6. AβID sites predicted in AD-related gene promoter sequences

Dynamic weight probing of the *APOE*, *APP*, *ASCL1*, *BACE1*, *IDE*, *MAPT*, *OLIG2*, and *SLC38A1* 5′-flanking regions found 11 AβIDs in APOE, 3 in APP, 5 in BACE1, and 2 in TP53 and found 6 in ASCL1, 1 in BACE2, 1 in IDE, 4 in MAPT, 1 in OLIG2, and 1 in SLC38A1 (Table 3, Fig. 9). The weight-based search of *APOE*, *APP*, and *BACE1* produced no "false negatives", and all AβIDs confirmed by EMSA had corresponding decamers from matrix-based search, with a 1 or 2-base "offset". In addition, no AβIDs determined by matrix search corresponded with any of the decamers that had been experimentally determined to lack Aβ-binding capacity. Comparable search was performed in genes unrelated to AD pathology. Frequency of the AβID within a search of over 1000 4 kb promoter sequence was significantly different from its calculated frequency in a random 4 kb sequence with the same base frequency distribution (data not shown).

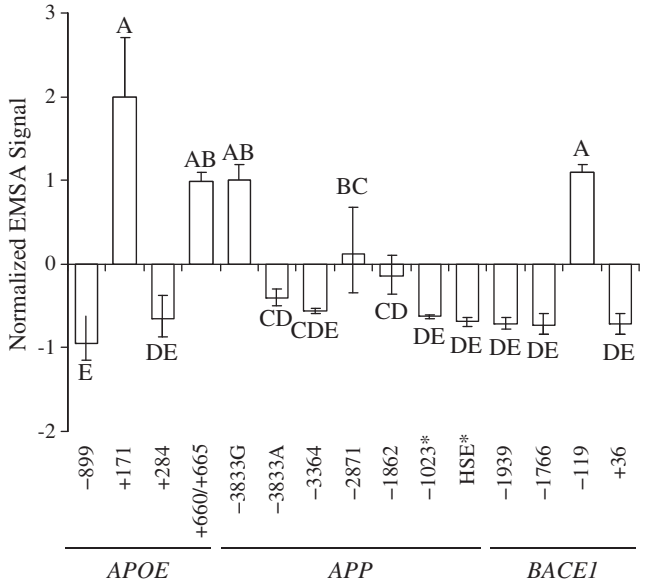


Fig. 3. Determination of "binding" *vs.* "non-binding oligomer pairs to Aβ peptides in EMSA. EMSA films were densitometrically scanned and signals within each film were normalized by subtracting the mean of that signal's film from the individual signal and dividing by the standard deviation of signals. Normalized signals were then analyzed by Waller–Duncan means separation, and categories indicated with letters on the figure. Samples sharing the same letter were not significantly different at k = 100 (analogous to α = 0.05). The two "background control" oligomer pairs, *APP*HSE and *APP* −1023, indicated by "*".

4. Discussion

A distinctive feature of AD is the accumulation of amyloid plaque in affected brains. The primary constituent of this plaque is Aβ peptide, derived from the much larger APP protein. Aβ is neurotoxic in monomers and oligomers (Walsh et al., 2002; Morgan et al., 2004; Ono et al., 2009). Therefore, research has concentrated on the "pathogenic function" of the peptides, especially the Aβ1–42 and Aβ1–40 peptides. However, there has been ongoing work on elucidating non-pathogenic function for the Aβ peptide. For example, Aβ modulates glutamatergic transmission in the rat basal forebrain (Chin et al., 2007). Its activity in kinase induction has also been investigated (Bogoyevitch et al., 2004). Aβ has also been shown to reduce metal-induced oxidative damage (Zou et al., 2002; Baruch-Suchodolsky and Fischer, 2009) and potentially to regulate cholesterol transport (Yao and Papadopoulos, 2002; Igbavboa et al., 2009).

Aβ has been shown to locate intracellularly in response to conditions such as oxidative stress (Bailey et al., 2011; Ohyagi and Tabira, 2006). These aspects of Aβ structure and location lead to considering whether some of Aβ's pathological activity may be related to direct peptide–DNA interaction with genes such as those that determine apoptosis. To test this hypothesis, Ohyagi et al. investigated whether or not intracellular Aβ peptide altered expression of the *TP53* apoptosis-associated protein gene (Ohyagi et al., 2005). What they determined was that, not only did Aβ enhance p53 levels, but that it did so through direct interaction with a region that contains an HSE on the *TP53* promoter/5′-UTR sequence. The transcription factors ASCL1 and OLIG2 have also shown to be differentially regulated in cell culture by Aβ (Uchida et al., 2007). Native *BACE1* gene transcription is upregulated in cell culture by addition of Aβ1–42 (Tamagno et al., 2009).

Another cleavage product of the APP protein, AICD, forms part of the AICD/KAT5/APBB1 transcription factory complex (ATF) complex, along with K (lysine) Acetyltransferase 5 (KAT5/TIP60) and the APP-Binding, Family B, Member 1 protein (APBB1/Fe65) (Konietzko et al., 2010). The ATF, in particular, can regulate *APP* gene transcription (von Rotz et al., 2004). However, it is unknown whether Aβ participates in this particular pathway as a possible transcription factor, especially since Aβ stimulation of *BACE1* RNA transcription occurred independently of addition of AICD (Giliberto et al., 2009).

Surface plasmon resonance has established that soluble Aβ1–42 readily binds DNA *in vitro* (Barrantes et al., 2007), although specificity of its binding site was not determined by that method. It has also been determined that p53, itself, downregulates expression of the APP gene (Cuesta et al., 2009). Aβ is likely to contain a helix–loop–helix structure common to certain transcription factors (Durell et al., 1994), and this theoretical model is similar to work that demonstrates a helix–kink–helix structure (Shao et al., 1999; Crescenzi et al., 2002) in apolar environments. In an aqueous environment, the Aβ peptide can transition between a β–turn–β structure and a more amorphous form with two small helices (Tomaselli et al., 2006).

To investigate the possibility of transcription factor-like sequence specific DNA–Aβ affinity, we have determined a consensus matrix for Aβ–DNA interaction. The consensus is a "G"-rich decamer with the IUPAC sequence 5′-KGGRKTGGGG-3′. However, we urge extreme caution in favoring this for motif searching instead of the sequence logo or a frequency matrix, given the severe limitations of consensus sequences (Schneider, 2002). The empirically determined decamer was "offset" from the original we used to search for oligomers used in EMSA. This might raise questions of actual specificity for Aβ–DNA interaction. However, our EMSA was not done solely with these decamers. Instead, we used 20-mers that contained native flanking sequences. In addition, the work done by Ohyagi et al. also used flanking sequences for DNA–protein binding assays. Thus, while the *TP53* HSE that happens to share sequence with the AβID might have one specific sequence, the actual AβID may only share partial overlap with the *TPE* HSE, and our use of multiple putative oligomers was able to more finely distinguish the difference. It bears note that the majority of our test oligomers did not have significant DNA–Aβ interaction by EMSA, giving our direct empirical evidence of Aβ interaction with specific DNA sequences and "rejection" of non-specific sequences.

We readily admit that non-specific interaction between AD plaque and oligonucleotide DNA has long been known (Syrjanen et al., 1991). We do not accept that this precludes sequence-specific interaction between intracellular Aβ and DNA, specifically because: i) "Amyloid plaque" does not exclusively consist of Aβ. Other proteins such as α-synuclein and ApoE are also plaque constituents (Sheng et al., 1996; Yokota et al., 2003); ii) amyloid plaque has a very different tertiary structure than do Aβ monomers, dimers, or oligomers, and properties resulting from higher-order structures, including significant change in DNA binding specificity, cannot be excluded; and iii) interaction with DNA in a non-specific manner does not automatically preclude a protein from being a transcription factor. It has been long established that transcription factors bind DNA in both sequence specific and non-specific fashions (Postel et al., 1993; von Hippel, 1994). Likewise, non-specific binding of a transcription factor to DNA is part of the initial process of its recognition of a specific binding site (von Hippel, 1994). Thus, we accept specificity for Aβ–AβID interaction. Significantly

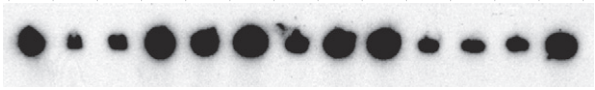
Table 2
Aligned Aβ-binding oligomers determined by semiquantitative EMSA.

Gene	+/−	Position[a]		Sequence[b]
		Predicted	Aligned	
APOE	+	+171	+170	-GGTC*GGGCTTGGGG*AGAGGA-
APOE	+	+660/665	+664	AGGGAA*TGGGTTGGGG*CGG-
APP	−	−2871	−2873	-AAC*TAGGATGGGG*ATGCTGT-
APP	−	−3833	−3831	-T*GGGGGTGGGGG*TACATAAT
BACE1	−	−119	−118	-GGG*CTGGAGAGGGG*TCTGGG-
TP53	+	−1	−2	-TGAT*GGGATTGGGG*TTTTCC-

[a] Position is relative to the +1 transcription start site.
[b] AβID decamers found by sequence alignment are italicized. Putative AβIDs predicted from 80% homology to the TP53 HSE are underlined.

A

Promoter	*APOE*				*APP*				*BACE1*				*TP53*
Site	+171*	−899	+284	+660/+665*	−1862*	−2871*	−3364	−3833G*	−119*	−1766	−1939*	+36	−1*
Peptide	← Aβ1–42 →												
Lane	1	2	3	4	5	6	7	8	9	10	11	12	13



B

Promoter	*APOE*				*APP*						*BACE1*				*TP53*	
Site	+171		+660/665		−1862		−2871		−3833G		−119		−1939		−1	
Competition	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
Peptide	← Aβ1–42 →															
Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

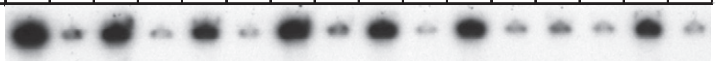


Fig. 4. Competition EMSA with "positive" oligomer pairs from *APOE*, *APP*, *BACE1* and *TP53* promoters *vs.* Aβ1–42 peptide. A. Aβ1–42 peptide was incubated with radiolabeled oligomers that contained the putative AβIDs in the *APOE* (lanes 1–4) 5′–flanking region at positions +171 (lane 1), −899 (lane 2), +284 (Lane 3), +660/+665 (lane 4); in the *APP* (lanes 5–8) 5′–flanking region at positions −1862 (lane 5), −2871 (lane 6), −3364 (lane 7), and −3833 (lane 8); and in the *BACE1* (lanes 9–12) 5′–flanking region at positions −119 (lane 9), −1766 (lane 10), −1939 (lane 11), and +36 (lane 12). In addition, an oligomer containing the Aβ-binding element from p53 was labeled and run (lane 13). The gel was dried and exposed to X-ray film for autoradiography. Specific oligomer pairs that were selected for competition EMSA are indicated with "*". B. Aβ1–42 was incubated with radiolabeled oligomers that had previously shown interaction with Aβ1–42 (lanes 1, 3, 5, 7, 9, 11, 13, 15). These reactions were repeated with the addition of 140× molar excess unlabeled oligomer (lanes 2, 4, 6, 8, 10, 12, 14, 16). Specificity of Aβ/oligomer interaction is demonstrated by a significant reduction of autoradiograph signal at top of lane. The gel was dried and exposed to X-ray film for autoradiography. DNA–peptide interactions appeared as dark signal near the top of the gel.

weaker interactions with sequences such as *APPHSE* and *APP* −1023 would be "background signal" from non-specific DNA–protein interaction.

We further determined that a single "G" DNA base within this decamer was required for DNA–protein interaction. The *APP* gene contains a previously characterized familial Alzheimer's disease (FAD) associated SNP at −3829 (Lahiri et al., 2005). The AβID at −3831 (which had a predicted position of −3833) includes this SNP site as the eighth base of its sequence. In the "sense" orientation for the *APP* gene, this is a C ↔ T polymorphism. We represent it herein as G ↔ A to preserve the orientation reading determined by Ohyagi et al. within *TP53*. The difference we observed between "G" and "A" response to Aβ 25–35 treatment was not dramatic, suggesting some possibility that this fragment of Aβ is sufficient for DNA binding, but lacks the specificity of the full-length Aβ peptides.

We investigated the 5′-flanking regions of selected genes involved in AD for potential AβIDs according to our weight matrix. In addition to conducting a search on the *APOE*, *APP*, and *BACE1* sequences, we also looked at the *ASCL1*, *BACE2*, *IDE*, *MAPT*, *OLIG2*, *SLC38A1*, and *TP53* 5′-flanking sequences. All sequences had predicted AβIDs. The *MAPT* sequence is of interest in AD research because it codes for microtubule-associated protein τ, the primary constituent in intraneuronal hyperphosphorylated τ tangles associated with AD (Iqbal et al., 2010). Overproduction of Aβ has already been shown to result in greater hyperphosphorylation of τ protein (Wang et al., 2006a, 2006b). The presence of AβIDs on the *MAPT* promoter suggests a further relationship between the two products. The *IDE* gene codes for insulysin/insulin-degrading enzyme, which has been shown to degrade Aβ *in vitro* and *in vivo* (Farris et al., 2003; Leissring et al., 2003; Eckman and Eckman, 2005). Presence of predicted AβID suggests further study in characterizing its role in AD. ASCL1 and OLIG2 have been shown to be regulated by Aβ, although the specific pathway of regulation was not suggested in that work (Uchida et al., 2007). The presence of AβIDs on both of the genes' sequences suggest

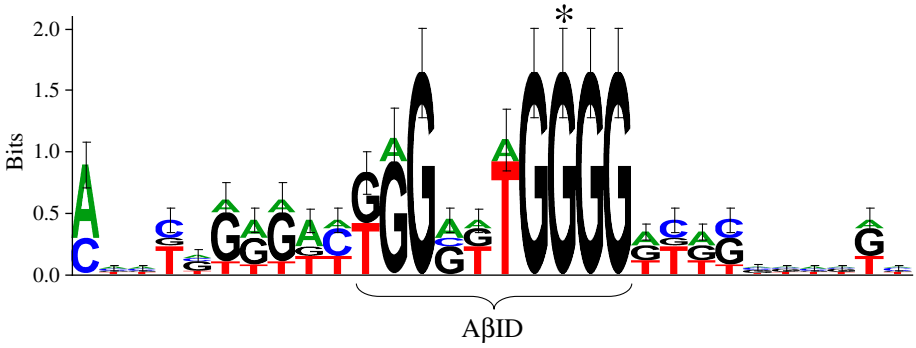


Fig. 5. Structure of the Aβ-binding domain (AβID) consensus motif. Combined height of stacked letters corresponds to bits of information (Shannon, 1997). An asterisk indicates the position of a "G" that may be critical for Aβ binding activity.

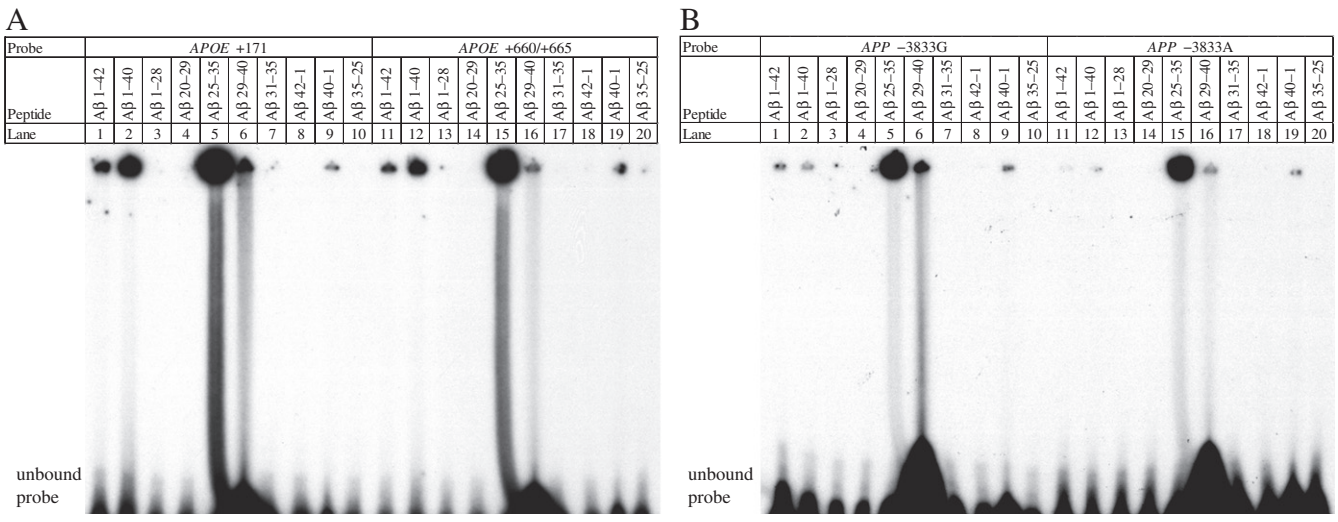


Fig. 6. EMSA of different fragments of the Aβ peptide *vs.* four different DNA probes. Fragments of Aβ peptide corresponding to residues 1–42, 1–40, 1–28, 20–29, 25–35, 29–40, and 31–35 were self-oligomerized. In addition, "reverse" fragments 42–1, 40–1, and 35–25 were prepared. A. Fragments were incubated against oligomers the contained the Aβ-binding sequence at *APOE* +171 (lanes 1–10) or *APOE* +660 (lanes 11–20). The gel was dried and exposed to X-ray film for autoradiography. DNA–peptide interactions appeared as dark signal near the top of the gel. B. Fragments were incubated against oligomers the contained the Aβ-binding sequence at *APP* −3833G (lanes 1–10) or *APP* −3833A (with a "G" → "A" substitution) (lanes 11–20). The gel was dried and exposed to X-ray film for autoradiography. DNA–peptide interactions appeared as dark signal near the top of the gel.

the possibility that intracellular Aβ may operate directly upon the promoters in a manner similar to that found for *TP53* by Ohyagi et al. (2005) and for *BACE1* and *APP* herein.

We also analyzed peptides within the Aβ1–42 peptide and determined that the Aβ25–35 peptide contains the seat for strongest DNA–protein interaction. However, the DNA-binding affinity of the

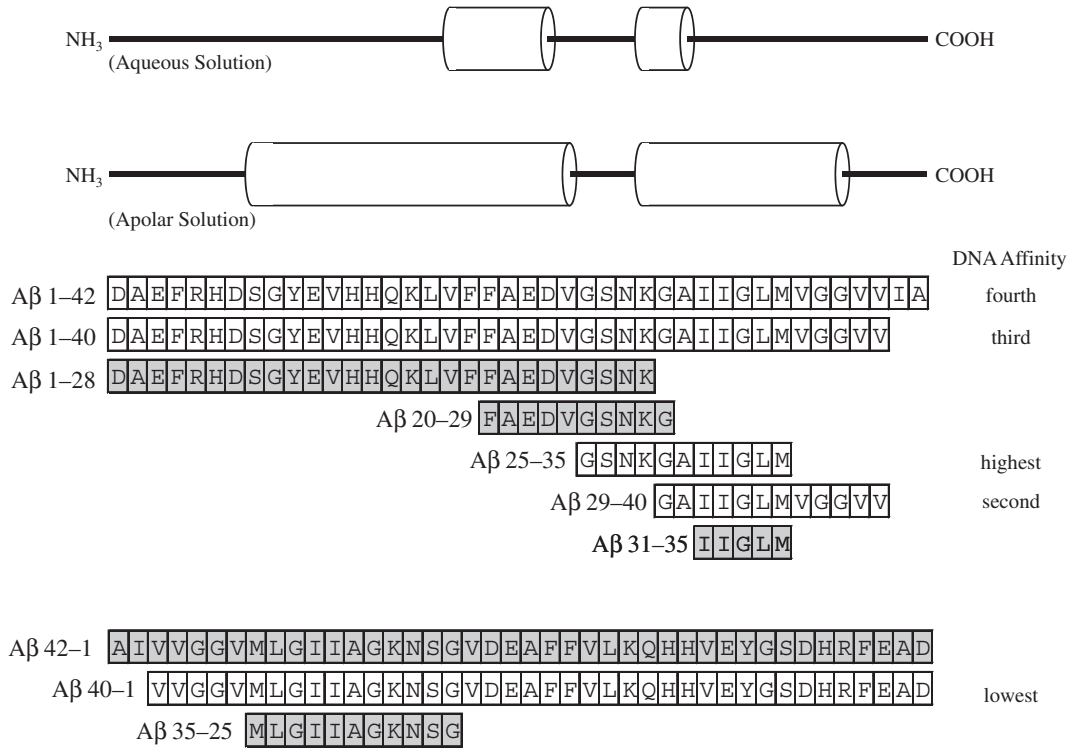
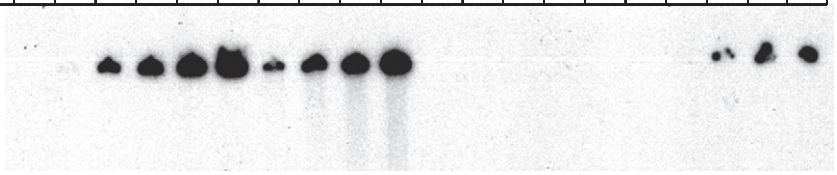


Fig. 7. Sequences and alignment of different Aβ fragments used in EMSA. All diagrams are aligned and to the same scale. A. "Helical-form" secondary structure of Aβ1–42 in aqueous environment (Tomaselli et al., 2006), helices indicated by cylinders. B. Secondary structure of Aβ1–42 in apolar solvent (Crescenzi et al., 2002), helices indicated by cylinders. C. Forward sequences. Sequences for Aβ 1–42, 1–40, 1–28, 20–29, 25–35, 29–40, and 31–35 are depicted as aligned amino acid residues. Those peptides with no apparent DNA-binding activity are in gray background. D. Reverse sequences. Sequences for Aβ42–1, 40–1, and 35–25 are depicted as aligned amino acid residues. Those peptides with no apparent DNA-binding activity are in gray background.

A

Probe	*APP* −3833G																			
Peptide	–	Aβ 1–40					Aβ 1–42				Aβ 4–21			Aβ 1–28			Aβ 25–35			
Amount		10 ng	100 ng	250 ng	500 ng	1 μg	500 ng	1 μg	2 μg	4 μg	2 μg	4 μg	10 μg	2 μg	4 μg	10 μg	10 ng	100 ng	250 ng	500 ng
Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20



B

Probe	*APOE* +660/+665										*BACE1* −119									
Peptide	Aβ 1–40		Aβ 1–42		Aβ 42–1		Aβ 1–28		Aβ 25–35		Aβ 1–40		Aβ 1–42		Aβ 42–1		Aβ 1–28		Aβ 25–35	
Amount	500 ng	1 μg	500 ng	1 μg	500 ng	1 μg	500 ng	1 μg	500 ng	1 μg	500 ng	1 μg	500 ng	1 μg	500 ng	1 μg	500 ng	1 μg	500 ng	1 μg
Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

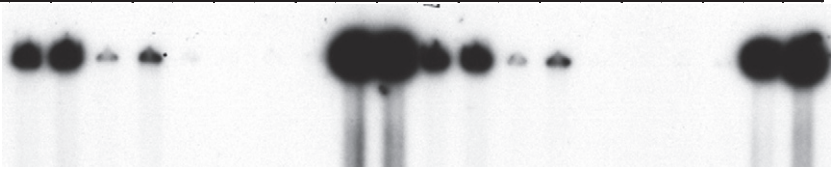


Fig. 8. Concentration dependency of Aβ–DNA interaction—A. The oligomer containing the Aβ-binding consensus at *APP* −3833G was incubated with fragments of the Aβ peptide, specifically Aβ1–40 (lanes 2–6), 1–42 (lanes 7–10), 42–1 (lanes 11–13), 1–28 (lanes 14–16), and 25–35 (lanes 17–20) at different peptide concentrations as indicated. The gel was dried and exposed to X-ray film for autoradiography. DNA–peptide interactions appeared as dark signal near top of gel. B. The oligomers containing the Aβ-binding consensus at *APOE* +660 (lanes 1–10) and at *BACE1* −119 (lanes 11–20) were incubated with fragments of the Aβ peptide, specifically Aβ1–40 (lanes 1–2, 11–12), 1–42 (lanes 3–4, 13–14), 42–1 (lanes 5–6, 15–16), 1–28 (lanes 7–8, 17–18), and 25–35 (lanes 9–10, 19–20) at different peptide concentrations as indicated. The gel was dried and exposed to X-ray film for autoradiography. DNA–peptide interactions appeared as dark signal near top of gel.

25–35 peptide is so high that it may reduce target sequence specificity. For example, if the Aβ25–35 peptide was incubated with the two *APP* FAD oligomer pairs, no apparent change in DNA–protein interaction occurred when qualitatively comparing the "A" oligomer pair with the "G" pair. This greater DNA affinity may explain the moderate differences that we observed when comparing the SNP -containing clones' responses to Aβ25 -35 treatment. Alternatively, amino acid sequences that are present in full-length Aβ, but absent in the Aβ25-35 fragment may confer DNA sequence specificity to the Aβ-DNA interaction could explain this apparent lack of specificity. Additional factors such as DNA modification of DNA secondary structure and histone association by the SNP could partially "overcome" the lower

Table 3
Aβ-binding DNA decamers predicted by dynamic weight search.

Gene	Location[a]	Orien.	Sequence[b]	Gene	Location	Orien.	Sequence
APOE	−3001	F	TAGGTTGGGG	*BACE1*	−193	R	GTCTGCCCTC
APOE	−2288	R	GGGGTAGGAG	***BACE1***	**−118**	**R**	**TCTCCCGTAG**
APOE	−2235	R	GGGGTAGGGG	*BACE1*	335	R	GACCGCAGGA
APOE	−847	F	TGGGGAGGGG	*BACE1*	823	R	CCTGTCGTCC
APOE	−325	R	GGGGAGAGGT	*BACE1*	881	R	TTCACAAGGG
APOE[c]	**170**	**F**	**GGGCTTGGGG**	*BACE2*	−591	F	GGGGGTGGGG
APOE	265	F	TGGGGTGGGG	*IDE*	−1229	R	GGGGTCGGGT
APOE	547	F	TGGGGAGGGG	*MAPT*	−2352	R	GGGGAAGGGT
APOE	553	F	GGGGGTGGGG	*MAPT*	−2069	F	GGGGTTGGGG
APOE	618	F	GGGGATGGGG	*MAPT*	614	F	GGGGGAGGGG
APOE	**664**	**F**	**TGGGTTGGGG**	*MAPT*	964	F	TGGGGAGGGG
APP[d]	**−3831**	**R**	**GGGGTGGGGG**	*OLIG2*	−1969	R	GGGGTGGGGG
APP	**−2873**	**F**	**TAGGATGGGG**	*SLC38A1*	93	R	GGGGTGGGGG
APP	745	R	GGGGTAGGGG	***TP53***	**−2**	**F**	**GGGATTGGGG**
ASCL1	−3871	R	GGGGTGGGAG	*TP53*	258	F	GGGGGTGGGG
ASCL1	−958	F	TGGGGAGGGG				
ASCL1	−504	R	GGGGTGAGGG				
ASCL1	−339	R	GGGGTGGGGG				
ASCL1	−116	F	GGGAGTGGGG				
ASCL1	−81	F	GGGAGTGGGG				

[a] Location is the 5′-most base of the decamer, relative to the +1 TSS.
[b] Sequence of reverse-orientation decamers is reverse-complemented.
[c] Boldface indicates confirmation of this sequence by EMSA.
[d] This decamer crosses a previously characterized *APP* single-nucleotide polymorphism (underlined).

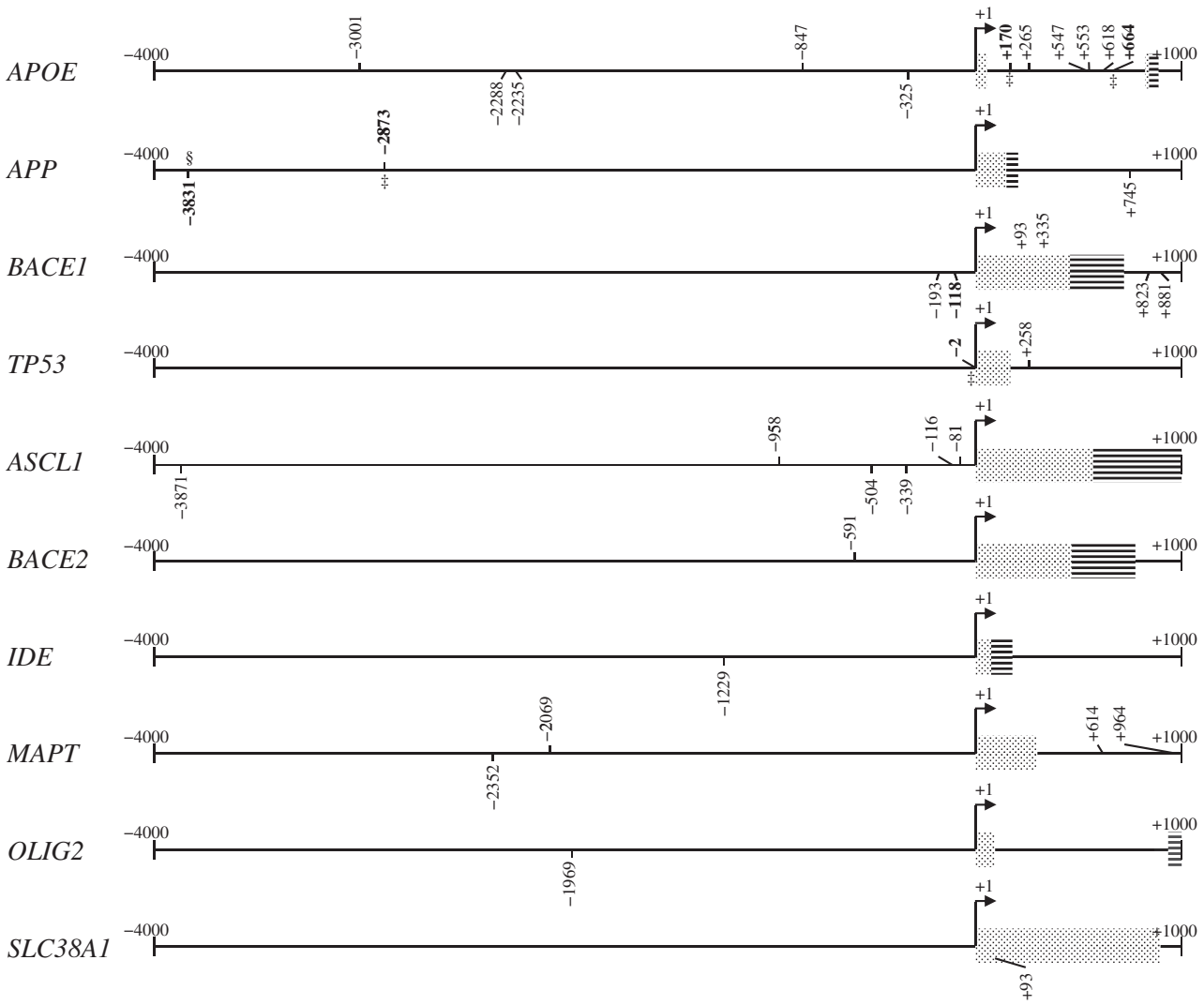


Fig. 9. Presence of confirmed AβIDs and putative motifs as predicted by dynamic weight scores on selected gene 5′-flanking regions. The 5′-flanking sequences of several genes were searched for decamers, using the weight matrix we generated. Matrix-matching sites are indicated on the sequences. Forward-orientation sites are above the sequence line. Reverse-orientation sites are below the sequence line. The 5′-flanking regions from 4 kb upstream to 1 kb downstream of the +1 transcription start site were analyzed for the *APOE*, *APP*, *BACE1*, *TP53*, *ASCL1*, *BACE2*, *IDE*, *MAPT*, *OLIG2*, and *SLC38A1* gene sequences. Positions that corresponded to oligomers for our EMSA herein, all positive on EMSA, are boldface. A site on the *APP* sequence marked with "§" crosses a previously-characterized *APP* polymorphism associated with late-onset familial AD.

specificity to produce a measurable functional difference in cell culture still dependent upon differences in overall transcription factor activity by Aβ on this site.

The EMSA results we present herein were obtained using Aβ peptides that were solubilized as aggregates. We have also performed EMSA with Aβ peptides generated from recombinant *E. coli* (rPeptide, Bogart, GA, USA). According to the manufacturer, these Aβ peptides were far more likely to be monomers if solubilized according to the manufacturer's instructions. EMSA assays performed with these peptides were universally negative (data not shown). Several transcription factors, such as NF-κB (Grilli et al., 1995), must consist of homo- or heterodimers of smaller protein subunits to bind DNA in a site-specific fashion. Individual Aβ peptides could function as such subunits for a higher-order transcription factor dimer/aggregate form. Such aggregation also would explain the very slow migration of our EMSA reactions in the gels.

We propose that, whatever other functions it may have, specific DNA–peptide interaction suggests that Aβ could be investigated as a possible transcription factor. Current treatments for AD concentrate upon remediation of cholinergic loss or other specific receptor-based treatments (Sambamurti et al., 2011) However, even if restricting AD etiology to effects of Aβ, evidence exists to suggest a wide variety of pathways that are worth therapeutic exploration (Lahiri and Maloney, 2010a). Furthermore, other pathways, such as loss of synaptic markers, may not be directly caused by Aβ and could be fruitfully treated on their own (Bailey and Lahiri, 2010). Given that the AβID is particularly "G" rich, oxidative damage of DNA may play a role in disrupting normal Aβ–DNA interaction. This would be compatible with a recently-proposed gene–environment interaction model of AD etiology, which proposes a specific mechanistic role for DNA oxidation and aberrant DNA methylation in early-life environmental induction of latent predisposition for AD and other idiopathic neurobiological disorders (Lahiri et al., 2009b).

We have determined herein that a degenerate DNA decamer that binds to the Aβ peptide in a sequence–specific manner. Furthermore, binding of Aβ to DNA requires a specific fragment of the Aβ peptide, between residues 25 and 35. These data would not be sufficient to propose that Aβ's very broad range of potential functions would include activity as a transcription factor, modifying expression of AD-related genes, such as *APP* and *BACE1* and glutamatergic pathway genes, such as *SLC38A1*. Functional studies would need to be performed. We have recently done such studies and determined that reporter clones

containing the AβID respond to treatment by Aβ peptides. Furthermore, this response was reduced significantly by mutation of the AβID at −3831 AβID site from −3829G to the −3833A SNP variant. In addition, chromatin immune precipitation assays on human neuroblastoma cell chromatin indicated specific binding of Aβ to AβID sequences within the *APP* and *BACE1* promoters (Bailey et al., 2011). The specificity of DNA interaction as we determined in the present work, coupled with the functional results we have also recently obtained, lead us to suggest the possibility that Aβ may function as a transcription factor and may further act to at least in part regulate its own precursor gene regulation.

Acknowledgments

This work was supported by the Alzheimer's Association and National Institutes of Health AG18379 and AG18884. Portions of this work have been presented as part of the proceedings of the 21st American Peptide Symposium (Lahiri et al., 2009a). We sincerely thank Yuan-Wen Ge for providing excellent technical assistance, and Jason Bailey for critically reading the manuscript.

References

Bailey, J.A., Lahiri, D.K., 2010. A novel effect of rivastigmine on pre-synaptic proteins and neuronal viability in a neurodegeneration model of fetal rat primary cortical cultures and its implication in Alzheimer's disease. J. Neurochem. 112, 843–853.
Bailey, J.A., Maloney, B., Ge, Y.-W., Lahiri, D.K., 2011. Functional activity of the novel Alzheimer's amyloid β-peptide interacting domain (AβID) in the APP and BACE1 promoter sequences and implications in activating apoptotic genes and in amyloidogenesis. Gene 488, 13–22.
Barrantes, A., Rejas, M.T., Benitez, M.J., Jimenez, J.S., 2007. Interaction between Alzheimer's Abeta1–42 peptide and DNA detected by surface plasmon resonance. J. Alzheimers Dis. 12, 345–355.
Baruch-Suchodolsky, R., Fischer, B., 2009. Abeta40, either soluble or aggregated, is a remarkably potent antioxidant in cell-free oxidative systems. Biochemistry 48, 4354–4370.
Bogoyevitch, M.A., Boehm, I., Oakley, A., Ketterman, A.J., Barr, R.K., 2004. Targeting the JNK MAPK cascade for inhibition: basic science and therapeutic potential. Biochim. Biophys. Acta 1697, 89–101.
Checler, F., Dunys, J., Pardossi-Piquard, R., Alves da Costa, C., 2010. p53 is regulated by and regulates members of the gamma-secretase complex. Neurodegener. Dis. 7, 50–55.
Chin, J.H., Ma, L., MacTavish, D., Jhamandas, J.H., 2007. Amyloid beta protein modulates glutamate-mediated neurotransmission in the rat basal forebrain: involvement of presynaptic neuronal nicotinic acetylcholine and metabotropic glutamate receptors. J. Neurosci. 27, 9262–9269.
Christensen, M.A., Zhou, W., Qing, H., Lehman, A., Philipsen, S., Song, W., 2004. Transcriptional regulation of BACE1, the beta-amyloid precursor protein beta-secretase, by Sp1. Mol. Cell Biol. 24, 865–874.
Crescenzi, O., Tomaselli, S., Guerrini, R., Salvadori, S., D'Ursi, A.M., Temussi, P.A., Picone, D., 2002. Solution structure of the Alzheimer amyloid beta-peptide (1–42) in an apolar microenvironment. Similarity with a virus fusion domain. Eur. J. Biochem. 269, 5642–5648.
Cuesta, A., Zambrano, A., Royo, M., Pascual, A., 2009. The tumour suppressor p53 regulates the expression of amyloid precursor protein (APP). Biochem. J. 418, 643–650.
De Strooper, B., 2010. Proteases and proteolysis in Alzheimer disease: a multifactorial view on the disease process. Physiol. Rev. 90, 465–494.
Dosunmu, R., Wu, J., Adwan, L., Maloney, B., Basha, M.R., McPherson, C.A., Harry, G.J., Rice, D.C., Zawia, N.H., Lahiri, D.K., 2009. Lifespan profiles of Alzheimer's disease-associated genes and products in monkeys and mice. J. Alzheimers Dis. 18, 211–230.
Du, Y., Chen, X., Wei, X., Ge, Y.-W., Bales, K.R., Berg, D.T., Paul, S.M., Farlow, M.R., Lahiri, D.K., 2005. NF-kappaB mediates amyloid beta peptide-stimulated activity of the human apolipoprotein E gene promoter in human astroglial cells. Brain Res. Mol. Brain Res. 136, 177–188.
Durell, S.R., Guy, H.R., Arispe, N., Rojas, E., Pollard, H.B., 1994. Theoretical models of the ion channel structure of amyloid beta-protein. Biophys. J. 67, 2137–2145.
Eckman, E.A., Eckman, C.B., 2005. Abeta-degrading enzymes: modulators of Alzheimer's disease pathogenesis and targets for therapeutic intervention. Biochem. Soc. Trans. 33, 1101–1105.
Farris, W., Mansourian, S., Leissring, M.A., Eckman, E.A., Bertram, L., Eckman, C.B., Tanzi, R.E., Selkoe, D., 2003. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc. Natl. Acad. Sci. U. S. A. 100, 4162–4167.
Ge, Y.-W., Ghosh, M., Song, W., Maloney, B., Lahiri, D., 2004. Mechanism of promoter activity of the beta-amyloid precursor protein gene in different cell types. Identification of a specific 30 bp fragment in the proximal promoter region. J. Neurochem. 90, 1432–1444.
Giliberto, L., et al., 2009. Mutant presenilin 1 increases the expression and activity of BACE1. J. Biol. Chem. 284, 9027–9038.
Girish, V., Vijayalakshmi, A., 2004. Affordable image analysis using NIH Image/ImageJ. Indian J. Cancer 41, 47.
Gouras, G.K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F., Greenfield, J.P., Haroutunian, V., Buxbaum, J.D., Xu, H., Greengard, P., Relkin, N.R., 2000. Intraneuronal Abeta42 accumulation in human brain. Am. J. Pathol. 156, 15–20.
Grilli, M., Ribola, M., Alberici, A., Valerio, A., Memo, M., Spano, P., 1995. Identification and characterization of a kappa B/Rel binding site in the regulatory region of the amyloid precursor protein gene. J. Biol. Chem. 270, 26774–26777.
Guyant-Marechal, L., Rovelet-Lecrux, A., Goumidi, L., Cousin, E., Hannequin, D., Raux, G., Penet, C., Ricard, S., Mace, S., Amouyel, P., Deleuze, J.F., Frebourg, T., Brice, A., Lambert, J.C., Campion, D., 2007. Variations in the APP gene promoter region and risk of Alzheimer disease. Neurology 68, 684–687.
Hattori, M., Tsukahara, F., Furuhata, Y., Tanahashi, H., Hirose, M., Saito, M., Tsukuni, S., Sakaki, Y., 1997. A novel method for making nested deletions and its application for sequencing of a 300 kb region of human APP locus. Nucleic Acids Res 25, 1802–1808.
Hebert, L.E., Scherr, P.A., Bienias, J.L., Bennett, D.A., Evans, D.A., 2003. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch. Neurol. 60, 1119–1122.
Hussain, I., et al., 1999. Identification of a novel aspartic protease (Asp 2) as beta-secretase. Mol. Cell. Neurosci. 14, 419–427.
Igbavboa, U., Sun, G.Y., Weisman, G.A., He, Y., Wood, W.G., 2009. Amyloid beta-protein stimulates trafficking of cholesterol and caveolin-1 from the plasma membrane to the Golgi complex in mouse primary astrocytes. Neuroscience 162, 328–338.
Iqbal, K., Liu, F., Gong, C.X., Grundke-Iqbal, I., 2010. Tau in Alzheimer disease and related tauopathies. Curr. Alzheimer Res. 7, 656–664.
Jang, H., et al., 2010. Truncated beta-amyloid peptide channels provide an alternative mechanism for Alzheimer's Disease and Down syndrome. Proc. Natl. Acad. Sci. U.S.A. 107, 6538–6543.
Kimberly, W.T., Zheng, J.B., Guenette, S.Y., Selkoe, D.J., 2001. The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a notch-like manner. J. Biol. Chem. 276, 40288–40292.
Konietzko, U., Goodger, Z., Meyer, M., Kohli, B., Bosset, J., Lahiri, D.K., Nitsch, R.M., 2010. Co-localization of the amyloid precursor protein and Notch intracellular domains in nuclear transcription factories. Neurobiol. Aging 31, 58–73.
Kovacs, D.M., Wasco, W., Witherby, J., Felsenstein, K.M., Brunel, F., Roeder, R.G., Tanzi, R.E., 1995. The upstream stimulatory factor functionally interacts with the Alzheimer amyloid beta-protein precursor gene. Human Molecular Genetics 4, 1527–1533.
La Fauci, G., Lahiri, D.K., Salton, S.R., Robakis, N.K., 1989. Characterization of the 5′-end region and the first two exons of the beta-protein precursor gene. Biochem. Biophys. Res. Commun. 159, 297–304.
Lahiri, D.K., Maloney, B., 2010a. Beyond the signaling effect role of amyloid-beta(42) on the processing of APP, and its clinical implications. Exp. Neurol. 225, 51–54.
Lahiri, D.K., Maloney, B., 2010b. The "LEARn" (Latent Early-life Associated Regulation) model integrates environmental risk factors and the developmental basis of Alzheimer's disease, and proposes remedial steps. Exp. Gerontol. 45, 291–296.
Lahiri, D.K., Robakis, N.K., 1991. The promoter activity of the gene encoding Alzheimer beta-amyloid precursor protein (APP) is regulated by two blocks of upstream sequences. Brain Res. Mol. Brain Res. 9, 253–257.
Lahiri, D.K., Nall, C., Ge, Y.W., 1999. Promoter activity of the beta-amyloid precursor protein gene is negatively modulated by an upstream regulatory element. Brain Res. Mol. Brain Res. 71, 32–41.
Lahiri, D.K., Song, W., Ge, Y.W., 2000. Analysis of the 5'-flanking region of the beta-amyloid precursor protein gene that contributes to increased promoter activity in differentiated neuronal cells. Brain Res. Mol. Brain Res. 77, 185–198.
Lahiri, D.K., Farlow, M.R., Greig, N.H., Sambamurti, K., 2002. Current drug targets for Alzheimer's disease treatment. Drug Dev. Res. 56, 267–281.
Lahiri, D.K., Farlow, M.R., Sambamurti, K., Greig, N.H., Giacobini, E., Schneider, L.S., 2003. A critical analysis of new molecular targets and strategies for drug developments in Alzheimer's disease. Curr. Drug Targets 4, 97–112.
Lahiri, D.K., Wavrant De-Vrieze, F., Ge, Y.-W., Maloney, B., Hardy, J., 2005. Characterization of two APP gene promoter polymorphisms that appear to influence risk of late-onset Alzheimer's disease. Neurobiol. Aging 26, 1329–1341.
Lahiri, D.K., Maloney, B., Bailey, J., Ge, Y.-W., 2009a. Role of alzheimer's amyloid-beta peptide as a putative transcription factor. In: Lebl, M. (Ed.), Peptides: Breaking Away, Proceedings of the Twenty-First American Peptide Symposium. Prompt Scientific Publishing, Bloomington, Indiana, USA, pp. 185–186.
Lahiri, D.K., Maloney, B., Zawia, N.H., 2009b. The LEARn model: an epigenetic explanation for idiopathic neurobiological diseases. Mol. Psychiatry 14, 992–1003.
Leissring, M.A., et al., 2003. Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. Neuron 40, 1087–1093.
Lu, T., et al., 2004. Gene regulation and DNA damage in the ageing human brain. Nature 429, 883–891.
Lukiw, W.J., Rogaev, E.I., Wong, L., Vaula, G., McLachlan, D.R., St George Hyslop, P., 1994. Protein-DNA interactions in the promoter region of the amyloid precursor protein (APP) gene in human neocortex. Brain Res. Mol. Brain Res. 22, 121–131.
Lv, H., Jia, L., Jia, J., 2008. Promoter polymorphisms which modulate APP expression may increase susceptibility to Alzheimer's disease. Neurobiol. Aging 29, 194–202.
Maloney, B., Ge, Y.W., Greig, N.H., Lahiri, D.K., 2006. Characterization of the human beta-secretase 2 (BACE2) 5′-Flanking region: identification of a 268-bp region as the basal BACE2 promoter. J. Mol. Neurosci. 29, 81–99.
Maloney, B., Ge, Y.W., Petersen, R.C., Hardy, J., Rogers, J.T., Perez-Tur, J., Lahiri, D.K., 2010. Functional characterization of three single-nucleotide polymorphisms present in the human APOE promoter sequence: Differential effects in neuronal

cells and on DNA-protein interactions. Am. J. Med. Genet. B Neuropsychiatr. Genet. 153B, 185–201.
Millucci, L., Ghezzi, L., Bernardini, G., Santucci, A., 2010. Conformations and biological activities of amyloid beta peptide 25–35. Curr. Protein Pept. Sci. 11, 54–67.
Morgan, C., Colombres, M., Nunez, M.T., Inestrosa, N.C., 2004. Structure and function of amyloid in Alzheimer's disease. Prog. Neurobiol. 74, 323–349.
Ohyagi, Y., Tabira, T., 2006. Intracellular amyloid beta-protein and its associated molecules in the pathogenesis of Alzheimer's disease. Mini. Rev. Med. Chem. 6, 1075–1080.
Ohyagi, Y., et al., 2005. Intracellular Abeta42 activates p53 promoter: a pathway to neurodegeneration in Alzheimer's disease. FASEB J. 19, 255–257.
Ohyagi, Y., et al., 2007. Intraneuronal amyloid beta42 enhanced by heating but counteracted by formic acid. J. Neurosci. Methods 159, 134–138.
Ono, K., Condron, M.M., Teplow, D.B., 2009. Structure–neurotoxicity relationships of amyloid beta-protein oligomers. Proc. Natl. Acad. Sci. U. S. A. 106, 14745–14750.
Paik, Y.K., Chang, D.J., Reardon, C.A., Davies, G.E., Mahley, R.W., Taylor, J.M., 1985. Nucleotide sequence and structure of the human apolipoprotein E gene. Proc. Natl. Acad. Sci. U. S. A. 82, 3445–3449.
Postel, E.H., Berberich, S.J., Flint, S.J., Ferrone, C.A., 1993. Human c-myc transcription factor PuF identified as nm23-H2 nucleoside diphosphate kinase, a candidate suppressor of tumor metastasis. Science 261, 478–480.
Sambamurti, K., Kinsey, R., Maloney, B., Ge, Y.W., Lahiri, D.K., 2004. Gene structure and organization of the human beta-secretase (BACE) promoter. FASEB J. 18, 1034–1036.
Sambamurti, K., Greig, N.H., Utsuki, T., Barnwell, E.L., Sharma, E., Mazell, C., Bhat, N.R., Kindy, M.S., Lahiri, D.K., Pappolla, M.A., 2011. Targets for AD treatment: conflicting messages from gamma-secretase inhibitors. J. Neurochem. 117, 359–374.
Sayers, E.W., et al., 2011. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 39, D38–D51.
Schmid, C.D., Perier, R., Praz, V., Bucher, P., 2006. EPD in its twentieth year: towards complete promoter coverage of selected model organisms. Nucleic Acids Res. 34, D82–D85.
Schneider, T.D., 2002. Consensus sequence Zen. Appl. Bioinformatics 1, 111–119.
Schneider, T.D., Stephens, R.M., 1990. Sequence logos: a new way to display consensus sequences. Nucleic Acids Res. 18, 6097–6100.
Selkoe, D.J., Schenk, D., 2003. Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. Annu. Rev. Pharmacol. Toxicol. 43, 545–584.
Shannon, C.E., 1997. The mathematical theory of communication. 1963. MD Comput. 14, 306–317.
Shao, H., Jao, S., Ma, K., Zagorski, M.G., 1999. Solution structures of micelle-bound amyloid beta-(1–40) and beta-(1–42) peptides of Alzheimer's disease. J. Mol. Biol. 285, 755–773.
Sheng, J.G., Mrak, R.E., Griffin, W.S., 1996. Apolipoprotein E distribution among different plaque types in Alzheimer's disease: implications for its role in plaque progression. Neuropathol. Appl. Neurobiol. 22, 334–341.
Shie, F.S., LeBoeuf, R.C., Jin, L.W., 2003. Early intraneuronal Abeta deposition in the hippocampus of APP transgenic mice. NeuroReport 14, 123–129.
Sinha, S., et al., 1999. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature 402, 537–540.
Sosinsky, A., Bonin, C.P., Mann, R.S., Honig, B., 2003. Target Explorer: an automated tool for the identification of new target genes for a specified set of transcription factors. Nucleic Acids Res. 31, 3589–3592.
Sun, X., Wang, Y., Qing, H., Christensen, M.A., Liu, Y., Zhou, W., Tong, Y., Xiao, C., Huang, Y., Zhang, S., Liu, X., Song, W., 2005. Distinct transcriptional regulation and function of the human BACE2 and BACE1 genes. Faseb. J. 19, 739–749.
Syrjanen, S., Heinonen, O., Miettinen, R., Paljarvi, L., Syrjanen, K., Riekkinen, P., 1991. Short biotinylated oligonucleotides bind non-specifically to senile plaques of Alzheimer's disease. Neurosci. Lett. 130, 89–91.
Tabaton, M., Zhu, X., Perry, G., Smith, M.A., Giliberto, L., 2010. Signaling effect of amyloid-beta(42) on the processing of AbetaPP. Exp. Neurol. 221, 18–25.
Tamagno, E., et al., 2009. JNK and ERK1/2 pathways have a dual opposite effect on the expression of BACE1. Neurobiol. Aging 30, 1563–1573.
Thies, W., Bleiler, L., 2011. 2011 Alzheimer's disease facts and figures. Alzheimers Dement. 7, 208–244.
Tomaselli, S., et al., 2006. The alpha-to-beta conformational transition of Alzheimer's Abeta-(1–42) peptide in aqueous media is reversible: a step by step conformational analysis suggests the location of beta conformation seeding. ChemBioChem 7, 257–267.
Uchida, Y., Nakano, S., Gomi, F., Takahashi, H., 2007. Differential regulation of basic helix–loop–helix factors Mash1 and Olig2 by beta-amyloid accelerates both differentiation and death of cultured neural stem/progenitor cells. J. Biol. Chem. 282, 19700–19709.
Vassar, R., et al., 1999. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286, 735–741.
von Hippel, P.H., 1994. Protein–DNA recognition: new perspectives and underlying themes. Science 263, 769–770.
von Rotz, R.C., et al., 2004. The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor. J. Cell Sci. 117, 4435–4448.
Walsh, D.M., et al., 2002. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416, 535–539.
Wang, S., Jia, J., 2010. Promoter polymorphisms which modulate BACE1 expression are associated with sporadic Alzheimer's disease. Am. J. Med. Genet. B Neuropsychiatr. Genet. 153B, 159–166.
Wang, Y.P., et al., 2006a. Endogenous overproduction of beta-amyloid induces tau hyperphosphorylation and decreases the solubility of tau in N2a cells. J. Neural Transm. 113, 1723–1732.
Wang, Z.F., et al., 2006b. Effects of endogenous beta-amyloid overproduction on tau phosphorylation in cell culture. J. Neurochem. 98, 1167–1175.
Yan, R., et al., 1999. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. Nature 402, 533–537.
Yao, Z.X., Papadopoulos, V., 2002. Function of beta-amyloid in cholesterol transport: a lead to neurotoxicity. FASEB J. 16, 1677–1679.
Yokota, O., et al., 2003. Variability and heterogeneity in Alzheimer's disease with cotton wool plaques: a clinicopathological study of four autopsy cases. Acta Neuropathol. 106, 348–356.
Zawia, N.H., Sharan, R., Brydie, M., Oyama, T., Crumpton, T., 1998. Sp1 as a target site for metal-induced perturbations of transcriptional regulation of developmental brain gene expression. Brain Res Dev Brain Res 107, 291–298.
Zhang, X., et al., 2007. Hypoxia-inducible factor 1alpha (HIF-1alpha)-mediated hypoxia increases BACE1 expression and beta-amyloid generation. J. Biol. Chem. 282, 10873–10880.
Zou, K., Gong, J.S., Yanagisawa, K., Michikawa, M., 2002. A novel function of monomeric amyloid beta-protein serving as an antioxidant molecule against metal-induced oxidative damage. J. Neurosci. 22, 4833–4841.