Localization of Genes Potentially Controlling Susceptibility to the Lethal Effects of Alzheimer's Amyloid Precursor Protein in Transgenic Mice

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ABSTRACT

Variation in the susceptibility to the lethal effects of Alzheimer's Amyloid Precursor Protein (APP) transgene exists among various mouse strains. Inbred FVB/N mice, expressing high levels of the transgene-encoded APP, die prior to 200 days, while inbred 129.Tg2576 mice carrying the transgene are far less susceptible. When the two strains are crossed, (FVB/Nx129.Tg2576) F1 mice survive, as does the 129.Tg2576 parent. Intercross and backcross offspring survived at rates of 60% and 35%, respectively, at 200 days signaling the presence of a polygenic trait. The goal of this study was to establish a linkage to genes affecting susceptibility to the APP transgene. The possible quantitative trait loci (QTL) were established using various genetic markers scattered throughout the genome. The presence of multiple QTLs is possible from the data obtained; however, an increased chance of type I errors (false positives) exists due to the large number of markers used for the genome scan.

INTRODUCTION

Build-up of a normal cleavage product of the human Alzheimer Amyloid Precursor Protein (APP) leads to the development of Alzheimer's Disease (AD) (Gravina et al., 1995). The function of APP, which is transcribed from a gene on chromosome 21, is unknown. The $A\beta_{1-42}$ peptide of APP makes up most of the Amyloid plaques (Jarrett et al., 1993) which are neurotoxic and cause the dementia and memory loss characteristic of Alzheimer's Disease (Hardy and Allsop, 1999). A diagram of the APP gene and protein are shown in Figure 1. While the plaques generally occur spontaneously, certain mutations in the APP locus have been shown to be involved in early onset or Familial Alzheimer's Disease (FAD) (Goate et al., 1991). Mutations in the presenilin genes (PS1 on chromosome 14 and PS2 on chromosome 1) account for most cases of early onset FAD (St. George-Hyslop et al., 1992; Levy-Lahad et al., 1995), suggesting that the pathogenic effects of APP are derived in processing (Scheuner et al., 1996). Mutations in the Swedish FAD gene occur next to the 5' and 3' ends of the Aβ-coding segment of the APP gene. The Swedish transgene with K670N and M671L mutations produces excesses of both $A\beta_{1-42}$ and $A\beta_{1-40}$ (Citron et al., 1992).

Memory loss correlates with the accumulation of detergent insoluble $A\beta_{1-42}$ peptide in transgenic mice (Westerman et al., 2002). Different forms of Aß are present in varying amounts throughout the lifetime of Tg2576 mice, with detergent soluble Aß being present throughout life, while $A\beta_{insol}$ is only present after 6 months (Kawarabayashi et al., 2001). Transgenic Tg2576 mice with the Swedish mutation show memory loss and develop Amyloid plaques with age similarly to humans (Hsaio et al.,

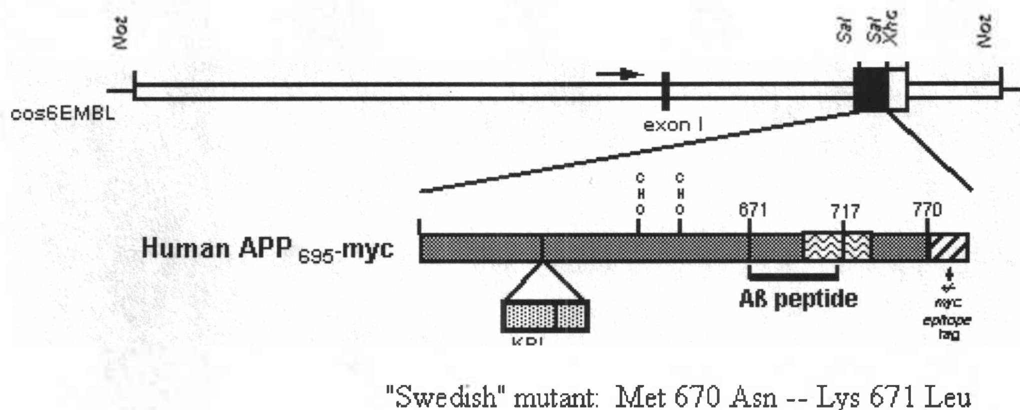

Figure 1. Diagram of the Alzheimer's Amyloid Precursor Protein and gene.

1996). Tg2576 mice show the first signs of memory loss at 6 months coinciding with the appearance of $Aß_{insol}$ and preceding plaque formation (Kawarabayashi et al, 2001 and Westerman et al, 2002). Memory loss tested in the Ashe lab focused on spatial reference learning and memory (Westerman et al., 2002) using a version of the Morris water maze (Morris 1984).

Survival rates vary among different strains of mice (Carlson et al., 1997). Inbred, FVB/N mice that over-express the APP transgene develop a CNS disorder and die before 200 days (Hsaio et al., 1995). The FVB/N mice fail to exhibit normal exploratory behavior when placed alone in a clean cage and develop an unusual frozen posture (Carlson et al., 1997). Meanwhile, 129.Tg2576 mice will survive well beyond 200 days, and develop Amyloid plaques and display the memory loss expected of the Alzheimer's mutant transgene at older ages. When these two inbred strains are crossed, they show survival curves similar to those of the pure inbred 129.Tg2576 line. Assuming that

survival is a monogenic trait, 25% of the F2 generation should die prior to 200 days, and 50% of the N2 backcross generation should die before 200 days. However, greater than 25% of the F2 generation survives, and 60% of the N2 generation survives. These survival rates indicate that survival and development of Alzheimer's in APP transgene mice are possibly affected by more than one gene. Survival rates of the F2 and N2 generations are not statistically different from a monogenic trait individually; however, the combination of both generations producing higher survival rates is significant enough to suggest the presence of more than one gene affecting susceptibility. Based on the percentages of mice dying young in FVBxCAST crosses, it is probable that there are three or fewer genes conferring susceptibility (Carlson et al., 1997). In this study, the hypothesis that the different survival rates are due to polygenic traits is tested. The transgenic mouse genome was scanned for loci that are linked to genes controlling the life span of APP transgenic mice.

MATERIALS AND METHODS

Tail Tip Collection. Tail tips from all mice positive for the APP transgene were snipped and stored at -80° C. To determine the presence of a possible polygenic trait, an initial sample of 88 N2 backcross mice positive for the transgene was obtained. Forty-four tails from mice found dead prior to 200 days and forty-four tails from mice surviving beyond 200 days were selected for DNA analysis. When selecting the mice that died prior to 200 days, mice with the youngest age at death were given preference. Two hundred days was chosen as the age marker, as all mice that show susceptibility to the APP transgene are dead prior to this age (Hsaio et al., 1995).

DNA Extraction. DNA was extracted from the tails using the Qiagen DNeasy Tissue Kit. The kit and protocols are available through Qiagen, through licensed distributors or the Qiagen website, http://www.qiagen.com.

Primer Selection and PCR. Primers were spaced between 15-20 cM apart to survey as much of the mouse genome as possible and primers had to have four base pair differences between the FVB and 129S6 alleles to be distinguishable during gel electrophoresis. Differences in locus size are due to the size of the microsatellites lying within the locus being amplified through PCR. The number of CA repeats in each microsatellite can differ among mouse strains. So in designing the primer set, proper microsatellite size differences were determined. Determining the location of specific primers and selecting primers were done using charts and tables available at McLaughlin Research Institute through Jackson Labs Mouse Genome Informatics (http://www.informatics.jax.org/) and through the National Human Genome Research Institute's Center for Inherited Disease-Mouse Genotyping (http://pages.cidr.nhgri.nih.gov/mouse/). Primers were designed

using the website for the Whitehead Institute at MIT. Once designed, Genosys synthesized the primers with the addition of an M13 tail on the forward strand.

In PCR samples, 0.2 μL of both the forward and reverse primers were used. 1.0 μL of dNTPs was used per sample, along with 0.1 μL of DNA Taq polymerase and 0.5 μL of wavelength marker. Each sample cocktail was brought up to 10 μL with 8.0 μL of picopure water. Ten μL of the cocktail were added to DNA dried into the PCR plate wells. The stock $MgCl_2$ concentration used was 15 μM. Primer was used in a 1:4 dilution of approximately 200 μM stock solutions. The stock dNTP concentration used was 1.25 μM. Dye-labeled primer complementary to the M13 tail was included to allow detection by the LiCor Sequencer.

Gel Electrophoresis and Data Collection. The amplified DNA was run through an acrylamide gel. Forty-eight of the wells were utilized with each sample run. The first 3 wells were used for standards. Well 1 was filled with amplified DNA that was homozygous FVB; in well 2 was DNA homozygous for 129S6; and in well 3 was DNA heterozygous for FVB and 129S6. In the following 44 wells sample DNA was used, with the DNA being ordered numerically, based upon the identification of each mouse the DNA was extracted from. The 48th well was a blank. On either side of the 48 wells a DNA ladder was placed in order to determine the fragment size of DNA running through the gel. A LiCor 4200 DNA Sequencer was used to electrophorese the gel and to collect the data. The homozygosity/heterozygosity of each animal was determined from printouts and entered into Map Manager QTX software developed by Jane M. Meer, Robert H. Cudmore, Jr., and Kenneth F. Manly at the Roswell Park Cancer Institute. Software and manuals can be found at http://mapmgr.roswellpark.org/mmQTX.html

Data Analysis. *Map Manager QTX* was used for all statistical analyses, which were run against genotypes. The genotype of each mouse was compared to its Age of Death, Age of Death/200, and the natural log (ln) of Age of Death/200 for significance leading to the identification of QTLs. In order to determine the presence of possible quantitative trait loci (QTL) for lethal susceptibility to the APP transgene, statistical values had to be determined that would provide markers to signal the presence of significant data. A permutation test was run using *Map Manager QTX*. The test was run using 10,000 permutations run over the entire marker set at 1 cM intervals. From the Permutation test the following statistical markers were determined: for a marker to be considered "suggestive," its statistical value had to be equal to or greater than 5.0; to be considered "significant," a marker needed a statistical value equal to or greater than 11.2; and to be considered "highly significant," a marker needed a statistical value equal to or greater than 20.2. All quantitative traits were analyzed against genotype for significance. *Map Manager QTX* compared the genotype of each mouse to the selected quantitative traits and searched for a correlation between genotype and each quantitative trait.

RESULTS

An example of the raw data collected from the LiCor 4200 DNA Sequencer is shown in Figure 2. The samples are aligned as explained in the Materials and Methods. Examples of data placed into Map Manager QTX are shown in Figure 3. "H" represents loci where the mice are heterozygous for FVB and 129S6 alleles. "F" represents loci where the mice are homozygous FVB. "x" represents points of crossover on the chromosomes.

Figure 2. Raw data displayed by LiCor 4200 DNA Sequencer at D1Mit236.

Chr1 P = 0.01
I: 5/5
p: 136/136 88 X 116.9 cM

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
c	D1Mit316	H	F	F	F	F	F	F	H	F	H	H	F	F	H	F	F	H	H	H	F	F	H	H	H	H	F	F	F	F	H
c	D1Mit236	H	F	F	x H	F	x H	F	H	F	H	H	F	x H	x F	F	F	H	H	x F	-	x H	H	H	H	x F	F	F	F	F	x F
c	D1Mit215	H	F	F	H	F	H	F	H	x H	H	H	F	H	F	F	F	H	H	F	F	H	H	x F	H	F	F	x H	F	x H	F
c	D1Mit102	H	F	x H	H	F	x F	F	H	x F	x F	H	x H	H	x H	F	F	H	H	F	F	x F	x F	F	x F	F	x H	H	F	x F	F
c	D1Mit155	H	x H	H	x F	F	F	F	H	F	F	x F	H	x F	H	F	F	H	H	F	F	F	F	x H	x H	F	H	x F	F	F	x H

Figure 3. Data placed into Map Manager QTX for Chromosome 1.

As seen in the data in Tables 1 and 2, the greatest statistical value among the backcross mice was found on chromosome 10, at D10Mit31. The statistical value at this marker is 8.0, and it has a p value of 0.00479. Values of the five most significant markers are shown in Table 1, using the Age of Death as the quantitative trait. Using Age of Death as the quantitative trait, D12Nds2 on Chromosome 12 is also suggestive. Its statistical value is 5.6, and it has a p value of 0.01803. Using Age of Death divided by 200 (age cutoff for finding mice dead) as the quantitative trait, statistical values were identical to the statistical values found using only Age of Death. The natural log of the previous quantitative trait (ln [Age of Death/200]) suggests D10Mit31 again. It has a statistical value of 8.0 and a p value of 0.00471. In this analysis, 12Nds2 is no longer at the suggestive level with a statistical value of 4.6. However, using this quantitative trait, D9Mit90 is suggestive with a statistical value of 5.8 and a p value of 0.01599. The five most suggestive markers at this quantitative trait are listed in Table 2.

Table 1. Statistical values for Age of Death analyzed against genotype

Chr.	Locus	Stat	p
3	D3Mit57	4.5	0.03295
9	D9Mit90	4.9	0.02667
10	D10Mit31	8.0	0.00479
12	D12Nds2	5.6	0.01803
19	D19Mit88	4.0	0.04637

Table 2. Statistical values for ln (Age of Death/200) analyzed against genotype

Chr	Locus	Stat	p
3	D3Mit57	3.3	0.06959
9	D9Mit90	5.8	0.01599
10	D10Mit31	8.0	0.00471
12	D12Nds2	4.6	0.03250
19	D19Mit88	4.1	0.04357

DISCUSSION

The purpose of this study was to demonstrate the presence of one or more Quantitative Trait Loci (QTLs) that affect susceptibility to the lethal effects of the Alzheimer's APP transgene. Inbred FVB/N mice positive for the Tg2576 transgene die prematurely due to a CNS disorder similar to one that occurs spontaneously in aged non-transgenic FVB mice (Carlson et al., 1997), while 129S6 mice with the Tg2576 transgene (129.Tg2576) show little premature death (Westerman et al., 2002). The point of interest then is to determine the factor, or factors, that affect susceptibility in these two strains. The two strains were crossed, and the heterozygous offspring were intercrossed and backcrossed to the FVB/N inbred line. Survival rates of these two generations of offspring signal that susceptibility is polygenic.

Individuals from the backcross generation, showing varied susceptibility, were searched for QTLs showing correlation between genotype and the age at which the mice died. The marker D10Mit31 showed the greatest significance, with a statistical value of 8.0 when Age of Death, Age of Death/200, and ln (Age of Death/200) were compared against genotype, making this region of the marker on chromosome 10 the most likely candidate for the presence of a QTL. On chromosome 12 at D12Nds2 and on chromosome 9 at D9Mit90, the low suggestive values signal that QTLs may lie in the regions around the markers or else a nearby locus weakly affects susceptibility.

Production of 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 (Carlson et al., 1997). The multiple genes necessary for Amyloid plaque production may mean that multiple genes may exist for

susceptibility and immunity to Amyloid plaque production. In highly polygenic traits, QTLs may be unidentifiable (Nadeau and Frankel, 2000), and genes affecting susceptibility may not show significance without massive sample sizes. Larger sample sizes will allow more QTLs to be identified. Also, highly polygenic traits require denser marker maps in order to identify all genes of interest; however denser marker maps also increase the chances of finding Type I errors (false positives) (Belknap et al., 1996). The running of additional markers on loci adjacent to or near the apparent QTL could verify the significance of the findings (Nadeau and Frankel, 2000). From the data collected, the hypothesis is accepted that survival is likely controlled by QTLs affecting susceptibility to Amyloid plaques due to the Alzheimer's APP, and they may be near the markers D10Mit31, D9Mit90, and D12Nds2.

Characterizing these QTLs affecting susceptibility to the Alzheimer's APP transgene may help provide the basis for similar studies of the disease in humans. Mice mimicking aspects of Alzheimer's Disease provide unprecedented opportunities in understanding the diagnostics of the disease (Carlson et al., 1997). The development of treatments for Alzheimer's Disease may benefit from understanding Aβ assemblies affecting learning and memory (Westerman et al., 2002). Using defined genetic crosses, researchers can identify candidate genes that are involved in sporadic Alzheimer's Disease that may be targets for therapeutic intervention or disease prevention (Carlson et al., 1997).

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