

Identification of loci determining susceptibility to the lethal effects of amyloid precursor protein transgene overexpression

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Phenotypes produced by expression of human amyloid precursor protein (APP) transgenes vary depending on the genetic background of the mouse. FVB/N mice overexpressing human APP₆₉₅ develop a central nervous system disorder and die prematurely, precluding development of A β peptide amyloid plaques. 129S6 mice are resistant to the lethal effects of APP overexpression, allowing sufficient levels of A β expression for the development of amyloid plaques and age-dependent memory deficits. To identify the genes that determine susceptibility or resistance to APP we analyzed crosses involving FVB/NCr and 129S6.Tg2576 mice that overexpress 'Swedish' mutant (K670N, M671L) APP₆₉₅. APP transgene-positive FVB129S6F1 (F1) mice are resistant to the lethal effects of APP overexpression, so FVB \times F1 backcross and F2 intercross offspring were produced. Analysis of age of death as a quantitative trait revealed significant linkage to loci on proximal chromosome 14 and on chromosome 9; 129S6 alleles protect against the lethal effects of APP. Within the chromosome 14 interval are segments homologous to regions on human chromosome 10 that have been linked to late onset Alzheimer's disease or to levels of A β peptide in plasma. However, analysis of plasma A β peptide concentrations at 6 weeks in backcross offspring produced no significant linkage. Similarly, elevation of human A β peptide concentrations by expression of mutant presenilin transgenes did not increase the proportion of mice dying prematurely, suggesting that early death reflects effects of APP or fragments other than A β .

INTRODUCTION

A β peptides, cleavage products of amyloid precursor protein (APP), are elevated and deposited as amyloid plaques in Alzheimer's disease (AD). Rare mutations flanking or within the A β encoding segment of APP are linked to early onset familial (F) AD and increase production of A β peptides. Mutations in the presenilin genes (*PS1* on chromosome 14 and *PS2* on chromosome 1) account for most cases of early onset FAD and cause disproportionate elevation of the highly amyloidogenic A β ₄₂ peptide.

Numerous lines of transgenic mice expressing mutant or wild-type human APP have been produced to provide animal models for AD with varying degrees of success (1,2). On the basis of its ability to drive position-independent and copy number-dependent transgene expression in the brain, we selected the hamster prion protein (PrP) gene-derived cosTet vector to produce APP₆₉₅ transgenic mice (3,4). Inbred FVB/N mice originally were selected as the background strain for transgene expression because of their fertility and highly visible pronuclei, which facilitate microinjection (5). As expected, levels of expression of wild-type or mutant

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APP increased in proportion to transgene copy number; unexpectedly, expression of high levels of APP₆₉₅ in FVB/N mice caused premature death and obvious behavioral abnormalities (6). Age at death and fraction of mice dying were proportional to the amount of APP that was expressed. Levels of APP approaching those now known to be necessary for amyloid plaque formation caused death of nearly all FVB/N transgenic mice before 100 days. Many lines of transgenic mice had been constructed previously using the cosTet vector to overexpress PrP to dissect susceptibility to transmissible prion disease, and early death was not observed (7). We also have used the hamster PrP-derived cosTet vector to produce high copy number FVB transgenic lines expressing PS1, PS2, nicastrin, tau, alpha synuclein or the PrP-like protein doppel, indicating that APP-induced premature death is not a non-specific effect of massive protein overexpression in this strain of mice (8,9 and unpublished data).

Other strains of mice also are susceptible to the lethal effects of APP transgene overexpression (10). F1 hybrids between FVB/N APP transgenic mice and some strains, such as C57BL/6J (B6), die prematurely whereas other backgrounds, such as SJL/J (SJL) or 129S6, are protective. Successful production of a line, Tg(APP_{NL})2576, developing amyloid plaques was achieved by microinjection of B6SJL embryos using cosTet PrP regulatory sequences to overexpresses 'Swedish' mutant (K670N, M671L) human APP₆₉₅ (6,11). Transfer of the Tg(APP_{NL})2576 transgene array onto a B6 background was abandoned when survival and proportion of transgene-positive offspring declined with each successive backcross generation (10).

The dependence of APP-induced premature death on the promoter used to drive expression has not been addressed systematically. *Thy1* promoter driven APP overexpression also caused premature death in FVB/N and B6 mice with FVB/N being more susceptible than B6 mice (12,13). Interestingly, though both B6 and FVB/N mice are susceptible to APP-induced lethality, the associated neurobehavioral abnormalities were much more severe in FVB/N mice (10,13). A *Thy1* promoter also was used to produce the amyloid plaque forming B6 line APP23 (14); a fraction of these transgene-positive mice die before 18 months, much later than FVB/N mice expressing APP driven by either PrP or *Thy1* sequences (15,16). However, the APP in this line encompasses the KPI domain, which may be protective. Similarly, transgenic B6 mice that were constructed with a yeast artificial chromosome and express APP under the control of its own promoter do not die prematurely (17). The first APP transgenic line to develop amyloid plaques was produced on a mixed genetic background with V717F mutant APP expression driven by the platelet-derived growth factor (*Pdgf*) promoter (18); premature death of this transgenic line, which also includes the KPI domain in APP, has not been reported. The roles of promoter and APP isoforms in premature death are discussed in more detail elsewhere (19).

Tg(APP_{NL})2576 is the most widely studied APP transgenic model for AD (6,11). The Tg2576 transgene array was transferred from the original mixed B6SJL background onto the 129S6 inbred background by repeated backcrossing to produce the 129S6.Tg2576 congenic strain; in addition to being resistant to the lethal effects of APP, 129S6 mice

perform well in spatial memory tests (20,21). Tg(APP_{NL})2576 mice recapitulate two key features of AD: amyloid plaque deposition and age-related memory deficits. The appearance of spatial learning and memory deficits in Tg2576 mice coincides with the formation of detergent insoluble forms of A β ₁₋₄₂ (A β 42), which is the longer and more amyloidogenic form of A β peptides (20). A β , plaque load and memory deficits in Tg2576 and other APP transgenic lines can be reduced by a variety of interventions (10,22-31). The reversibility of age-related memory deficits and lack of neuronal loss suggests that Tg2576 may represent a 'pre-Alzheimers' like condition that fails to convert to the inexorable progression seen in AD patients.

Identification of genes that modify phenotypes in APP transgenic mice could lead to identification of new AD susceptibility genes, advancing our understanding of AD and providing new opportunities for therapeutic intervention. Although the relevance of APP-induced premature death to AD is unclear, understanding the genetic control of this phenomenon could help elucidate the physiological functions of APP. Towards these ends, we applied quantitative trait analysis to age at death in FVB \times 129S6.Tg2576 crosses. Regions on mouse chromosomes 9 and 14 showed significant linkage to modifiers of survival. In contrast, no significant linkage to plasma A β peptide levels was detected with this strain combination. Elevation of A β peptide levels by mutant PS1 or PS2 transgenes in FVB/N mice did not increase the proportion or age at death of mice that died prematurely, suggesting that APP-induced lethality is not mediated by A β peptides.

RESULTS

Resistance to APP-induced premature death is a dominant genetic trait

In earlier work, it proved impossible to construct mice expressing high levels of APP₆₉₅ and developing amyloid plaques using the FVB/NCr inbred strain (6). The highest level of APP₆₉₅ expression in FVB/NCr mice that could be maintained in a permanent line was \sim 3.6 times the amount of endogenous APP. This line of mice, designated Tg(APP)1130H, harbored over 70 copies of V717I mutant APP₆₉₅, which also carried inadvertently introduced V721A and M722A changes. More than 95% of Tg1130H mice died before 150 days, with half the mice dead at 87 ± 4 days. Several FVB/NCr high copy number founders died before producing offspring; production of Swedish mutant APP transgenic FVB/N lines was particularly problematic with only one success. Microinjection of B6SJL F2 embryos resulted in a viable, amyloid plaque-producing transgenic line, Tg2576, expressing \sim 5.6 times endogenous levels of Swedish mutant APP. We assume that expression of the Tg2576 transgene array would kill FVB/N mice; attempts to transfer the Tg2576 array to B6 mice, which also are susceptible to the lethal effects of APP overexpression, were unsuccessful because of early death (10).

The Tg2576 transgene array was transferred onto the 129S6 inbred background by repeated backcrossing; this strain was selected because F1 hybrids between FVB-Tg1130H and 129 substrains survived and because 129S6 performs well in tests of spatial learning and memory (20,21). Only seven of

Table 1. Low rates of APP-induced premature death (<200 days) in 129S6 and (FVB × 129S6)F1 mice

Mice	Number dead	Number at risk	% dead	Ages (days) at death
Transgene-negative 129S6	1	231	0.4	67
Transgene-positive 129S6.Tg2576	7	170	4.1	68, 102, 110, 112, 117, 167, 194
Transgene-positive (FVB × 129S6.Tg2576)F1	4	59	6.8	79, 92, 139, 157

170 (4.1%) 129S6.Tg2576 from the eighth to fifteenth backcross (N8 to N15) died younger than 200 days; only one of 231 (0.4%) transgene-negative 129S6 littermates died prior to 200 days. Four of 59 (6.8%) transgene-positive F1 offspring of FVB/NCr females mated with 129S6.Tg2576 males died prematurely. These results, including individual ages at death, are presented in Table 1. Although the fractions of 129S6 and FVBS6F1 APP transgenic mice dying are low, they are significantly greater than that for non-transgenic mice ($P < 0.001$). Therefore, although 129S6 alleles are dominant in protecting against early death due to expression of APP transgenes, the protection is not completely penetrant.

Survival of FVB × (FVB × 129S6.Tg2576)F1 backcross and (FVB × 129S6.Tg2576)F2 intercross offspring

FVB/NCr females were used to produce F1 and backcross offspring because of their high fertility and poor maternal behavior in mice overexpressing APP (unpublished data). Transgene-negative F1 females were mated with transgene-positive F1 males to produce intercross offspring. All transgene-positive mice were bled at 6 weeks, and plasma for assaying A β peptide concentrations stored at -80°C . The mice were observed daily. Dates of death not due to obvious extraneous causes, such as a leaky water bottle, were recorded. No obvious signs of illness preceding death were noted. Mice that were injured by fighting (a common phenomenon among transgene-positive males) were removed from the study. When fighting injuries were noted in a cage, the uninjured males were separated and housed individually. Mice surviving to 200 days were euthanized.

Cumulative survival (Kaplan–Meier plot) of 136 transgene-positive backcross offspring born within a 110 day interval is presented in Figure 1. Birth cohorts grouped by litter or month of birth did not differ significantly in proportions of mice dying prematurely (ANOVA). Data from male and female mice are combined; 21 of 51 (41.2%) male mice died prior to 200 days compared with 46 of 85 (54.1%) females. This difference may reflect exclusion of males injured in fights. Among mice dying prematurely, there was no difference between male and female mice in mean age at death or the 50% survival time. The mean age at death for male mice was 109 ± 9 days with 50% dead at 99 ± 11 days when compared with a mean of 111 ± 5 days and 50% survival time of 99 ± 4 days for female mice.

Cumulative survival of 88 transgene-positive F2 offspring born over the course of 331 days is also presented in Figure 1. Premature death occurred in birth cohorts throughout

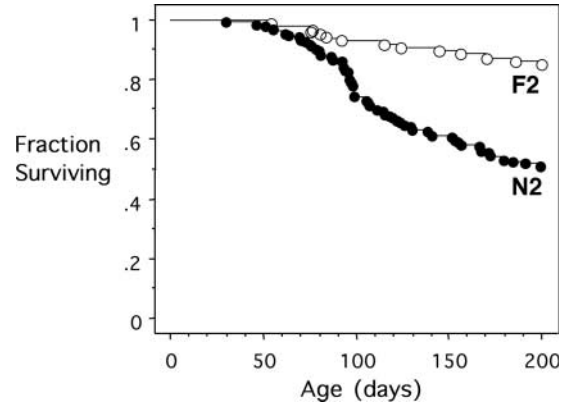


Figure 1. Survival of APP transgene-positive FVB × (FVB × 129S6.Tg2576)F1 backcross (N2) and (FVB × 129S6.Tg2576)F2 intercross (F2) offspring. Kaplan–Meier survival plots for 136 backcross offspring (filled circles) and 88 intercross offspring (open circles) are shown. Observation ended at 200 days and surviving mice were euthanized.

the course of the observation period, but only 13 mice (14.8%) died before or at 200 days; seven of 39 (17.9%) were males and six of 49 (12.2%) were females. The mean age at death of those dying before the experiment was terminated was 120 ± 13 days, with 50% of these mice surviving to 115 ± 29 days.

Identification of chromosomal regions harboring genes modifying susceptibility to APP-induced premature death

Backcross. An initial genome scan of backcross offspring included 44 mice that died before 200 days and 44 mice that survived to 200 days. The mice dying prematurely were those with the shortest lifespans; age at death ranged from 46 to 119 days. The early death group consisted of 14 male and 30 female mice; the 200 day survivors included 19 male and 25 female mice. Genotyping mice at the extremes of a distribution, which are most likely to share genotypes, has power comparable to genotyping all individuals (32,33). The mice were typed for 76 markers; the average spacing determined from our data was 22.4 ± 8.6 cM. The largest interval between any two markers was 38.8 cM on chromosome 15 and the smallest was 3.8 cM on chromosome 10. Only single markers were used for chromosomes X and 18. Fluorescence *in situ* hybridization previously had localized the Tg2576 transgene array to proximal chromosome 18, reducing the ability to detect modifiers on this chromosome. The excess of *D18Mit48* heterozygous backcross offspring (62 heterozygous versus 22 mice homozygous for the FVB allele) is compatible with linkage to the transgene insertion site. The extent of the 129S6.Tg2576 congenic interval on chromosome 18 containing B6- and/or SJL-derived alleles has not been determined. Genotypes for the 76 markers were obtained for 78–88 of the mice; individual samples that failed to amplify or that gave ambiguous results were not rerun unless there was suggestive linkage in the region. The mean number of informative offspring for all markers was 86.1 ± 2.1 . Only

Table 2. Loci showing suggestive linkage to genes modifying susceptibility to the lethal effects of APP in initial genome scan of 88 mice

Locus	MGD ^a map position	Empirical map position ^b	LRS ^c (<i>P</i>)	Percent contribution to variance
<i>D9Mit285</i>	21.0	24.5	11.8 (0.0006)	13
<i>D10Mit31</i>	36.0	32.7	6.9 (0.0087)	8
<i>D10Mit14</i>	65.0	69.1	6.9 (0.0085)	8
<i>D14Mit98</i>	3.0	(3.0)	12.1 (0.0005)	13
<i>D14Mit127</i>	10.0	16.1	12.3 (0.0005)	13

^aCumulative map distance in centimorgans from the Mouse Genome Database (<http://www.informatics.jax.org/>).

^bCumulative map distance in centimorgans calculated from the 88 mouse genome scan using MapManager QTXb19. The proximal marker on each chromosome was assigned the distance from the centromere reported in MGD. The most proximal chromosome 9 marker is *D10Mit90* at 9 cM; on chromosome 10 the most proximal marker is *D10Mit49* at 2 cM; on chromosome 14 the proximal marker is *D14Mit98* at 3.0 cM, thus 'MGD' and 'empirical' values are equivalent at this locus.

^cLikelihood ratio statistic.

one (*D2Mit148*) of the 66 markers was informative on fewer than 80 mice.

Data from the initial scan of 88 mice were analyzed for linkage to genes modifying the effects of APP expression using MapManager QTXb19. As 200 days was the endpoint for the experiment, the survival times did not approximate a normal distribution. The data were analyzed as the ln of the fraction 'age at death/200', which more closely approximates a normal distribution than age at death. Markers showing suggestive linkage to genes modifying susceptibility to APP-induced lethality as indicated by a χ^2 probability of <0.01 are presented in Table 2. Chromosome 9 had one of these markers and chromosomes 10 and 14 had two markers each. Homozygosity for FVB alleles of these markers was over-represented in the dead mouse population, with heterozygosity more frequent in the survivors.

To empirically determine significance levels, 10 000 permutations of the data were run using MapManager (34–36). The likelihood ratio statistics (LRS) for ln(age/200) corresponding to suggestive, significant and highly significant linkage were 6.5, 12.6 and 20.5. The remaining 48 offspring from the backcross were typed for markers on chromosomes 9, 10 and 14. Interval mapping in 1 cM steps produced plots of LRS against the maximum likelihood linkage maps. LRS on chromosome 10 when all 136 mice were genotyped for all five markers used for this chromosome did not reach the criteria for suggestive linkage (data not shown). In contrast, the peak LRS on chromosome 9 had a value of 14.0 and was located 1 cM proximal from *D9Mit285*; a probability plot for ln(age/200) against a linkage map of chromosome 9 is presented in Figure 2A. Homologous human chromosomal segments also are illustrated. The highest probability of linkage was to a mouse locus or loci on chromosome 14. As illustrated in Figure 2B, the peak LRS (18.3) was located between *D14Mit98* and *D14Mit127* at ~11 cM. The probability of obtaining an LRS of 18.3 in 10 000 random permutations of the data is 0.0027 ± 0.0005 .

A search for additional QTL by composite interval analysis controlling for chromosomes 9 and 14 markers individually and in combination did not reveal any new regions linked to survival. Controlling for chromosome 9 or chromosome 14 slightly increased the peak LRS for the other QTL (chromosome 9, LRS = 14.8; chromosome 14, LRS = 19.7) but not the location of the peak.

Intercross. Only 13 of 88 F2 mice died at 200 days or younger. Seventy-nine of these F2 offspring, including 13 that died, were typed for markers on chromosomes 9 and 14. Linkage to survival was not significant. However, on chromosome 14, the distribution of genotypes for *D14Mit127* in 13 mice that died differed significantly from the expected 1:2:1 ratio ($\chi^2 = 7.62$, $P < 0.05$). Seven mice were homozygous for the FVB allele, six were heterozygous and none were homozygous for the 129 allele. The distribution of *D9Mit285* genotypes on chromosome 9 in the mice dying prematurely did not differ from that expected.

Plasma A β peptide levels at 6 weeks did not correlate with survival

The mouse chromosome 14 interval contains a segment homologous to the region on human chromosome 10 identified as containing modifiers of plasma A β peptide levels and risk for late-onset AD (37–39). We measured A β 40 and A β 42 concentrations in plasma obtained from all backcross offspring at 6 weeks of age. Levels of A β peptides did not differ significantly from those in 129S6.Tg2576 or (FVB \times 129S6.Tg2576)F1 mice. QTL analysis did not suggest any chromosomal regions containing genes influencing plasma A β levels nor was there any suggestion of a correlation between age at death or survival to 200 days and either A β 40 or A β 42 concentration (data not shown).

Elevation of A β 42 by mutant PS transgene expression does not alter survival of APP transgenic mice

Effects of PS-induced elevation of A β 42 on survival were assessed in the FVB inbred background. FVB–Tg(APP_{WT})6209 mice express ~1.5 times endogenous levels of myc-tagged wild-type human APP₆₉₅ (6,10). On average, half of the APP transgene-positive mice in this line die prematurely. Tg(APP_{WT})6209 were crossed to FVB transgenic lines expressing wild-type or mutant human presenilin-1 (PS1) or mutant human presenilin-2 (PS2) transgenes (8). The offspring were genotyped for both the APP and PS transgenes and all APP-positive mice were aged. The fraction of mice dying younger than 200 days, along with the mean age at death and 50% survival time for those that died, are presented in Table 3. Mice expressing mutant PS transgenes, which

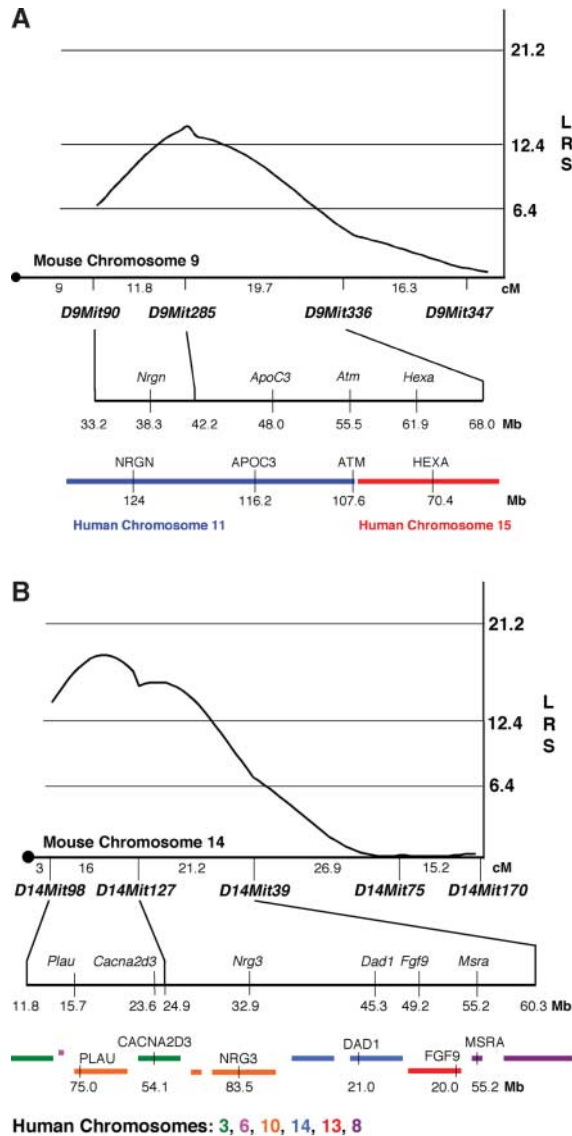


Figure 2. Evidence for loci on mouse chromosomes 9 and 14 modifying susceptibility to APP overexpression. The LRS is plotted against the empirical map distance between markers calculated using MapManager QTXb19. Horizontal lines and LRS values indicate suggestive, significant and highly significant linkage determined by permutation analysis of the data from 136 backcross offspring; 10 000 permutations were run. (A) Chromosome 9. The distance from the centromere to *D9Mit90* is the value cited in the Mouse Genome Database (<http://www.informatics.jax.org/>). A physical map of the *D9Mit90* and *D9Mit336* interval (~34.8 Mb) based on Build 32 presented on the Ensembl Mouse Genome Server (http://www.ensembl.org/Mus_musculus/) is presented along with the locations of *Nrgn* (neurogranin), *ApoC3* (apolipoprotein-C3), *Atm* (ataxia telangiectasia mutated homologue) and *Hexa* (hexosaminidase A) as landmarks. The blue (chromosome 11) and red (chromosome 15) lines illustrate the homologous regions and physical location of the corresponding human genes. (B) Chromosome 14. The distance from the centromere to *D14Mit98* is the value cited in the Mouse Genome Database. A physical map of the *D14Mit98* and *D14Mit39* interval (~48.5 Mb) based on Build 32 presented on the Ensembl Mouse Genome Server is presented along with the locations of *Plau* (plasminogen activator, urokinase), *Cacna2d3* (calcium channel, voltage dependent, alpha2/delta subunit 3), *Nrg3* (neuregulin 3), *Dad1* (defender against death 1), *Fgf9* (fibroblast growth factor 9) and *Msra* (methionine sulfoxide reductase A) as landmarks. Colored lines indicate regions of homology to segments of human chromosomes 3 (green), 6 (pink), 10 (orange), 14 (blue), 13 (red) and 8 (purple).

elevate the proportion of human APP-derived A β 42 as shown in Table 4, did not die in greater numbers or sooner than their PS transgene-negative siblings and cage mates. Note that although all APP-positive, PS-negative mice should be genetically equivalent, there is considerable variation in survival from experiment to experiment; this suggests environmental effects. In no case, however, was elevation of A β associated with an increase in premature death when comparing PS transgene-positive and transgene-negative offspring of any individual cross.

DISCUSSION

Identification of loci modifying susceptibility to the lethal effects of APP overexpression poses particular challenges due both to the incomplete penetrance of resistance and susceptibility genes and to potential environmental effects. Resistant inbred strains or F1 hybrids have a small, but significant, fraction of individuals dying prematurely; the fraction dying and age at death differs among strains showing resistance to APP transgene overexpression (10). Similarly, the fraction and age of animals dying young among inbred FVB mice expressing subamyloidogenic levels of APP is proportional to the level of APP expression (6) and can vary from experiment to experiment (Table 3). Therefore, genetic background is not the sole determinant of survival in the face of the Tg2576 transgene array with indeterminate fractions of genetically resistant mice dying early and of susceptible mice surviving.

Previous analysis of survival of APP transgenic inbred FVB mice suggested that individuals surviving beyond 200 days were at no greater risk of dying than their non-transgenic littermates (6,10). The window of lethality in the crosses reported here may differ from that in inbred FVB/N mice. As illustrated in Figure 1, death occurred among FVB-129S6 backcross and intercross offspring right up to the termination of the experiment at 200 days. Not all the surviving animals were euthanized at exactly 200 days and some mice died after this arbitrary cutoff. APP-induced death at earlier and later times conceivably could involve different mechanisms.

Environmental effects are suggested by differences in survival rates of Tg(APP)6209 mice as shown in Table 3. Although PS transgene expression did not affect survival, there was considerable variation among experiments. The experiments were not done concurrently and numerous variables, such as location of the cage rack, level of activity in the room, changes in caretakers or seasonal variables, could have influenced survival in different experiments. Though such variables did not appear to have profound effects on survival in the face of the much higher level of expression in the Tg2576 crosses, it is likely that environmental-genetic interactions are a complicating factor for identifying genes controlling the complex trait of APP-induced premature death.

In spite of the influence of non-genetic determinants of survival, QTL analysis revealed significant linkage to loci on chromosomes 9 and 14. Linkage to the chromosome 9 region barely reached the level of significance determined by permutation analysis. No genes known to be involved in AD are located within the corresponding human intervals,

Table 3. Expression of mutant PS transgenes does not increase susceptibility to APP-induced lethality

Transgene ^a	Transgenic line	Presenilin transgene	Fraction dead (<i>n</i>)	Age at death ± SE (days)	50% survival age (days)
PS1 _{WT}	Tg195	Positive	0.286 (14)	136 ± 22	145
		Negative	0.667 (15)	112 ± 10	103
PS1 _{L286V}	Tg198	Positive	0.661 (18)	124 ± 15	119
		Negative	0.538 (13)	98 ± 17	96
PS1 _{L286V}	Tg1274	Positive	0.444 (9)	125 ± 13	123
		Negative	0.538 (13)	122 ± 13	127
PS1 _{M146L}	Tg1	Positive	0.667 (15)	125 ± 12	106
		Negative	0.458 (24)	115 ± 10	108
PS1 _{M146L}	Tg29	Positive	0.938 (32)	106 ± 5	95
		Negative	0.917 (36)	107 ± 6	96
PS1 _{M146L}	Tg34	Positive	0.556 (9)	109 ± 6	101
		Negative	0.750 (12)	103 ± 13	99
PS2 _{M239V}	Tg1379	Positive	0.455 (11)	128 ± 23	157
		Negative	0.455 (11)	126 ± 13	121
PS2 _{N141I}	Tg1032	Positive	0.300 (10)	124 ± 18	124
		Negative	0.570 (7)	132 ± 24	106

^aAll survival data are for mice positive for the APP_{wt} transgene carried by Tg(APP)6209. The PS and APP transgenes are expressed on the inbred FVB/NCr background.

Table 4. Mutant PS transgene expression elevates Aβ peptide levels in Tg(APP)6209 mice expressing human wild-type APP

Presenilin transgene	Transgenic line ^a	<i>n</i>	Aβ42 ± SE (ng/g)	Total Aβ ± SE (ng/g)	Aβ42/Total Aβ
None ^b		9	3.86 ± 0.1	45.3 ± 1.5	0.085
PS1 _{WT}	Tg195	6	4.1 ± 0.1	51.2 ± 3.1	0.082
PS1 _{WT}	Tg672	5	4.0 ± 0.1	53.5 ± 2.1	0.075
PS1 _{L286V}	Tg198	5	6.7 ± 0.4	50.6 ± 4.1	0.134
PS1 _{L286V}	Tg1274	5	12.4 ± 0.5	59.9 ± 2.0	0.207
PS1 _{M146L}	Tg1	6	12.1 ± 1.7	61.9 ± 4.5	0.190
PS1 _{M146L}	Tg29	4	13.0 ± 0.5	59.9 ± 2.7	0.211
PS1 _{M146L}	Tg34	9	9.9 ± 0.3	59.1 ± 2.7	0.165
PS2 _{M239V}	Tg1379	6	15.7 ± 3.5	47.1 ± 3.7	0.324
PS2 _{N141I}	Tg1032	6	13.1 ± 2.5	44.8 ± 3.1	0.291

^aAβ levels indicated for each PS transgenic line are for PS transgene-positive, APP transgene-positive offspring of the line crossed to FVB-Tg(APP)6209.

^bAβ levels in Tg(APP)6209 mice expressing APP only.

nor has linkage to late-onset AD been suggested. Interestingly, this chromosome 9 interval overlaps with one exhibiting highly significant linkage to incubation time for experimental scrapie, a neurodegenerative disease caused by prions (40).

Evidence for linkage on mouse chromosome 14 is more compelling. In addition to a broad peak of significant linkage in QTL analysis of backcross offspring, the absence of mice homozygous for the 129S6 alleles of *D14Mit98* and *D14Mit127* among the 13 F2 mice dying prematurely supports the presence of a gene(s) linked to survival. Within this region are segments showing synteny with segments on several human chromosomes. The most intriguing homology is to the proximal region of human chromosome 10 that has been linked to late-onset AD (37,39) and to elevation of Aβ42 concentrations in plasma (38). The region of human chromosome 10 exhibiting synteny with mouse chromosome 14 includes the urokinase-type plasminogen activator (PLAU), which can degrade Aβ aggregates by increasing plasmin generation (41). Although no genetic linkage to plasma Aβ levels was detected in our QTL analysis, *Plau* cannot be eliminated as a candidate gene underlying the chromosome 14 QTL.

Elevation of Aβ42 by co-expression of PS and APP transgenes did not increase the age at death or proportion of mice dying prematurely (Tables 3 and 4), even though the level of elevation of Aβ peptides achieved by mutant PS1 has been demonstrated to have significant biological effects. Overexpression of APP causes impaired vasodilation in response to pharmacological (28) or physiological (42,43) stimulation. Co-expression of mutant PS1 and APP transgenes demonstrated that the degree of impairment correlated with levels of Aβ40 rather than Aβ42 (42). Thus, even the modest elevation in Aβ40 induced by mutant PS1 expression had significant functional effects. Mutant PS1 expression also accelerates the appearance of memory deficits and detergent-insoluble forms of Aβ in Tg2576 mice (20). It is likely, though not formally proven, that aggregates of Aβ42, rather than Aβ40, are responsible for memory loss in Tg2576 mice. Overexpression of APP itself clearly is not sufficient to induce age-dependent memory deficits as shown by the lack of memory loss in aged Tg5469 mice that express wild-type APP at levels similar to those in Tg2576 animals. As the fraction of transgenic mice dying prematurely increases

and age at death decreases with APP concentration but not with mutant PS-mediated elevation of A β peptides, it is likely that APP itself is responsible for premature death in Tg(APP) mice.

Beyond the association with APP levels themselves, attempts to determine whether specific APP metabolites are responsible for premature death and behavioral deficits in FVB/N APP transgenic mice have been inconclusive (13). The same constellation of early phenotypic changes were observed in mice that express various wild-type and mutant transgenes and that differ in proportions of secreted APP and its CTF fragments. Though genetic effects on levels of specific APP metabolites are amenable to investigation, it is more likely that modifiers of APP lethality reflect differences in host response to APP.

Confounding prioritization of genes as candidates underlying the QTLs is our ignorance on the cause of APP-induced premature death. No obvious pathological change in the brain or elsewhere has been found consistently that distinguishes APP-susceptible from APP-resistant strains of mice. Astrocytic gliosis was noted in some mouse strains of each susceptibility type (10). We and others have occasionally observed seizures in FVB/N APP transgenic mice (6,13). Enhanced susceptibility to kainic acid-induced seizures was observed in young transgenic mice expressing either mutant or wild-type APP transgenes (13), in keeping with other observations of perturbations in the glutamate system (44). On the basis of the predominant expression in CNS neurons in our transgenic mice and the severe behavioral abnormalities in FVB-Tg(APP) mice it seems likely that premature death has a neurological basis. Other causes, such as cardiac failure, have not been eliminated, however.

Clues to the mechanism may be provided by transgenes that modulate APP-induced premature death. Overexpression of human basic fibroblast growth factor (FGF2) transgenes potentiates the lethal effects of APP overexpression without altering APP processing (10). In addition to premature closure of the long bones, Tg(FGF2) transgenic mice have hypertrophy of vascular smooth muscle (45), raising the possibility of a connection with APP-induced cerebrovascular dysfunction.

Overexpression of superoxide dismutase-1 (SOD1) protects against APP-induced lethality (10) and prevents A β 40-induced cerebrovascular dysfunction (28). Imbalanced Cu homeostasis in Tg(APP) mice might explain some of the protective effects of SOD1 overexpression. APP null mice have elevated brain Cu levels whereas transgenic mice that overexpress APP have reduced amounts of Cu in the brain (16,46,47). Reduced activity of SOD1 accompanies APP transgene overexpression; increasing brain Cu levels by addition of CuSO₄ to the drinking water restores SOD1 enzymatic activity (16). Elevating Cu levels either in the diet or genetically by the toxic milk mutation of the *ATPase7b* gene also protects against premature death in Tg(APP) mice (16,47). Although scenarios for involvement of several genes in SOD1-related pathways within the large QTL interval on chromosome 14 could be envisioned, no candidate among the 400 plus known mouse genes with human equivalents stands out.

A recent study reports production of a B6.Tg2576 congenic strain for studies on the association of atherosclerosis with

cerebral amyloidosis and learning deficits (48). As B6 mice are susceptible to the lethal effects of APP overexpression, successful production of this congenic line may provide a new tool to identify chromosomal subregions harboring genes determining resistance to the lethal effects of APP overexpression. Congenic strain production was originally developed as a technique to isolate novel chromosomal regions harboring histocompatibility genes based on resistance to tumor growth (49). Breeding selects for genes enhancing fertility and survival along with the target locus. We suspect that SJL-derived alleles protecting against APP-induced lethality were co-selected when transferring the Tg2576 transgene array to the B6 background. Genetic analysis of the new B6.Tg2576 congenic strain could reveal additional loci influencing APP-susceptibility or narrow the intervals identified here.

The genetic malleability of phenotypes produced by APP overexpression in mice may be both an impediment and a benefit to the use of transgenic mice as models for AD. Although the potential involvement of these genes as modifiers of AD or as therapeutic targets is unknown, their identification may provide new insight into the physiological functions of APP and to the development of improved models for this human disease.

MATERIALS AND METHODS

Mice

All mice were produced at the McLaughlin Research Institute and are free of mouse viral pathogens and endo- and ectoparasites. The institute's animal resource center is fully accredited by AAALAC International. Tg(APP_{NL})2576 mice express Swedish mutant APP₆₉₅ under control of hamster PrP regulatory sequences as previously described (6,11). Tg2576 mice were produced by microinjection of (B6SJL)F2 embryos and originally maintained on this mixed genetic background. The transgene array was transferred onto the inbred 129S6 background by repeated backcrossing; the 129S6.Tg2576 congenic line was in the tenth or eleventh backcross generation when used as breeders for these experiments. FVB/NCr (FVB) mice were originally obtained as pedigreed stock from the National Cancer Institute, Frederick, MD, USA and are maintained by brother-sister mating. FVB-Tg6209 mice expressing myc-tagged wild-type human APP₆₉₅ under control of hamster PrP sequences have been previously described (6). The same hamster PrP-derived cosmid expression vector was used to construct FVB transgenic lines expressing human PS1 or PS2 transgenes (8). Tg1, Tg29 and Tg34 express PS1_{M146L}, Tg198 and Tg1274 express PS1_{L286V}, Tg195 and Tg1098 express PS1_{WT}, Tg1379 expresses PS2_{M239V} and Tg1032 expresses PS2_{N141I}.

Genotyping

DNA was extracted from tail snips using the Qiagen DNeasy tissue kit. APP transgene-positive individuals were identified using APP-specific oligonucleotide primers in PCR as described previously (10). Transgene-positive mice were typed for microsatellite loci by PCR amplification using

M13-tailed primers and including IRD-700 or IRD-800 dye-labeled M13-29 oligonucleotide (LiCor, Lincoln, NE, USA) in the PCR reaction mixture. The PCR amplified fragments were resolved by electrophoresis on denaturing polyacrylamide gels using LiCor GeneReadIR 4200 systems. Details of these procedures have been presented previously (40). Primer pairs for microsatellite markers that produce fragments with at least a two basepair difference were selected from the list of markers discriminating FVB/NJ and 129S6 provided by the Center for Inherited Disease Research (<http://pages.cidr.nhgri.nih.gov/mouse/queries.html>). Although FVB/NJ and FVB/NCr are distinct substrains, the markers selected were informative. The list of markers used is available on request. Genotypes were scored independently by at least two individuals.

A β peptide measurement

Sandwich ELISA assays to detect the effects of PS transgene expression on levels of total A β peptide and A β _{1–42} (A β 42) have been previously described (8). Briefly, total A β was detected using monoclonal antibody (mAb) 266 against A β residues 13–28 as capture and mAb 3D6 to residues 1–5 as reporter. To specifically measure A β 42, mAb21F12 against residues 33–42 was used for capture. Plasma A β 40 and A β 42 concentrations were determined using the BAN-50/BA-27 and BAN-50/BC005 sandwich ELISA assay as previously described (50).

Linkage and statistical analyses

Linkage analysis, data permutation and associated statistical tests employed MapManager QTXb19, which is available at <http://mapmgr.roswellpark.org/mmQTX.html> (36). Statistical comparisons of survival with birth cohort or A β peptide levels were performed using Statview 5.0 (SAS Institute, Cary, NC, USA).

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