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Human Molecular Genetics, 1997, Vol. 6, No. 11 **1951–1959**

Genetic modification of the phenotypes produced by amyloid precursor protein overexpression in transgenic mice

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Received June 13, 1997; Revised and Accepted July 18, 1997

Overexpression of Alzheimer amyloid precursor protein (APP) produces dramatically different phenotypes in transgenic mice depending on the genetic background. For example, concentrations of APP that produce amyloid plaques in outbred transgenic lines are lethal for inbred FVB/N or C57BL/6J mice. Expression of *SOD1* transgenes is protective, suggesting involvement of oxidative damage in premature death, but ablation of *Apoe* had no significant effect. In contrast, *FGF2* transgene overexpression enhances the lethal effects of APP. Differential survival does not appear to reflect genetic differences in APP processing, but rather host responses to APP or its derivatives.

INTRODUCTION

The human Alzheimer amyloid precursor protein (APP) gene on chromosome 21 encodes a transmembrane protein of unknown function that is intimately involved in the pathogenesis of Alzheimer's disease (AD). The amyloid plaques that are a pathognomic feature of AD consist primarily of $A\beta_{1-42}$ peptide, a normal APP cleavage product produced at lower levels than the less amyloidogenic $A\beta_{1-40}$ peptide (1–4). Overexpression of APP due to trisomy 21 (Down's syndrome) leads to AD-like pathology and cognitive decline in middle age (5). Mutations in the *APP* locus have been linked to early-onset familial AD (FAD); these missense mutations lie adjacent to the 5′ and 3′ ends of the Aβ peptide-coding segment (6–10). APP with the K670N and M671L amino acid substitutions (Swedish mutant) just amino-terminal to Aβ shows increased production of both $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides (11,12), while substitutions at codon 717 that lies carboxy-terminal to Aβ selectively increase the proportion of $A\beta_{1-42}$ (13).

Mutations in presenilin genes (*PS1* on chromosome 14 and *PS2* on chromosome 1) account for most cases of early-onset FAD (14–17). Sera and skin fibroblasts from *PS1* and *PS2* mutation carriers secrete elevated amounts of $A\beta_{1-42}$, suggesting that the pathogenic effects of these are mediated through effects on APP processing (18). Similarly, expression of mutant, but not wild-type, human PS1 in transgenic mice increases the brain concentrations of $A\beta_{1-42}$ encoded by the endogenous mouse *App* gene (19) or by mutant (20) or wild-type (21) human *APP* transgenes.

Although APP and its Aβ cleavage product have been implicated in AD for many years, development of transgenic mouse models for AD that clearly recapitulate features of the human disorder, such as plaque formation, has proved elusive until recently (22,23). Overexpression of V717F mutant human APP from a platelet-derived growth factor (PDGF) promoter-driven construct, allowing production of 695, 751 and 770 amino acid isoforms, led to production of cortico-limbic amyloid plaques and neuritic changes at approximately one year (22). Driving Swedish-mutant APP_{695} overexpression with the prion protein (PrP) gene-derived cosTet transgene expression vector (24) also produced a line (Tg2576) that developed amyloid plaques at approximately one year, indicating that the Kunitz-like proteinase inhibitor domain in the 751 and 771 isoforms was not required for plaque deposition (23). Correlative memory deficits were also documented in the Swedish mutant line Tg2576. It is noteworthy that the *APP* transgenic lines that develop Aβ amyloid plaques were produced using outbred mice; the first (22) was a mixture of outbred Swiss, DBA/2 (D2) and C57BL/6 (B6), while Tg2576 was produced from B6×SJL/J hybrids (23).

A variety of inbred FVB/N transgenic lines that expressed wild-type (wt) or mutant forms of APP_{695} were produced with the same PrP-derived expression vector used for the amyloid plaque-producing Tg2576 line (24,25). FVB/N (FVB) mice expressing supraendogenous concentrations of human (Hu) or mouse (Mo) APP_{695}-encoding transgenes died prematurely and developed a central nervous system (CNS) disorder similar to one that occurs spontaneously in some aged non-transgenic FVB

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mice. The CNS disorder includes a behavioral pattern suggestive of neophobia, decreased spontaneous alternation in a Y maze and diminished glucose utilization with astrocytic gliosis in the cerebrum. Increasing levels of brain APP increased the proportion of mice dying and produced earlier and more severe disease. FAD-linked mutations were not required for disease; wtAPP$_{695}$ transgene overexpression induced the CNS disorder, but possibly less efficiently than mutant transgenes. In contrast to outbred Tg2576, FVB/N transgenics developed no amyloid plaques nor extracellular deposits of Aβ peptide, even when surviving beyond 1 year of age. However, all permanent FVB transgenic lines had lower concentrations of APP in their brains than did Tg2576, and several high transgene copy number FVB founders failed to establish pemanent lines.

This study demonstrates the profound effect of host genotype on phenotypes induced by APP. It remains to be determined whether modifier genes identified using transgenic mice will prove relevant to AD in humans and offer new targets for therapeutic intervention.

RESULTS

The phenotypes produced by amyloid plaque-producing concentrations of APP change with genetic background

Tg(HuAPP$_{695}$.SWE)2576 mice were produced by micro-injecting (B6×SJL)F2 eggs with a hamster prion protein-derived cosmid insert in which the PrP open reading frame was replaced by a human *APP* cDNA with the K670N and M671L substitutions (23). APP concentrations in the transgenic lines used in these studies have been reported (25) and were assessed by quantitative immunoblotting using [^{125}I]protein A and phosphor imaging on at least three brains from young mice (1–4 months old). Tg2576 mice expressed 5.6 ± 0.3 (SEM, $n = 3$) times more transgene-encoded than endogenous APP (25) and developed amyloid plaques and impairments in correlative memory tests by 1 year (23). We originally had planned to transfer the Tg2576 transgene array onto the B6 inbred background to avoid the problems inherent with using outbred mice. It quickly became apparent that the proportion of mice dying prematurely increased as the percentage of B6-derived alleles increased; the proportion of transgene-positive mice that were weaned from the N3 and N4 generations also was significantly less than the expected 50%. Table 1 summarizes these results. Results from outcrossing N2 mice to B6SJLF1, rather than continuing the backcross to B6 to produce N3 offspring, are also shown. The fraction of transgene-positive offspring from the outcross to B6SJLF1 mice was 50%, suggesting that pre-weaning and *in utero* death, rather than transmission distortion, was responsible for the deficiency of transgene-positive mice in the N3 and N4 backcross offspring of B6. The fraction of mice from the N2×B6SJLF1 cross surviving long-term also increased, indicating that SJL-derived alleles protect against the lethal effects of APP overexpression.

The mice with mixed B6×SJL backgrounds that died prematurely showed no obvious behavioral abnormalities. In contrast, when *APP* transgenes were overexpressed in inbred FVB mice, premature death was usually preceded by a variety of neurologic signs (25). FVB transgenic mice fail to exhibit normal exploratory behavior when placed alone into a clean cage and adopt an unusual frozen posture. Mice that failed to explore their environment in three consecutive examinations are considered neophobic. Thigmotaxis (slinking around the perimeter of the cage) also is frequent in FVB mice with supraendogenous levels of APP. Neither neophobia nor thigmotaxis were observed during routine handling or neurologic examination of any Tg2576 mice produced from crosses to B6 or B6SJLF1.

To test whether the behaviors that had been observed in FVB could be elicited by addition of alleles from FVB, male N1 sires were mated with either FVB or B6. The two sets of mice were examined twice per week starting at 80 days, until death or 160 days. Results are shown in Table 2. Though affecting a minority of the mice, only offspring of the cross to FVB showed neophobic or thigmotaxic behavior. The absence of behavioral abnormalities among the mice with varying mixtures of B6 and SJL genes (summarized in Table 1) makes it likely that the exclusive appearance of these phenotypes in mice with a genetic contribution from FVB is not due to chance. A greater fraction of mice with FVB in their background also died prematurely than did offspring of the cross to B6 but, because each Tg2576 breeder is unique, comparison of their offspring is difficult.

Table 1. Decreased transgene transmission and survival with backcrossing the Tg2576 HuAPP.SWE transgene array onto C57BL/6 mice

Generation[a]	Fraction[b] transgene positive (n)	Fraction dead[c] (%)	Mean age (days) at death ± SE[d]
N1	0.54 (33)	1/3 (33%)	107
N2	0.44 (335)	40/111 (36%)	87 ± 4
N3	0.19 (90)[e]	6/12 (50%)	65 ± 7
N4	0.24 (33)[f]	4/4 (100%)	65 ± 4
N2×B6SJLF1	0.50 (534)	47/286 (16%)	74 ± 3

[a]The founder mouse was a (B6×SJL)F2 animal. The N1 generation represents offspring of the founder and C57BL/6J.
[b]Transgene-positive divided by number of mice weaned; (n) is the number weaned.
[c]Number dying prematurely (<150 days) over number of mice at risk. Mice killed for pathological or other analyses are not included.
[d]Mean age of death for those mice dying prematurely. Individual values are shown when fewer than three mice.
[e]Significantly different from the expected 50:50 ratio ($\chi^2 = 20.9$, $P < 0.0001$). The results combine data from McLaughlin Research Institute (6/17 transgene-positive) and University of Minnesota (11/73 transgene-positive).
[f]Different from expected 50:50 ratio at marginal significance ($P < 0.04$, Fisher's exact test).

Table 2. Appearance of neophobia and thigmotaxis among offspring of FVB, but not B6, mice mated with Tg(HuAPP.SWE)2576

Female parent	Premature death[a] (P[b])	Neophobia (P)	Thigmotaxis (P)
FVB	11/25 (0.02)	5/20 (0.05)	7/20 (0.02)
B6[c]	3/20	0/13	0/13

[a]Number dying prematurely (<150 days) over number of mice at risk.
[b]Significance by χ^2 analysis.
[c]Offspring of B6 shown here are included among the N2 mice shown in Table 1.

Genetic background modulates the phenotypes produced by transgene arrays causing APP overexpression disease in FVB

To explore further the hypothesis that host genes determine susceptibility to the lethal effects of APP overexpression, inbred FVB transgenics were used to avoid the complications inherent with outbred stocks. Two FVB transgenic lines and three phenotypes formed the basis for most of these studies. Line Tg1130H expresses 3.6 ± 0.5 times more mutant (V717I, V721A and M722V) $HuAPP_{695}$ than endogenous MoAPP, and line Tg6209 expresses wtHuAPP at 1.6 ± 0.4 times endogenous APP levels (25). The transgenes in both lines encode a carboxy-terminal myc epitope tag that has no apparent effect on phenotype (25) nor on processing to Aβ peptides in *PS1* transgenic mice (21). The most dramatic phenotype is premature death of unknown cause. Nearly all Tg1130H mice die by 150 days, compared with ~50% of Tg6209 mice. There are also clear behavioral abnormalities as discussed above. FVB mice that show neophobic behavior and fail to explore their environment also exhibit a third phenotype, cortical hypertrophic astrocytic gliosis (25).

Male Tg1130H mice were mated with B6, D2 or the wild-derived CAST/Ei (CAST) inbred strains, and three times a week their offspring were given a brief neurological examination and the 'corner test' as described in Materials and Methods. When mice began to die, brains from surviving animals were evaluated for pathological changes in hematoxylin–eosin- or silver (Hirano)-stained sections. Immunostaining with anti-glial fibrillary acidic protein aided evaluation of gliosis. Survival results are summarized in Figure 1. Transgene-positive (B6×FVB)F1 and (D2×FVB)F1 mice died at least as early as the FVB parent, but all (CAST×FVB)F1 mice that were not killed for other studies remained alive; the oldest animal was killed at 407 days. None of the 110 F1 hybrid mice examined failed to explore their environment when placed in a clean cage or showed any evidence of the abnormal behaviors peculiar to FVB transgenic mice, clearly dissociating these behavioral phenotypes from premature death. Cortical gliosis also was not a necessary concomitant of premature death (data not shown). (D2×FVB)F1 transgenics did not exhibit a gliotic response, in spite of being highly susceptible to the lethal effects of APP overexpression, while susceptible (B6×FVB)F1 mice exhibited florid cortical gliosis similar to that seen in FVB/N mice. No other pathological lesions were observed.

Similar differences in survival were observed using Tg6209 mice to produce F1 hybrids. Twenty four of 52 transgene-positive B6 hybrids died prematurely (99 ± 5 days), as did eight of 17 D2 hybrids (94.8 ± 9.3 days); no (Tg6209×CAST)F1 mice died young (n = 14).

These results are consistent with those described above using non-inbred Tg2576 mice. When transferred to susceptible backgrounds, the Tg2576 transgene array appears to be more detrimental than the Tg1130H array transmitted from an inbred FVB/N founder. This difference may be attributed to the greater production of APP in the brains of Tg2576 mice than in Tg1130H mice. The differences in APP production also are reflected in the brain concentrations of Aβ peptides, with 6-fold more $A\beta_{1-40}$ and 2.7-fold more $A\beta_{1-42}$ found in comparably aged young Tg2576 than in Tg1130H mice.

Two issues make premature death the premier phenotype for further study. First, although clearly due to APP overexpression, the relevance of this phenotype to AD in humans is unknown. If premature death is a mouse genotype-specific response to biochemical processes causing AD in humans, use of strains susceptible to the lethal effects of APP overexpression could provide a rapid assay for mechanistic studies or therapeutic interventions. Second, development of amyloid plaques (22,23) and memory deficits (23) in mice requires both high levels of APP and survival for approximately one year. If these late events, but not premature death, are relevant to AD, identification and elimination of alleles of genes conferring susceptibility to premature death would be very important for the development of genetically defined models.

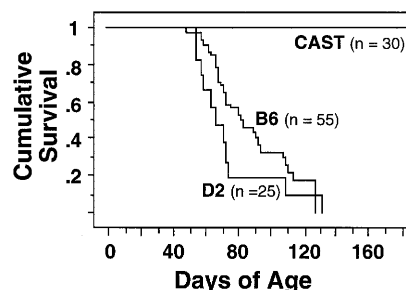

Figure 1. Survival in three different F1 hybrids overexpressing mutant *APP*. Male FVB Tg1130H were mated with B6, D2 or CAST to produce F1 hybrid offspring. Kaplan–Meier survival curves for each hybrid are shown. The B6, D2 and CAST curves represent 55, 25 and 30 mice. No (CAST×Tg1130H)F1 mice died, but were sacrificed for other studies with the oldest being 407 days old. Neurologic examination and a corner test were done three times weekly on groups of transgene-positive and transgene-negative mice. None of the hybrids showed the behavioral deficits observed in FVB/N (data not shown).

Genetic differences in APP lethality and formation of Aβ peptides

To test the hypothesis that increased survival time reflects decreased production of toxic Aβ peptides, we measured brain Aβ levels in APP transgenic (D2×FVB)F1, (B6×FVB)F1 and (CAST×FVB)F1 mice using an enzyme-linked immunosorbent assay (ELISA) capture assay (13). Concentrations of $A\beta_{1-40}$, $A\beta_{1-42}$ and the $A\beta_{1-42}/A\beta_{1-40}$ ratio in the brains of F1 mice and inbred FVB/N animals expressing the Tg6209 wtAPP transgenes are shown in Figure 2a and b. Differences in brain concentrations of Aβ peptides among FVB and its three F1 hybrids were not dramatic and did not correlate with survival. The ratio of $A\beta_{1-42}$ to the predominant $A\beta_{1-40}$ peptide also did not correlate with survival. No obvious differences in APP concentration were apparent in Western blots.

Although the genetic differences in survival do not correlate with differences in brain concentrations of $A\beta_{1-40}$ or $A\beta_{1-42}$, the possibility that Aβ peptides, rather than unprocessed APP, are the mediators of the lethal effects of transgene overexpression should not be dismissed.

Segregation of alleles modifying APP-induced phenotype

Transgene-positive (CAST×FVB)F2 and (CAST×FVB)F1×FVB backcross offspring positive for the Tg1130H transgene array were aged,and survival curves are presented in Figure 3. Thirteen of 34 F2 offspring (38%) died prematurely, as did 15/57 backcross mice (26%), suggesting that more than one gene is involved (for a single

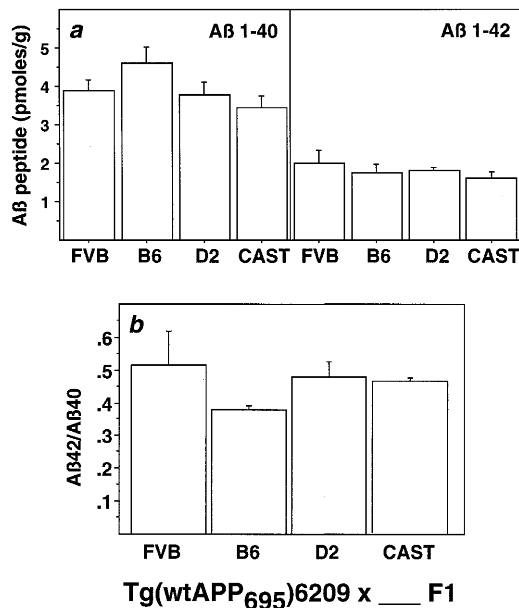

Figure 2. Effect of genetic background on resistance to lethal effects of APP overexpression is not mediated by increased production of Aβ peptides. (**a**) Concentrations (pmol/g of brain) of $A\beta_{1-40}$ and $A\beta_{1-42}$ in the brains of mice expressing wtHuAPP from the Tg6209 transgene array. Concentrations in inbred FVB/N transgenic mice and their F1 hybrids with B6, D2 and CAST are shown (three mice per group). There were no significant differences among the groups by analysis of variance. (**b**) The proportions of brain $A\beta_{1-42}$ in mice dying prematurely are not higher than in (FVB/N×CAST)F1 transgene-positive mice that are resistant to the lethal effects of APP overexpression.

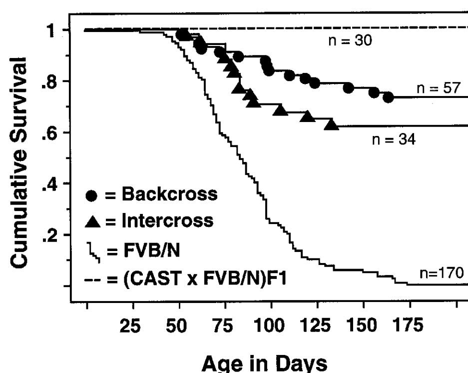

Figure 3. Survival curves for (CAST×Tg1130H)F2 (triangles) and (CAST × Tg1130H)F1×FVB backcross (circles) transgene-positive offspring. Survival curves for (CAST×Tg1130H)F1 (from Fig. 1) and for FVB/N Tg1130H also are presented. The 13 F2 animals represented are from a total population of 34. Of the 21 animals that survived long term, two have died (one at 314 days and the other at 373 days) and three have been killed for histology; the survivors are older than 1 year. Among the backcross offspring, 15 have died, with the remaining 42 animals >200 days old.

recessive gene, 25% expected in F2 and 50% expected in backcross).

Assuming a simple model in which FVB alleles are associated with susceptibility and CAST alleles with resistance, the finding of increased death in F2 mice would be unexpected. However, both parents in the F2 cross were transgene-positive, so approximately one-third of the transgenic offspring would be expected to carry two copies of the transgene array. The difference in APP concentrations between transgene homozygous and hemizygous mice raises an important issue for interpreting results from these crosses. Even in the inbred FVB strain, APP overexpression is not uniformly lethal, except, perhaps, at very high levels. For example, only half of transgene-positive Tg6209 mice die prematurely. Offspring of long-term survivors are as likely to die prematurely as offspring of mice that died early, indicating partial penetrance rather than loss of transgene copies and lowered APP expression. It seems probable that penetrance will vary with genetic background and that some fraction of the mice dying prematurely will share relevant genotypes with mice surviving. Based on the percentages of mice dying young in the FVB×CAST crosses, it is probable that there are three or fewer genes conferring susceptibility. A single, fully penetrant recessive gene would result in 50% death among backcross offspring, while two genes would produce ~25% of the mice dying young.

Because of the effects of partial penetrance, mapping the genes modulating survival will require greater numbers of mice; such studies are in progress. In addition to a genome-wide approach to search for genes modifying the response to APP, candidate genes were also selected and evaluated.

Absence of ApoE has little, if any, effect on APP-induced lethality

Homozygosity for the *APOE4* haplotype of apolipoprotein E greatly increases the risk of developing AD, while the *E2* haplotype is protective (26,27). Although the relevance of *APP* transgene-induced premature death to AD is unknown, interactions of ApoE with APP or its derivatives have been proposed as one mechanism for its effects on AD (28–30).

Initial intercrosses and backcrosses involving outbred *Apoe*-null (31) and Tg1130H mice revealed a striking deficiency in transgene-positive, *Apoe*-null homozygotes. Inclusion of microsatellite markers in the analyses indicated that the insertion site for the Tg1130H transgene array is located ~7 cM distal from *Apoe* on chromosome 7. Mice carrying the *Apoe*-null allele and the transgene array on the same recombinant chromosome were selected as breeders for subsequent studies.

Two transgene-positive males homozygous for the null allele of *Apoe* mated with *Apoe* heterozygous mice produced the offspring whose survival is shown in Figure 4. The outbred *Apoe* null stock contains a mixture of genes from 129/Sv, the original source of the embryonic stem cells used in gene targeting, and B6. The 129/Sv strain is the likely source of alleles that protect against the lethal effects of APP in this cross, providing yet another example of modulation of the APP-induced phenotype. Survival of mice lacking ApoE did not differ significantly from mice expressing the apolipoprotein. However, given the large influence of background genes on survival, only dramatic effects of ApoE would be detected in this experiment.

SOD1 overexpression protects against premature death

Mice overexpressing a human superoxide dismutase-1 (*SOD1*) transgene were also outbred (C3H/HeJ and B6) (32), but the effects of background genes on survival did not mask the strong protection against APP-induced premature death conferred by SOD1

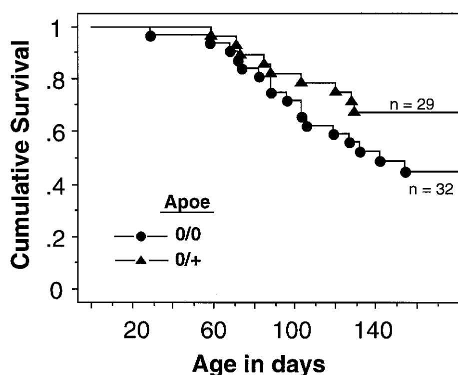

Figure 4. Lack of expression of ApoE does not modify survival of outbred mice overexpressing APP. There were 32 $Apoe^0/Apoe^0$ and 29 heterozygous mice. The slight difference between the groups was not statistically significant.

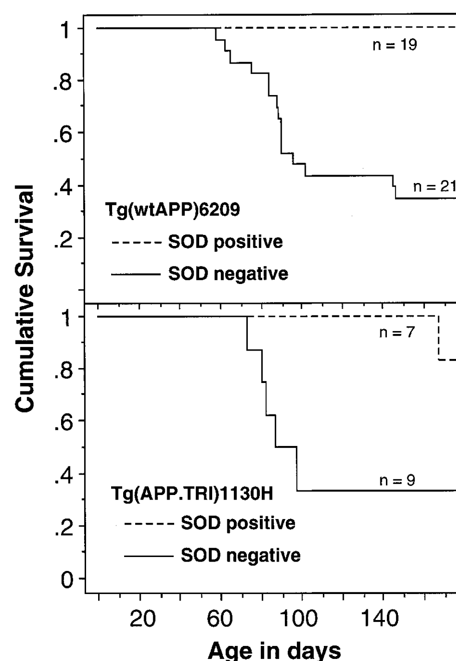

Figure 5. Overexpression of SOD1 protects against APP-induced death. Transgene hemizygous Tg6209 or Tg1130H FVB/N mice were crossed to outbred hemizygous transgenic mice overexpressing human *SOD1* under the control of its own promoter. Kaplan–Meier survival curves for mice carrying *APP* transgenes with or without *SOD1* transgenes are shown. The curves represent 19 or seven *SOD1*-positive mice and 21 or nine *SOD1*-negative mice with the Tg6209 or Tg1130H transgene arrays.

overexpression. The Tg(SOD1)76 line used in these experiments was constructed with a 12 kb genomic clone containing the entire human gene and produces 8-fold more SOD1 activity in the brain than non-transgenic mice; the enzyme also is overexpressed in other tissues (32). Tg1130H and Tg6209 mice were crossed with TgSOD1 mice and typed for both the *APP* and *SOD1* transgenes. The results for *APP* transgene-positive mice are presented in Figure 5; as reported previously, SOD1 overexpression had no effect on survival in the absence of *APP* transgenes (32). Co-expression of high levels of human SOD1 with the 1130H or 6209 *APP* transgene arrays was highly protective. Only one of 26 mice positive for the *SOD1* transgene died prior to 170 days. In contrast, all deaths in mice overexpressing APP without SOD1 occurred before 150 days. These data dramatically indicate that increasing the levels of superoxide-scavenging activity protects against the lethal effects of APP.

Once again, the effects of genetic background were apparent in this experiment. For example, only 70% of mice with the Tg1130H transgene array died prematurely, compared with 100% of inbred FVB transgenic mice. In spite of the variability inherent in using non-inbred mice, the protective effect of SOD1 was obvious.

Overexpression of FGF2 heightens the lethal effects of APP overexpression but does not alter APP processing

Basic fibroblast growth factor (FGF2) has been reported to be neuroprotective (33), and its effect on APP-induced death was tested using both Tg6209 and Tg1130H mice. Mice expressing HuFGF2 driven by the phosphoglycerate kinase promoter were produced on the same inbred FVB/N background as our *APP* transgenic lines, and their only external phenotype is dwarfism due to premature closure of the long bones (34). Mice expressing *FGF2* and *APP* transgenes died sooner than mice expressing APP alone. As shown in Figure 6, the effect was particularly striking with Tg6209 where wtAPP transgene-induced lethality is partially penetrant; FGF2 expression in conjunction with APP caused all mice to die prematurely. Similar results were obtained with a second *FGF2* transgenic line, eliminating an insertional mutation, rather than FGF2, as the cause. FGF2 expression does not act by increasing the processing of APP to Aβ peptides. Aβ peptide concentrations were measured in brains from three mice in each group. *FGF2* transgene expression caused no significant change in concentrations of Aβ peptides in APP transgenic mice; again, no obvious changes in APP were observed in immunoblots. As indicated in Figure 6, the ratio of $A\beta_{1-42}$ to $A\beta_{1-40}$ also was not altered by FGF2 expression.

DISCUSSION

Our studies demonstrate both rescue from APP-induced death by genes from resistant backgrounds and increased lethality by plaque-producing concentrations of APP by transfer of the transgene to susceptible backgrounds. The susceptibility of common mouse strains to the lethal effects of APP overexpression can account in large part for the rarity of past success in producing transgenic mice that develop Aβ amyloid plaques. In most laboratories, transgenic mice are produced by microinjecting either inbred FVB or F1 hybrid embryos. B6 is commonly one of the parents of the F1; transgene-positive outbred founders are often crossed to B6 to perpetuate a line. As suggested previously (23,25) and documented here, concentrations of APP sufficient for amyloid plaque deposition at 1 year are lethal on susceptible backgrounds. Therefore, production of Aβ amyloid plaques in mice requires not only strong transgene promoters but also a combination of genes conferring viability in the face of high concentrations of APP or its derivatives.

A key question is whether alleles conferring susceptibility to the lethal effects of APP have any relevance to AD or are interesting nuisances impeding study of AD-related phenotypes such as amyloid plaque deposition. Evidence suggests that

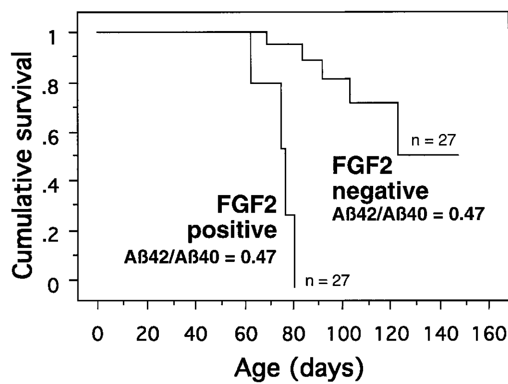

Figure 6. Overexpression of human *FGF2* transgenes potentiates the lethal effects of APP overexpression. Tg6209 mice were used in this experiment and were crossed to a FVB/N transgenic line overexpressing FGF2. Kaplan–Meier curves (including mice killed for other analyses) for *APP*-positive mice with ($n = 27$) or without ($n = 27$) *FGF2* transgenes are shown. Brain Aβ peptide concentrations in three mice from each group were determined and the $A\beta_{1-42}$ to $A\beta_{1-40}$ ratios are included in the figure. $A\beta_{1-40}$ concentrations in *FGF2*-positive mice were 7.0 ± 1.1 (± SEM) pmol/g of brain and in *FGF2*-negative mice they were 6.7 ± 1; $A\beta_{1-42}$ concentrations in *FGF2*-positive and -negative mice were 3.3 ± 0.4 and 3.2 ± 0.3.

elevation of Aβ peptides, rather than APP, is lethal in susceptible mouse strains. The Swedish K670N, M671L APP mutation dramatically elevates production of both $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides; only a single permanent FVB line expressing this transgene has been produced despite identification of four potential founders (25). Mutant transgenes also produced premature death at lower concentrations of APP than did wild-type transgenes. In another study, overexpression of transgenes encoding Aβ peptide without a signal sequence, rather than APP, in FVB mice led to premature death (35), but the relationship to the present work is not clear. An interesting comparison comes from very recent work demonstrating deposition of Aβ peptide, neuronal cell loss, impairment of learning and long-term potentiation in transgenic mice overexpressing a C-terminal 104 (CT104) amino acid fragment of APP, again without a signal sequence (36). These transgenics were produced using outbred mice (B6×C3H). The possibility that the different phenotypes produced intracellular expression of CT104 (36) or Aβ (35) could be background dependent is intriguing. If further research demonstrates that Aβ peptides are responsible for APP's lethal effects, premature death in susceptible strains could provide a convenient assay for alterations in APP processing.

Although Aβ peptide, rather than APP, may induce premature death, genetic modulation of survival did not seem to act by altering the Aβ peptide concentration in the brain. Only small differences in Aβ peptide levels were observed among FVB and the three F1 hybrids tested here; (CAST×1130H)F1 hybrids were not susceptible to the lethal effects of APP overexpression but had concentrations of Aβ comparable with those in susceptible mice. Similarly, *FGF2* transgenes appeared to enhance the lethal effects of APP overexpression but the Aβ peptide concentration in the brain remained unchanged. Although unlikely, the cause of death in *FGF2–APP* double transgenic mice may differ from that in mice expressing only *APP* transgenes, however.

Although cortical gliosis and behavioral abnormalities suggest an effect in the CNS, these phenotypes were dissociated in our analysis from early death, which may be due entirely to non-neurologic causes. Although gliosis was observed on some genetic backgrounds, there was no evidence for neuronal loss or other brain pathology. Necropsy and examination of multiple organs, including lungs and heart, similarly revealed no abnormalities at the light microscope level. Differences in survival reflect the host response to APP or its derivatives; determining the genes involved in this modulation and their modes of action may provide clues to the causes of death. Identification of chromosomal regions containing 'premature death genes' awaits typing and survival results from larger numbers of mice.

A body of evidence suggests that Aβ peptide's neurotoxic effects are mediated by peroxides (37,38). The protective effect of SOD1 overexpression is consistent with a role for oxidative damage in premature death, but this result does not indicate that neurotoxicity is involved because SOD1 is expressed systemically in the transgenic mice used in these experiments. The *APOE4* allele enhances the risk of Alzheimer's disease while the *E2* allele is protective (26,27). One hypothesis invokes the difference in the ability of the ApoE isoforms to protect against Aβ-induced oxidative damage *in vitro* (39). Ablation of *Apoe* had little effect on survival of *APP* transgenic mice. Segregation of background genes from the ablated stock could have masked small effects, however. The lack of an effect of *Apoe* ablation on survival in mice does not negate the possibility that ApoE protects against oxidative damage to neurons, however. We stress again that the induction of CNS dysfunction by APP overexpression does not necessarily indicate that CNS dysfunction causes premature death.

We tested the effects of FGF2 expression on premature death based on its neurotrophic effects protecting against cerebral hypoxic or ischemic damage (33). Rather than protecting against the lethal effects of APP overexpression, the susceptibility of *FGF2* transgene-positive mice was enhanced. Necropsy and histology of the double transgenic mice were as uninformative for the cause of death as it was for mice expressing only APP.

An intriguing, and testable, hypothesis for the cause of APP-induced death is suggested by recent work showing that Aβ peptide causes vascular dysfunction *in vitro* by damaging the endothelium (40). SOD1 prevented endothelial damage and increased contractility in cultured aortic rings, a parallel with our transgenic mouse results. Interestingly, although producing no obvious phenotype, *FGF2* transgenic mice have hypertrophy of vascular smooth muscle compared with non-transgenic FVB littermates (34). If Aβ peptide causes endothelial damage leading to lowered levels of endothelial-derived vascular relaxing factor (40), the increased vascular smooth muscle of *FGF2* transgenic mice could lead to further decreases in blood flow due to enhanced vasoconstriction. Under this scenario, the cause of death would be hypoxia producing no obvious pathological hallmarks. If this were the case, one of the genes determining susceptibility to the lethal effects of APP might exert its effects on blood vessels. This is compatible with our results suggesting that genes protecting against APP-induced death act by controlling the host response to Aβ rather than by modulating Aβ peptide concentrations. If mechanisms such as those above are involved in early death, FVB transgenic mice overexpressing APP could be an important model for the role of vascular changes in AD dementia (41).

Neither inbred FVB nor outbred transgenic lines provide a complete model for AD. The Tg2576 line that develops Aβ amyloid plaques at 1 year shows no neuronal loss or neurofibrillary tangles, features that characterize the human disease; FVB transgenic mice exhibit none of the pathological features of AD. However, the genetic malleability of the phenotypes produced by APP overexpression argues that modeling different aspects of AD will require different combinations of genes in addition to the transgene. Other human transgenes, such as those encoding neurofilament subunits, might be required to produce phenotypes similar to the neurofibrillary tangles observed in AD patients.

The availability of mice that mimic aspects of AD provides an unprecedented opportunity to dissect individual features of the human disease and to apply the power of defined genetic crosses to identify candidate genes that may be involved in sporadic AD or that may serve as targets for therapeutic intervention or disease prevention.

MATERIALS AND METHODS

Mice

The production of all transgenic mice used in these studies has been described previously (23,25,32,34). Dr N. Maeda (University of North Carolina, Chapel Hill) kindly provided *Apoe*-ablated breeding stock for these experiments (31). Mice were produced both at McLaughlin Research Institute (MRI) and at the University of Minnesota (U MN). Although the MRI colony is free of mouse viral pathogens while mice in the U MN colony are seropositive for mouse hepatitis virus and infantile diarrhea virus, results were similar in the two laboratories.

Neurologic testing

The corner test has been described in detail elsewhere (25) and was performed on some experimental groups as noted in Results. Obvious neurologic abnormalities also were noted. A brief neurologic examination was performed at the time of corner testing or weekly. Animal research technicians observe each animal's activity level, gait and presence or absence of tremors, head-bobbing or other unusual behaviors. The mouse is then picked up by the tail to check for paw-clasping behavior suggestive of proprioceptive deficits. Following a brief tail pinch to test deep pain sensation, the animal is returned to its cage and flipped on its back to test its righting ability.

Genotyping

The polymerase chain reaction (PCR) was used as described previously to identify transgene-positive mice (25,32,34). DNA samples were prepared from the tail tips (>0.25 cm) of individual mice. Each tail tip was placed in 200 µl of tail digestion buffer (20 mM Tris pH 8.0, 1 mM $MgCl_2$, 0.5% NP-40 and 0.5% Tween-20) with proteinase K at 0.5 µg/µl. The samples were digested for 3 h to overnight, heat-inactivated for 15 min at 95°C, and centrifuged to remove debris. The samples were diluted 1:20 in distilled H_2O before adding 10 µl to each PCR reaction (total reaction volume = 20 µl).

Oligonucleotide primers and conditions for each transgene are described briefly. *APP* transgenes were detected with primers A4-901-930 (5′-GACAAGTATCTCGAGACACCTGGGGATGAGC) and A4-3′-2070-2041 (5′-AAAGAACTTGTAGGTTGGATTTTCGTAGCC). Introns separating these sequences preclude detection of the endogenous gene. Annealing temperature during the cycle was 50°C. A three primer PCR reaction was used to discriminate the human *SOD1* transgene from the endogenous mouse *Sod1* gene. The primers used were HSODI3S (CCAAGATGCTTAACTCTTGTAATCAATGGC), H/MSODE4AS (CAGCAGTCACATTGCCCAAGTCTCCAACATG) and MSODI2S (GTTACATATAGGGGTTTACTTCATAATCTG), with annealing at 50°C. Detection of *FGF2* transgenes used a forward primer specific for the phosphoglycerate kinase promoter in the construct (5′-CTTCAAAAGCGCACGTCTGC) and a reverse primer within the artificial intron (5′-GCCTGCCACACCTCAAGCTT) with annealing at 65°C. In all cases, amplification involved 30–35 cycles at 94°C for 30 s, the annealing temperature for 30 s and 72°C for 30 s, with a final cycle at 72°C for 15 min. Following amplification, electrophoresis through a 1.2% agarose gel and staining with ethidium bromide, bands were photographed under UV light.

Southern analysis using mouse cDNA clone p2C1-apoE was used to discriminate wild-type and ablated *Apoe* alleles as described (31,42). More recent experiments used PCR for *Apoe* typing. Primers and conditions for detecting the null allele are posted by the Induced Mutant Resource of The Jackson Laboratory (http://lena.jax.org/resources/ documents/imr/)

Simple sequence length polymorphisms were identified by PCR and gel electrophoresis for use in mapping studies by commonly used procedures using primers purchased from Research Genetics (Huntsville, Alabama) (43).

Aβ peptide determinations

Methods for preparing brain and quantifying Aβ have been described previously (13,18). Briefly, brains were harvested and snap-frozen by placing on an aluminum-foil boat floating on a dry ice–ethanol mixture. The brains were Dounce homogenized at 0.15 g/1 ml of 70% formic acid and centrifuged at 100 000 *g* for 1 h to remove debris. Following neutralization by 20-fold dilution into 1 M Tris base, the sample was diluted in EC buffer (0.02 M sodium phosphate, 0.2 mM EDTA, 0.4 M NaCl, 0.2% bovine serum albumin, 0.05% CHAPS, 0.4% Block-Ace, 0.05% sodium azide at pH 7.0) and analyzed using the BAN-50/BA-27 or BAN-50/BC-05 sandwich ELISA assay. The values were compared with a standard curve of synthetic $A\beta_{1-40}$ and $A\beta_{1-42}$ and converted to pmol/g wet tissue.

Statistics

Statistical differences between group means were determined using Students *t*-test, Fisher's exact test or analysis of variance using the Statview statistical analysis program (version 4.5, Abacus Concepts, Inc.) for the Macintosh computer. Construction of Kaplan–Meier survival curves and non-parametric statistical analysis of survival data used the same program. Genetic linkage data were analyzed using MapManager QTLb developed by Kenneth Manly and available at http://mcbio.med.buffalo.edu/mapmgr.html.

ACKNOWLEDGEMENTS

We thank Stanley B. Prusiner and Michael Scott for the cosTet transgene expression vector and for their advice. We are very grateful to Philip Wong for providing the human SOD1

transgenic animals, and to Debbie Swing, Nancy Jenkins and Neal Copeland for their assistance in generating these mice. Gratitude is also due to Donald Price and Judy Van Lare for their assistance in neuropathology, and to William R. Crain and John Mercer for helpful discussions. This work was supported by grants AG10681 (G.A.C.), AG07914 (D.R.B.), AG12685 (S.Y.) and NS33249 (K.H.) from the National Institutes of Health, from the Alzheimer's Association (D.R.B. and S.Y.), from the Fraternal Order of Eagles (G.A.C.) and from the American Health Assistance Foundation (S.Y.). V.D. was awarded a summer research fellowship from the Dennis and Phyllis Washington Foundation.

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