Review

Bridging integrator 1 (BIN1): form, function, and Alzheimer's disease

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The bridging integrator 1 (*BIN1*) gene, also known as amphiphysin 2, has recently been identified as the most important risk locus for late onset Alzheimer's disease (LOAD), after apolipoprotein E (*APOE*). Here, we summarize the known functions of BIN1 and discuss the polymorphisms associated with LOAD, as well as their possible physiological effects. Emerging data suggest that BIN1 affects AD risk primarily by modulating tau pathology, but other affected cellular functions are discussed, including endocytosis/trafficking, inflammation, calcium homeostasis, and apoptosis. Epigenetic modifications are important for AD pathogenesis, and we review data that suggests the possible DNA methylation of the *BIN1* promoter. Finally, given the potential contributions of BIN1 to AD pathogenesis, targeting BIN1 might present novel opportunities for AD therapy.

Alzheimer's disease and the susceptibility gene *BIN1*

Alzheimer's disease (AD; see Glossary) is a complex, multifactorial neurodegenerative disease that is the leading cause of dementia in the elderly, creating a great burden for affected individuals, their caregivers, and society. Genetic susceptibility at multiple loci and interactions among these genes influence the risk of developing AD; recent estimates of heritability range from 58% to 79% [1]. For many years, amyloid precursor protein (*APP*) and the presenilin genes 1 and 2 (*PSEN1*, *PSEN2*) have been the only unequivocally established susceptibility genes for early onset familial AD, and apolipoprotein E (*APOE*) the only confirmed susceptibility gene for common late onset AD (LOAD). In recent years, large genome-wide association studies (GWAS) have identified nine other genes/loci [2–6] that, along with *APOE4*, contribute to a high proportion of genetic risk for LOAD (Box 1).

Among them, the bridging integrator 1 (*BIN1* or amphiphysin 2/*AMPH2*) gene is currently identified as the most important genetic susceptibility locus in LOAD after *APOE*, according to the Alzgene database (http://www.alzgene.org/) [7,8]. Meanwhile, a study has shown that the odds ratio (OR) and population attributable fraction (PAF) of the significantly associated single nucleotide polymorphism (SNP) at *BIN1* are estimated to be 1.20% and 6%, respectively, which are the highest among the effects of non-*ApoE* risk loci [5]. The altered expression of BIN1 has been demonstrated in aging mice, transgenic mouse models of AD, and in AD brains [8,9], and higher levels of BIN1 expression have recently been reported to be associated with later age at onset and shorter disease duration in AD patients [10]. Similar to APOE, numerous studies have presented compelling evidence implicating BIN1 in the pathogenesis of AD, and although the mechanisms that underlie the contributions of BIN1 to AD pathogenesis are still not fully understood, several pathways have been discussed. In addition to its potential interactions with tau pathology [8], BIN1 may also be involved in regulating endocytosis and trafficking, immunity and inflammation, calcium homeostasis, and apoptosis. Through further investigation of the potential molecular pathways by which BIN1 affects AD risk and its detailed mechanisms in AD pathogenesis, we have reason to believe that these new interactions will revolutionize our understanding of the biology of LOAD and provide new targets for treatment.

Glossary

Alzheimer's disease (AD): the most common cause of dementia, characterized clinically by a progressive and irreversible loss of cognitive functions and pathologically by the loss of synapses and neurons, as well as the formation of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs).
Apolipoprotein E4 (ApoE4): the major known genetic risk factor for LOAD and plays both Aβ-dependent and Aβ-independent roles in the pathogenesis of AD.
Bridging integrator 1 (BIN1): identified initially as an MYC-interacting proapoptotic tumor suppressor, which is currently the most important genetic susceptibility locus in LOAD after ApoE, which may affect AD risk mainly through the tau pathology pathway.
Epigenetics: the heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence.
Linkage disequilibrium (LD): the occurrence of some combinations of alleles or genetic markers in a population more often or less often than would be expected from a random formation of haplotypes from alleles based on their frequencies.
Mini-mental state examination (MMSE): a brief 30-point questionnaire test that is commonly used to screen for cognitive impairment and dementia.
Receptor-mediated endocytosis (RME): a process by which external ligands bind to receptors on the external cell surface and the plasma membrane invaginates to form a clathrin-coated pit which is eventually snipped from the membrane and internalized in the cell.
Retinoblastoma protein (RB): a tumor suppressor protein that is dysfunctional in several major cancers, which prevents excessive cell growth by inhibiting cell cycle progression. This protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure.
Tau: a microtubule-associated protein that is abundant in neurons in the CNS, which interacts with tubulin to stabilize microtubules and promotes tubulin assembly into microtubules. Hyperphosphorylation can induce self-assembly of tau into paired helical filaments, called NFTs.
T-tubule: a deep invagination of the sarcolemma, which is the plasma membrane, only found in skeletal and cardiac muscle cells. These invaginations allow depolarization of the membrane to quickly penetrate to the interior of the cell.

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Box 1. Novel susceptibility genes for LOAD identified by GWAS

To identify the novel genes for common LOAD, efforts have been focused on conducting GWAS because this approach is hypothesis-free and conceptually would identify all known and unknown genes. Recent large GWAS have identified nine additional genes/loci that, along with *APOE4*, contribute a high proportion of genetic risk for LOAD. These include clusterin (*CLU*), phosphatidylinositol-binding clathrin assembly protein (*PICALM*), and complement receptor 1 (*CR1*), as well as the ATP cassette transporter *ABCA7*, bridging integrator *BIN1*, a CD2-associated protein (*CD2AP*), *CD33*, ephrin A1 (*EPHA1*), and a cluster of membrane-spanning 4-domains, subfamily A (*MS4A*) genes recently honed down to *MS4A2*, although other genes within this cluster may also be relevant. Most of these susceptibility genes have already been systematically replicated in large case–control studies in ethnically distinct populations.

It has been demonstrated that these new identified susceptibility genes for AD are not random but show patterns of putative functional relationships. Some evidence already exists that allow the speculation about potential disease-related function effects. Briefly, the genome-wide significant genes and their possible pathways implicated in AD [53,55,95] are summarized as follows:

- Endocytosis pathways – BIN1, PICALM, and CD2AP;
- Immune system function – CLU, CR1, CD33, MS4A, ABCA7, and EPHA1;
- Lipid-processing pathway – APOE, CLU, and ABCA7;
- Complement system – CR1, CLU, CD2AP, and ABCA7.

Biochemical properties of BIN1

BIN1 is located on chromosome 2q14.3 and encodes at least 20 exons [11]. Initially, 19 exons were identified by Wechsler-Reya *et al.* [12], although subsequently an overlooked exon was located between exons 6 and 7, called exon 6a. Because BIN1 transcripts are subject to extensive differential splicing, a diverse array of BIN1 splice variants is generated with different tissue distributions (Figure 1) [13,14]. However, the major forms vary primarily in their inclusion of four exons: 6a, 10, 12 (including a series of alternative brain-specific exons, 12A–D), and 13.

BIN1 was initially identified as a tumor suppressor with a MYC-interacting domain, a C-terminal SH3 domain, and an N-terminal BAR (Bin1/Amphiphysin/RVS167) domain [11,15]. Splice variants that include a central insert domain, encoded by exon 12, can interact with clathrin and AP2/α-adaptin [16,17], and in some brain splice variants, a 31-residue insert (N-terminal insert domain) encoded by exon 6a is included within a putative coiled-coil region in the BAR domain [18]. In the muscle-specific isoform, a 15-residue domain encoded by exon 10 includes a putative nuclear localization sequence and lipid-binding sequence [15,19]. In addition, part of the MYC-binding domain is encoded by exon 13, which is differentially spliced in a tissue-independent manner. Interestingly, one study has observed that two of the BIN1 cDNAs appear to encode splice variants that lack a C-terminal SH3 domain [14].

The various BIN1 splice variants exhibit anomalous electrophoretic mobilities, as shown by migration during polyacrylamide gel electrophoresis (PAGE), indicating the potential contribution of post-translational modification of this protein. Despite the longest BIN1 transcript in human, rat, or mouse having a predicted mass of only 65 kDa [14,20,21], in humans the brain isoform of BIN1 migrates at 85 kDa [20], and in rats it migrates at 92 kDa [14]. Similarly, the muscle isoform migrates within polyacrylamide gels at a position equivalent to 60–70 kDa, although its predicted size is 50 kDa [15]. The protein sequence encoded by exon 12 appears to confer this aberrant electrophoretic mobility [14].

Although ubiquitous, BIN1 is expressed most abundantly in the brain and muscle [12]. Judging by the numerous splice variants produced by this gene, its functions are complex and likely to be highly regulated. BIN1 has been implicated in the process of clathrin-mediated endocytosis and intracellular endosome trafficking [11,22]. Interestingly, endocytosis is also related to another gene strongly associated with AD: PICALM. And both the mean expression of BIN1 and PICALM were recently found to be higher in white matter of the central nervous system (CNS) [23]. Moreover, although several alternatively spliced BIN1 forms exist [11], all appear to contain the N-terminal BAR domain that binds lipid membranes and induces membrane curvature in sites such as T-tubules in muscular cells, endocytic pits in neuronal as well as non-neuronal cells, or possibly cytoplasmic endosomes. *BIN1* knockout mice have disruptions to T-tubules causing cardiac deficiencies from birth [24]. BIN1 also appears to link the microtubule cytoskeleton with the cellular membrane via the tubular membrane structures it forms [25], and this may influence the formation of one major pathological feature of AD brains, neurofibrillary tangles. Finally, BIN1 is also crucial for the function of pathways leading to cell senescence and apoptosis [26,27]. In chick embryo cells, a

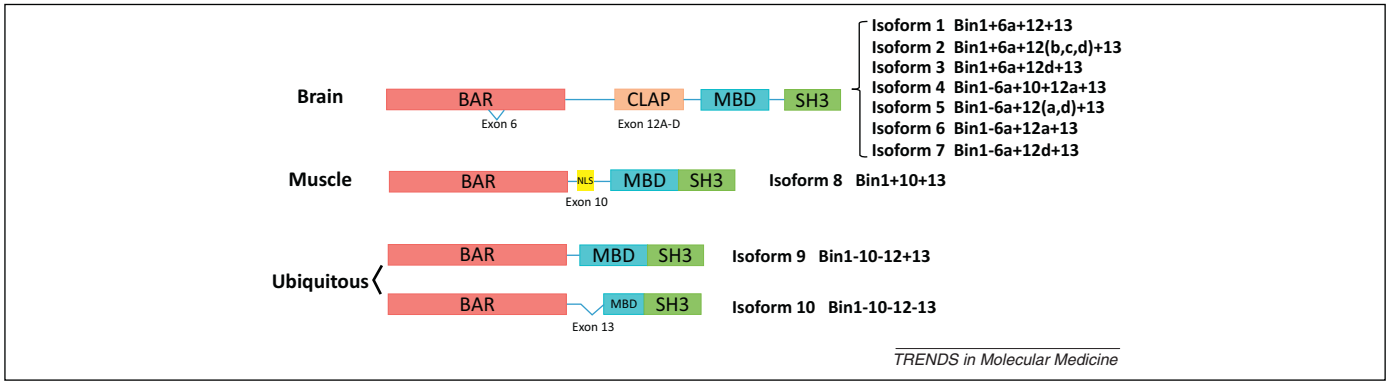

Figure 1. Domain structure of human BIN1/Amphiphysin2. The primary tissue-specific and ubiquitous splice variants are shown here. Exons 6, 10, 13, and 12A–D are alternately spliced. Abbreviations: BIN1, bridging integrator 1; BAR, Bin1/Amphiphysin/RVS167 domain; CLAP, clathrin-AP2 binding region; MBD, Myc-binding domain; SH3, Src homology 3 domain; NLS, nuclear localization sequence.