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Genetic loci modulating amyloid-beta levels in a mouse model of Alzheimer's disease

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Abstract

Genetic studies have demonstrated very high heritability for Alzheimer's disease (AD) risk in humans; however, these genetic contributions have proven extremely challenging to map in large studies of AD patients. Processing of the amyloid precursor protein (APP) to produce amyloid-beta (Aβ) peptide is increasingly believed to be of central importance in AD pathogenesis. Intriguingly, mice from the C57BL/6J and DBA2/J inbred strains carrying the R1.40 *APP* transgene produce identical levels of unprocessed APP, but demonstrate significant, heritable differences in Aβ levels. To identify specific loci responsible for the observed genetic control of Aβ metabolism in this model system, we have performed a whole-genome quantitative trait locus (QTL) mapping experiment on a total of 516 animals from a C57BL/6J × DBA/2J intercross using a dense set of SNP genetic markers. Our studies have identified three loci on mouse chromosomes 1, 2, and 7 showing significant or suggestive associations with brain Aβ levels, several of which contain regions syntenic to previous reports of linkage in human AD.

Keywords: APP; Beta-amyloid; Abeta; Alzheimer; Genetics; QTL; Mouse; Intercross

1. Introduction

Alzheimer's disease (AD) is a debilitating and increasingly prevalent neurodegenerative disease with significant genetic contributions that are incompletely understood. A family history of AD diagnosis is second only to age as the single most important known risk factor for developing the disease. Several human genetic studies have demonstrated high heritability for AD risk and age at onset (Gatz et al., 2005), with the largest study to date of AD risk in twin pairs (Gatz et al., 2006) recently estimating heritability for AD at a striking 79% after accounting for shared environmental influences.

Pathologically, AD is defined by the presence of extracellular plaques in the brain formed by aggregation of the amyloid-β (Aβ) peptide, and intracellular neuronal tangles composed of hyperphosphorylated tau protein. Aβ is a derived from the proteolytic processing of the amyloid precursor protein (APP), a transmembrane protein of unknown function, and is increasingly believed to be of central importance in AD pathogenesis (Hardy and Selkoe, 2002). APP can be cleaved through a series of alternative proteolytic events, the relative efficiency of which is a strong determinant of Aβ production. Initial cleavage by the enzyme α-secretase at position 687 (within the Aβ region itself) releases a truncated non-amyloidogenic peptide known as C-terminal fragment α (CTF-α), while initial cleavage by the β-secretase enzyme at position 671 produces a longer C-terminal fragment β (CTF-β), which can then be acted upon by γ-secretase, an intramembraneous protease complex, to release the pathogenic Aβ peptide.

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Positional cloning studies have identified several of the genes directly involved in APP processing, and have linked rare autosomal dominant mutations in these genes with highly penetrant familial AD (FAD) in a small but significant subset of patients with unusually early onset of disease (<65 years of age). A number of mutations in the *APP* gene affecting relative lability to β and γ secretase cleavage have been linked with dominant early-onset FAD syndromes, including the Swedish (K670M/N671L) mutant form used as a transgene in the present study (Goate et al., 1991). Mutations in the presenilins *PSEN1* and *PSEN2*, members of the γ-secretase complex, have also been shown to be causative for early-onset FAD (Rogaev et al., 1995; Sherrington et al., 1995). Although identification of these rare autosomal dominant early-onset FAD syndromes has been crucial to reaching a greater understanding of basic mechanisms of the disease, early-onset FAD accounts for a vanishingly small subset (<1%) of AD cases overall. The vast majority of AD cases are late-onset in nature, with a significant genetic risk component that appears to be highly complex.

Allelic status at the apolipoprotein E (ApoE) gene has been identified as a significant risk factor for late-onset AD, but is believed to account for only a minority of the observed heritability of AD risk (Steffens et al., 2000; Warwick Daw et al., 2000). Several whole-genome screens of AD patient populations using both risk of developing the disease and age at disease onset as outcome measures have failed to uncover any additional linkage logarithm-of-odds (LOD) scores of 3 or greater, although multiple loci were found with weakly suggestive LOD scores greater than 1 (Pericak-Vance et al., 1998, 2000; Kehoe et al., 1999; Li et al., 2002; Myers et al., 2002; Blacker et al., 2003), reviewed in (Kamboh, 2004), with broad intervals on chromosomes 9, 10, and 12 showing the most consistent evidence for linkage in multiple studies.

Because of the significant challenges encountered in human AD linkage studies, we and others have turned to the mouse as a powerful model system for exploring the complex genetics of AD-associated traits. Prominent advantages of modeling AD genetics in the mouse include its short generation time and early disease onset, its similarity to human neurophysiology, the utility of genetically defined inbred strains in creating highly informative intercross populations, and the ability to isolate and reproducibly study specific candidate genes and regions through the eventual construction of congenic strains for defined genomic intervals. Krezowski et al. (2004) used a mouse intercross QTL mapping approach to identify genetic loci modifying susceptibility to APP transgene-induced lethality, Brich et al. (2003) performed a mouse genome scan for QTLs modifying tau phosphorylation, and Sebastiani et al. (2006) recently published a mouse genome scan for amyloid pathology, detecting several QTLs modifying plaque deposition in an intercross using TgCRND8 transgenic mice from the C57BL/6J and A/J inbred strains.

Unlike other transgenic mouse models of AD, many of which drastically overexpress cDNA constructs of mutant *APP* using exogenous promoter constructs, the R1.40 mouse model used in the current study employs a full genomic copy of the FAD-associated Swedish mutant (K670M/N671L) form of human *APP* under the control of its native promoter. The intact splicing and regulatory structures of the R1.40 transgene preserve its native physiology as much as is possible in a mouse model, allowing experimenters to make fewer assumptions about the role of transgene-specific expression and splicing patterns and making it well suited to the investigation of genetic modifiers of Aβ production likely to be relevant to the human disease.

By over 12 generations of repeated backcrossing, the R1.40 transgene was established at an identical integration site on the genetic backgrounds of several different inbred strains of laboratory mice. Analysis of these R1.40 congenic animals revealed that APP processing and Aβ production are significantly modified by genetic background, with the most pronounced differences in Aβ level being present between the C57BL/6J and DBA/2J congenic strains (Lehman et al., 2003). Although each strain has similar levels of transgene expression and production of unprocessed APP, R1.40 animals on the C57BL/6J genetic background demonstrate Aβ levels over 20% higher than their DBA/2J counterparts as early as 28 days of age. In addition, C57BL/6J R1.40 animals develop amyloid plaques at 14 months of age, while DBA/2J animals fail to develop any detectable neuropathology as late as 24 months of age. Our results suggest that similarly to many FAD mutations observed in humans, a moderate but chronic increase in Aβ production eventually leads to completely penetrant AD-like neuropathology in the C57BL/6J R1.40 congenic strain.

In order to map the genetic loci responsible for the observed heritable differences in brain Aβ levels between the C57BL/6J and DBA/2J R1.40 inbred strains, we performed a whole-genome QTL mapping experiment on a 516-animal B6D2F2 intercross using a dense set of 909 informative SNP markers, and detected suggestive or significant linkage to regions on mouse chromosomes 1, 2, and 7 containing a number of interesting candidate genes that may modulate brain Aβ levels.

2. Materials and methods

2.1. Generation and phenotypic characterization of transgenic mice

The R1.40 transgene is a full genomic copy of human *APP* carrying the Swedish (K670M/N671L) mutation associated with early-onset FAD. Creation of R1.40 transgenic animals and backcrossing of the R1.40 transgene onto the C57BL6/J and DBA2/J inbred strains are described in (Lamb et al., 1993). The backcrossed B6.129-Tg(APPSw)40Btla/J and DBA.129-Tg(APPSw)40Btla/J inbred strains are hereinafter referred to as B6 R1.40 and D2 R1.40. To generate an F2 population for the current study, female B6 R1.40 (+/+)