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

Davis Ryman [a,b], Yuan Gao [b], Bruce T. Lamb [a,b,c,*]

[a] *Lerner Research Institute, Cleveland Clinic Foundation, Department of Neurosciences, NC3-164, 9500 Euclid Avenue, Cleveland, OH 44195 USA*
[b] *Case Western Reserve University School of Medicine, Department of Genetics, 10900 Euclid Avenue, Cleveland, OH 44106-4955, USA*
[c] *Case Western Reserve University School of Medicine, Department of Neuroscience, 10900 Euclid Avenue, Cleveland, OH 44106-4955, USA*

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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.
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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.

* Corresponding author at: Lerner Research Institute, Cleveland Clinic Foundation, Department of Neurosciences, NC3-164, 9500 Euclid Avenue, Cleveland, OH 44195, USA. Tel.: +1 216 444 3592; fax: +1 216 444 7927.
E-mail address: lambb@ccf.org (B.T. Lamb).

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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 (+/+)

and male D2 R1.40 (+/+) transgenic animals were mated to produce B6D2F1 R1.40 (+/+) offspring, which were then mated to female B6D2F1 non-transgenic animals from a separate C57BL/6J female × DBA/2J male cross.

516 B6D2F2 R1.40 (+/−) transgenic animals from this intercross were sacrificed and dissected at 28 days of age, and whole brain samples were extracted by polytron homogenization and 4-h incubation at room temperature in an 8 M guanidine/1 M hydrochloric acid solution. Because initial studies found that brain levels of Aβ40 and Aβ42, the 40 and 42 amino acid isoforms of Aβ, were strongly correlated in the B6D2F2 population ($r = 0.9$, two-tailed Pearson test, $p < 0.0001$) (Lehman et al., 2003), and because of the significantly better reproducibility of the Aβ40 ELISA, we selected brain Aβ40 level as a quantitative trait for this study. Brain extracts were assayed in duplicate for Aβ40 levels by an ELISA assay (Biosource International, Camarillo, CA). To further control for inter-assay variability, guanidine/HCl brain extracts from a single control animal from each of the C57BL/6J R1.40 (+/−), DBA/2J R1.40 (+/−), and B6D2F1 R1.40 (+/−) strains were also prepared, and an aliquot of each control sample was included in duplicate in each ELISA assay plate. Aβ values for each F2 animal on an ELISA plate were then normalized to an average of the three constant controls on each plate.

2.2. Determination of genotype at informative SNP markers and analysis of QTLs

For each F2 animal, genomic DNA was extracted from 0.5 cm tail snips using a standard TNES/proteinase K digestion and NaCl/ethanol precipitation protocol (MD Anderson Cancer Center). DNA extracts were resuspended in a 10 mM Tris–HCl/1 mM EDTA solution, measured for DNA concentration by PicoGreen fluorescence assays (Invitrogen Corporation, Carlsbad, CA), and adjusted to a concentration range of 50–150 ng/μL before genotyping on GoldenGate™ Mouse MD SNP Genotyping Arrays (Illumina Corporation, San Diego, CA). SNP genotypes were called separately for each array plate by automated clustering using the BeadStudio software package (Illumina Corporation).

Using scripts written in the Perl programming language (available upon request), we identified 909 SNP markers with informative allele differences between the C57BL/6J and DBA/2J strains, and evaluated the genotype data at these markers for potential genotyping errors by searching for apparent double-crossover events at closely linked SNP markers, and searching for focal deviations from Hardy–Weinberg equilibrium present at a single SNP but absent at surrounding markers. We resolved most of the apparent errors by manually correcting genotype-calling clusters in BeadStudio, and excluded 7 of the 909 informative markers due to genotyping problems.

After identifying and removing probable genotyping errors, we evaluated the remaining set of SNP markers for consistent regional deviations from Hardy–Weinberg equilibrium that may represent selective pressure on loci modifying survival in the transgenic background. Genotype frequencies in the F2 population were subjected to chi-square analysis versus the expected 1:2:1 genotype ratios using 2 degrees of freedom.

Quantitative trait loci were evaluated by performing marker regression analysis and interval mapping with the MapManager QTX software suite (Manly and Olson, 1999). To assess whether additional linkage regions could be discovered after controlling for the effects of detected QTLs, composite marker regression analyses were performed using the SNP markers with peak association scores as a background. A series of 100,000 random permutations were performed on the data to generate empirically determined, dataset-specific threshold values for suggestive and significant QTLs (Churchill and Doerge, 1994).

3. Results

3.1. Mapping of transgenic interval

By comparing the SNP genotype data with alleles from the 129S1 strain on which the R1.40 transgene was originally established prior to backcrossing, the congenic interval containing the R1.40 transgene insertion was mapped to the interval between markers rs3688207 and gnf13.093.328 on mouse chromosome 13 (44933576–88907941 bp). This insertion was present in all F2 animals examined, and SNP markers spanning this interval were excluded from QTL analysis. SNP genotyping of C57BL6/J R1.40 and DBA2/J R1.40 transgenic animals confirmed that no passenger loci outside the chromosome 13 interval were present for the set of all SNP markers examined.

3.2. Phenotyping of F2 animals

B6D2F2 animals showed a wide range of brain Aβ levels, with a more than fivefold difference between the maximum and minimum values observed (Fig. 1), demonstrating that segregating genetic differences between these strains exert

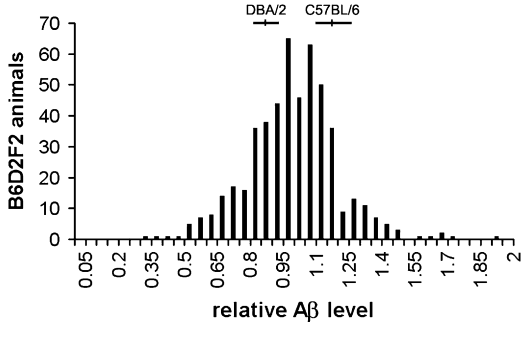


Fig. 1. Histogram of brain Aβ40 levels in the B6D2F2 R1.40 population by ELISA assay of whole-brain homogenates relative to three constant controls. C57BL6/J and DBA2/J parental strain means ± S.D. are shown above.

a significant degree of control over brain Aβ levels. Trait values for the F2 population matched a normal distribution, indicating that contributions from multiple QTLs are likely involved in modifying Aβ levels in this population, and that the observed genetic control of Aβ level is not explained by one or few QTLs with a large individual effect. A substantial number of F2 individuals had Aβ levels that were higher than the C57BL/6 parental strain or lower than the DBA2/J parental strain, demonstrating that in spite of the overall higher Aβ levels seen in C57BL6/J animals, some C57BL6/J QTLs act to reduce Aβ production while some DBA2/J QTLs act to increase it.

No significant difference in Aβ level between males and female animals was detected in the F2 population. Interestingly, DBA2/J R1.40 animals demonstrate a slight but significant increase in brain Aβ40 levels in males compared to females, while C57BL6/J R1.40 animals show no gender difference in brain Aβ levels in spite of significantly increased relative levels of the amyloidogenic APP processing product CTF-β in female animals (Lehman et al., 2003).

3.3. QTL analysis

Marker regression analysis using Aβ40 levels as a quantitative trait detected 38 SNP markers with association LOD scores greater than 2. By permutation analysis, nine of these markers on chromosome 2 met statistical criteria for genome-wide significance, and 14 markers on chromosomes 1, 2, and 7 met criteria for suggestive linkage (Table 1). Because of the high marker density used in this experiment, interval mapping methods reported similar results to direct marker regression, with linkage peaks directly underlying the markers detected by simple regression. Peak LOD scores for the principal regions detected on chromosomes 1, 2, and 7 were respectively identified at SNP markers rs13476273, rs13476454, and rs13479319. Linkage maps of each chromosome are displayed in Fig. 2, and QTL support regions having LOD scores within 1.5 of the peak value are indicated above (Lynch and Walsh, 1998).

Table 1
Results of marker regression analysis on the set of 909 informative SNP markers using relative brain Aβ levels as a quantitative trait

Chr	Marker ID	Position	*P* value	LOD score
1	rs13474399	180555357	0.00167	2.782608696*
1	rs13476272	181371085	0.00072	3.152173913*
1	rs13476273	182049374	0.00061	3.217391304*
1	mCV22849619	186392359	0.00415	2.391304348*
2	CEL-2_32981944	32954115	0.00012	3.913043478*
2	gnf02.035.469	33926782	0.00018	3.760869565*
2	rs13476439	38043281	0.00003	4.543478261**
2	rs13476454	41216315	0.00001	4.891304348**
2	rs13476467	44333936	0.00031	3.52173913**
2	rs13476472	45625389	0.00013	3.869565217**
2	rs6265423	47096774	0.00006	4.217391304**
2	CEL-2_50605053	50577224	0.00008	4.086956522**
2	rs3725341	51397858	0.00006	4.217391304**
2	rs13476503	53115772	0.00006	4.239130435**
2	rs3718711	56461355	0.00013	3.913043478**
7	rs13479251	46574481	0.00255	2.586956522*
7	rs3718641	47876082	0.00174	2.760869565*
7	rs13479277	52987588	0.00189	2.717391304*
7	mCV23423763	54746470	0.00132	2.891304348*
7	rs6160140	60165513	0.00041	3.391304348*
7	rs13479319	63680922	0.00021	3.695652174*
7	rs3676254	66569463	0.00085	3.065217391*
7	rs13479338	67953758	0.00289	2.543478261*

Permutation analysis—*: suggestive, **: significant.

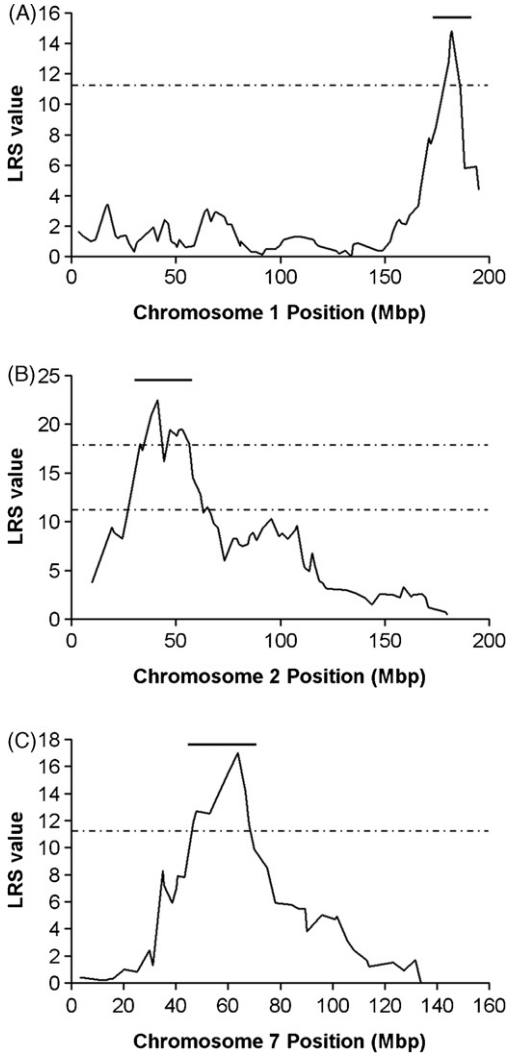


Fig. 2. Maps of genetic linkage to brain Aβ level in the B6D2F2 population for chromosomes 1 (A), 2 (B), and 7 (C), plotted by chromosomal location in millions of base pairs (Mbp). Likelihood ratio statistic (LRS) = LOD × 4.6. LOD-1.5 support intervals for peaks of maximum linkage are indicated above.

The principal chromosome 2 linkage area consists of two separate linkage peaks centered at markers rs13476454 and rs13476503, which are separated by a short region of decreased linkage but have overlapping support intervals. A composite marker regression analysis controlling for the effect of the most strongly associated QTL at rs13476454 did not report an independent association with the closely linked rs13476503 marker or identify any previously undetected

linkage regions. Likewise, composite marker regressions controlling for the chromosome 1 peak at rs13476273 and the chromosome 7 peak at rs13479319 did not identify any additional associations.

For each of the loci detected, DBA/2J alleles were protective and decreased Aβ levels in F2 animals. Mean Aβ40 values by genotype for each of the most strongly associated SNPs are displayed in Fig. 3. As anticipated from the wide normal distribution of trait values in the F2 population, these loci individually explained only a minority of the total variance, with the individual effect sizes estimated by MapManager at approximately 4% of total variance for each locus. C57BL/6 alleles at the loci on chromosome 2 and 7 appear to act in a partially but incompletely dominant manner to increase Aβ level, while C57BL/6 alleles at the chromosome 1 locus exert a partially recessive effect.

3.4. Analysis of Hardy–Weinberg equilibrium

Overexpression of APP transgenes such as TgCRND8 has been shown to have potentially lethal effects in mice, which appear to be specific to the APP protein itself and are strongly modified by genetic background. In one recent study, a QTL mapping approach was used to identify several loci modifying APP-dependent lethality (Krezowski et al., 2004). To assess the F2 population for regional divergences from expected Hardy–Weinberg genotype ratios that may result from genes modifying survival, we performed a chi-square analysis comparing prevalence of observed genotypes with the expected values. A region on the q arm of chromosome 19 centered at the rs6304326 SNP marker demonstrated a chi-square statistic of 14.033 ($p = 0.0009$) due to a moderate excess of both homozygous genotypes compared to heterozygotes (27.8% C57BL/6, 30.3% DBA/2, 41.9% B6D2). Because this finding was not linked to the chromosome 13 transgene site or to any of the detected loci, it is unlikely to influence the linkage results.

4. Discussion

Genetic factors exert a highly significant degree of control over AD risk in the human population that remains poorly understood. Discovery of rare dominant mutations leading to early-onset familial AD syndromes has led to the identification of key mechanistic players in Aβ production and greatly expanded understanding of the disease; however, large and comprehensive genetic mapping efforts in humans have so far failed to fully characterize the observed significant genetic control of late-onset AD risk. The R1.40 mouse model system contains an intact human genomic *APP* transgene and promoter present at identical integration sites on the genetic background of different mouse inbred strains, and provides an alternative method for analyzing genetic modifiers of this complex disease. In this study, we performed a dense whole-genome scan of an intercross between two strains with

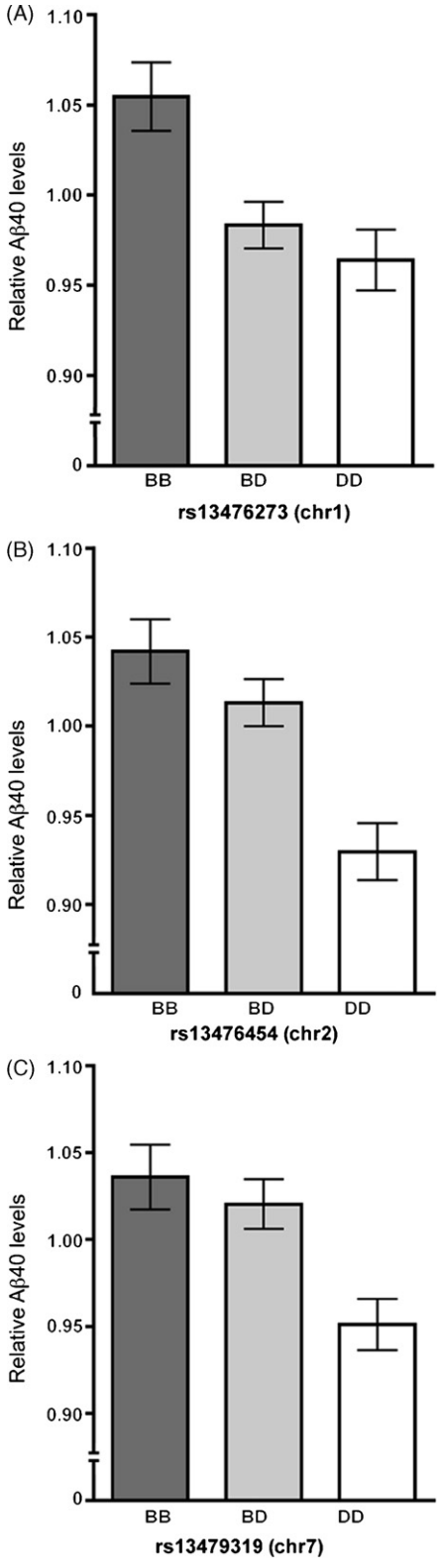


Fig. 3. Average brain Aβ levels by ELISA assay of whole-brain homogenates for all B6D2F2 animals, grouped by genotype at the most strongly associated SNP markers (panel A = rs13476273, chromosome 1. Panel B = rs13476454, chromosome 2. Panel C = rs13479319, chromosome 7). BB = C57BL/6J homozygotes, BD = heterozygotes, DD = DBA/2J homozygotes. Means ± S.E.M.

significant genetic differences in Aβ production, and identified several QTLs showing associations with brain Aβ levels, several of which contain biologically interesting AD candidate genes and some previous reports of linkage to human AD risk.

The normal distribution of trait values observed in the F2 population in the current study strongly suggests that multiple loci are involved in modifying this phenotype; even in this model system, the genetics of APP metabolism appear to be significantly complex. For each of the significant and suggestive QTLs detected, C57BL/6J genotype was positively correlated with Aβ level, in accordance with the observed higher Aβ levels in C57BL/6J animals. However, the existence of some B6D2F2 animals with Aβ levels lower than both the C57BL/6J and DBA/2J parental strains suggests the existence of at least some additional QTLs with protective effects on reducing Aβ level in the C57BL/6J strain. Regions on chromosomes 3, 9, 10, and 16 showed weak negative associations with Aβ in the C57BL/6J strain, although these did not reach a suggestive level.

Despite the complex genetic control of brain Aβ levels observed in this intercross, the high marker density and large F2 population analyzed enabled detection of several suggestive and significant QTLs. Publicly available sequence data enabled comparison of the detected loci to the equivalent syntenic regions on the human genome. The chromosome 1 region is syntenic to human chromosome 1q43-44, while the significantly associated chromosome 2 locus contains regions syntenic to human 9q33 and 2q22-24, and the chromosome 7 region is syntenic to areas of 11p14-15 and 15q12-13. Interestingly, several of these human genomic regions have been independently reported in patient-based linkage and linkage disequilibrium studies of late-onset AD (Table 2). Linkage disequilibrium at 1q44 was reported in a study of 100 AD cases and 100 controls (Zubenko et al., 1998), linkage at 2q22 and 2q24 was detected in a linkage study of 88 LOAD families (Lee et al., 2004), and linkage at 9q34 was detected in a study of 466 families (Pericak-Vance et al., 2000), close to the 9q33 syntenic region reported here. This 9q33 syntenic region is also close to a major region of interest in human AD on chromosome 9q, but lies slightly outside the 9q21-31 region typically identified with the strongest support, and does not contain the *DAPK1* gene recently implicated in an association study (Li et al., 2006). Unlike the more extensively reported areas of linkage on human chromosomes 9, 10, and 12, these syntenic human linkage findings have not been replicated by multiple studies, suggesting that they are unlikely to explain a major part of the observed heritability of AD risk. However, the independent detection in the current study of syntenic loci linked to brain Aβ levels raises the interesting possibility that these regions may contain genes with a relationship to human AD, even if the genetic variation present in these genes in the human population creates subtle variations in AD risk that have been relatively difficult to detect (Table 3).

Table 2
Human genomic regions with synteny to the detected chromosome 1, 2, and 7 mouse QTLs for brain Aβ level, listed with previous reports in human linkage mapping or linkage disequilibrium studies of AD

Chr	Human synteny	AD linkage/LD reports
1	1q43-44	1q44: Zubenko et al. (1998)
2	9q33	9q31: Myers et al. (2002), 9q34: Pericak-Vance et al. (2000)
	2q22-24	2q22, 2q24: Lee et al. (2004)
7	11p14-15	None
	15q12-13	None

Table 3
Genes in the significantly linked chromosome 2 interval containing validated non-synonymous coding polymorphisms between the C57BL/6J and DBA/2J strains (http://www.ensembl.org/biomart, Sanger dbSNP build 126)

Gene	Position (bp)	OMIM Gene Function
Hc	34805340–34883405	Inflammation; complement cascade
Zbtb6	37248854–37252872	Unknown; zinc finger containing
Nr5a1	38514636–38536509	Corticosteroid synthesis; gonadal differentiation
Lrp1b	40418782–42475607	Tumor suppressor; APP binding activity
Zfhx1b	44809132–44935025	Neural crest development
Nmi	51770516–51795097	NMYC and STAT interactor; IL-2 and IFN-g signaling
Rif1	51894841–51944065	Telomere-associated protein
Neb	51959251–52157768	Myofibril-associated protein
Arl6ip6	53014120–53041035	Unknown; ADP-ribosylation-like factor 6-interacting

A recent mapping study by Sebastiani et al. (2006) analyzed QTLs linked to amyloid pathology and Aβ levels in an intercross of mice from the A/J and C57BL/6J backgrounds carrying the TgCRND8 transgenic construct. Interestingly, this study detected a QTL for Aβ levels and pathology on chromosome 11 that was not found in the current experiment, but also detected a QTL significantly linked to plasma Aβ40 levels (but not brain Aβ or pathology) underlying our peak for brain Aβ40 levels on the q arm of mouse chromosome 1. Other pathology QTLs identified by this study appeared not to be linked to Aβ levels, perhaps due to the action of alleles specific for plaque deposition or clearance. There are a number of explanations as to why the results from the two studies do not directly coincide.

First, the two studies involved different pairs of inbred strains, and the set of genetic loci responsible for differences in Aβ phenotypes between the C57BL/6J and DBA2/J inbred strains utilized in the current study are likely not identical to those between the C57BL/6J and A/J strains utilized in the Sebastiani study. Indeed, similar mapping studies involving a wide variety of phenotypes in mouse F2 intercrosses, backcrosses and recombinant inbred lines have often led to the identification of unique QTLs depending upon the strain pair analyzed (see http://pga.jax.org/qtl/index.html, Martin et al., 2006). Second, the animals in the two studies were evaluated at different ages (28 days versus 21 weeks) and examined for different phenotypes (steady state Aβ lev-

els via ELISA versus Aβ deposition via ThioS and 6E10 staining). Modifiers affecting Aβ levels after the onset of pathology will likely be different from those acting earlier in the disease process. Third, the transgenic construct employed in the two studies also was significantly different; the current study used a full genomic copy of human APP^{Swe} under control of the native human promoter, while the Sebastiani study utilized an APP^{Swe}/APP^{Ind} 695 cDNA overexpressed from the hamster PrP promoter, resulting in substantial differences in the levels and spatial and temporal control of APP expression as well as the alternative splice forms and specific FAD mutations involved. Finally, significant APP overexpression lethality was noted in TgCRND8, which appeared to be powerfully modified by genetic background and could complicate mapping of other phenotypes. Similarly to the analysis of other complex traits in the mouse, we believe that genetic analysis of multiple mouse model strains has the potential to sequentially add to the understanding of AD genetics, as each inbred strain pair may harbor a unique set of strain–strain polymorphisms modifying AD phenotypes. We have recently established the R1.40 transgene on the A/J genetic background, and ongoing comparisons with other R1.40 transgenic strains should prove enlightening in the context of results from the TgCRND8 model.

To identify preliminary sets of candidate genes within the detected QTLs with known polymorphisms resulting in differences in protein sequence between these strains, the linked regions were searched for validated SNPs with informative non-synonymous differences in the coding region of genes between the C57BL/6J and DBA/2J strains using the Ensembl BioMart data mining tool (Sanger mouse dbSNP database build 126). While recognizing that the future study of non-coding polymorphisms modifying splicing or gene expression patterns is also likely to be of significant importance, we performed an initial screen for non-synonymous coding differences to obtain a manageable initial set of genes that can be shown to be attractive candidates based on known sequence data. A list of non-synonymous C57BL/6J × DBA/2J coding polymorphisms in the significantly associated chromosome 2 interval is displayed in Table 2. The specific linkage regions detected contain a number of biologically interesting candidate genes.

The haplotype of the chromosome 2 locus present in the DBA/2J strain is known to contain a 2 base pair deletion in the gene for complement component 5 (*Hc*), which results in a frameshift and a truncated, non-functional translation product (Wetsel et al., 1990). The *Hc* gene is an essential part of the complement cascade, a primary mechanism of the innate immune system that may play an important role in the neuroinflammation observed in AD and some other neurodegenerative diseases (reviewed in Bonifati and Kishore, 2007). Modulation of inflammatory pathways is believed to be of significant importance in AD pathogenesis, as demonstrated by the involvement of microglial activation and neuroinflammation in AD pathology and the association of non-steroidal anti-inflammatory drug (NSAID) treatment with a significant reduction in AD risk (reviewed in McGeer et al., 2006; Wyss-Coray, 2006). Aβ has been demonstrated to induce the complement cascade (Fan and Tenner, 2004), and a wide variety of complement proteins, including component 5, are specifically upregulated in AD brains (Yasojima et al., 1999). Interestingly, the *Hc* gene has recently been associated with several other diseases in mouse and human studies, including liver fibrosis (Hillebrandt et al., 2005), glomerulonephritis (Peng et al., 2005), and airway hyperresponsiveness in asthma (Pickering et al., 2006). The specific *Hc* truncation present in the current study has been shown to have dramatic effects on inflammation in mice, and lies squarely within the region significantly associated with lower Aβ levels in the DBA/2J strain, making it an attractive candidate for further study.

Downstream of the *Hc* deficiency, the significantly linked region of chromosome 2 also contains multiple validated C57BL/6J versus DBA/2J polymorphisms in the gene for LDL receptor-like protein 1B (*Lrp1b*), including three non-synonymous coding polymorphisms and over 1000 polymorphisms in intronic and untranslated regions. LRP1B is highly expressed in brain tissue (Marschang et al., 2004), and has structural similarities to LDL-receptor related protein (LRP), which is known to affect Aβ production through a direct interaction with APP. LRP1B was found to bind specifically to APP isoforms containing a Kunitz proteinase inhibitor (KPI) domain and facilitate their degradation, and has been implicated in decreasing Aβ production (Cam et al., 2004). Notably, the R1.40 transgene used in the current study is an intact genomic copy of human *APP* containing all alternatively spliced exons, including those of the rarely studied KPI-containing 751 and 770 amino acid isoforms of APP. *Lrp1b* is another attractive potential candidate for the observed linkage to Aβ production on chromosome 2.

The chromosome 1 locus contains presenilin-2 (*PSEN2*), a member of the γ-secretase complex responsible for production of Aβ. Nicastrin, another γ-secretase complex member, lies 9 Mb upstream of the linked interval. No non-synonymous coding polymorphisms between the C57BL/6J and DBA/2J strains were detected within the *PSEN2* gene, although 15 validated polymorphisms were found within the introns and 3′ and 5′ untranslated regions.

The chromosome 7 locus contains *Apba2*, also known as X11β, an APP-binding protein family member which is expressed in neurons and has been implicated in modulating Aβ production (Lee et al., 2004). The mechanism by which X11β acts on Aβ production is not known, but has been hypothesized to involve a variety of pathways including Fe65 nuclear signaling, APP trafficking, or copper metabolism (reviewed in Miller et al., 2006). The coding polymorphism observed in X11β appears to be a strong potential candidate for the suggestive linkage observed at this locus.

Human AD risk has a very significant unexplained genetic component, which appears unlikely to be due to a small number of powerful QTLs. With exhaustive QTL mapping efforts in human populations consistently encountering

great difficulty in detecting significant linkage and narrowly defining linked regions, mapping of AD-associated traits using transgenic mouse model intercrosses can be a fruitful strategy to detect genetic modifiers of AD pathogenesis. In this study, genetic factors were observed to have a high degree of control of brain Aβ levels, which appears to result from the action of a substantial number of independent QTLs. The use of a large F2 population, densely-spaced SNP markers, and methods to ensure high quality of genotype and phenotype data enabled the detection of genomic regions associated with brain Aβ levels in B6D2F2 R1.40 animals, several of which contain biologically interesting candidate genes and are syntenic to regions with indications of potential linkage to human AD risk.

The genetic mapping of complex traits is complicated by small individual allele effect sizes and the difficulty of distinguishing false positives from significant results. Associations with AD risk identified in large and well-designed human mapping studies have often proved difficult to replicate. Although complex trait mapping can prove equally difficult in a mouse intercross, the defined genetic background of mouse inbred strains provides a number of advantages for the intensive further study of identified QTLs, including knowledge of full genomic sequence data for multiple strains, improving technology for large-scale analysis of gene expression, availability of congenic strains for isolation and refinement of detected intervals, and the potential for knockout or transgenesis experiments to directly test the action of individual candidate genes. Genetic studies for a wide variety of traits have mapped QTLs to homologous regions in the mouse and human genomes, suggesting a strong predictive value for QTL mapping in mice, and multiple disease genes identified by mouse QTL studies have been confirmed to be involved in humans (reviewed in Peters et al., 2007). With the complex genetics of human AD proving remarkably difficult to elucidate, identification of QTLs acting on AD phenotypes in mouse models provides a powerful way to isolate and reproducibly study genetic modifiers that are detected.

Conflict of interest

The authors report no conflicts of interest.

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