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Genomic Imprinting and Audiogenic Seizures in Mice

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Audiogenic seizure (AGS) susceptibility in mice is a multifactorial behavioral disorder that involves severe generalized convulsions in response to loud, high-frequency sound. The inheritance of AGS susceptibility was examined in crosses between AGS-susceptible DBA/2J (D2) mice and epilepsy-prone (EP) mice. The EP mice were selected for high AGS susceptibility in a BALB/c-derived line. The AGS phenotype was similar in the EP and D2 mice at 30 days of age. The frequency of generalized clonic–tonic AGS was high in both the D2 and the EP mice (53 and 83%, respectively) but was low in the reciprocal EPD2F1 and D2EPF1 hybrids (14 and 19%, respectively). In the backcross to the EP parent, no significant associations were found between AGS susceptibility and microsatellite markers linked to *Asp1* or *Asp2*, AGS genes located on Chromosomes 12 and 4, respectively. Significant associations were found for markers linked to *Asp3*, which is located in the proximal region of Chromosome 7. The influence of *Asp3* on AGS susceptibility was seen in the EP × EPD2F1 backcross but not in the reciprocal EPD2F1 × EP backcross, suggesting that *Asp3* expression is influenced by genomic imprinting. A model is proposed where genomic imprinting represses the maternal *Asp3* allele, providing an influence largely from the paternal allele.

KEY WORDS: Genomic imprinting; audiogenic seizures; multifactorial traits; epilepsy.

INTRODUCTION

Audiogenic seizure (AGS) susceptibility in mice is a multifactorial behavioral disorder that involves severe generalized convulsions in response to loud, high-frequency sound. Marked interstrain variability occurs for the onset, incidence, and severity of AGS (Seyfried, 1982a). DBA/2J (D2) mice are maximally susceptible at 21 days of age and gradually become resistant by adulthood (Seyfried, 1982, 1983). The influence of genetic heterogeneity and various environmental factors have hindered progress in defining the genetic architecture of AGS susceptibility (Seyfried *et al.*, 1980, 1982a, b, 1983). Nevertheless, three *audiogenic seizure prone* genes have been identified, i.e., *Asp1*, *Asp2*, and *Asp3*. These AGS genes were found in crosses between the D2 and the AGS-resistant C57BL/6J (B6) strains. *Asp1* (formally *las*) was mapped to Chromosome 12, tightly linked to the *Ah* locus (Seyfried *et al.*, 1980; Seyfried and Glaser, 1981), and *Asp2*, a modifier of AGS (formally *asp*), was mapped to Chromosome 4, tightly linked to the *b* coat color locus (Collins, 1970; Neumann and Seyfried, 1990; Neumann and Collins, 1991). *Asp3* was found through its linkage with the albino (*c*) locus in the midregion of Chromosome 7 (Neumann and Collins, 1991, 1992).

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Epilepsy-prone (EP) and epilepsy-resistant (ER) mice were derived from a line of BALB/c mice and have been selectively bred for susceptibility and resistance, respectively, to audiogenic seizures (Dolina *et al.*, 1992). The AGS phenotype is similar in the EP and D2 mice and is recessive to the seizure-resistant phenotype in the ER and B6 mice (Banko *et al.*, 1995). In addition to AGS susceptibility, EP mice also express several neuroanatomical abnormalities, including absent or partial corpus callosum and aberrant projections to the basal forebrain (Morin *et al.*, 1994).

Recent studies suggest that genomic imprinting can influence aspects of brain development and behavior (Durcan and Goldman, 1993; Allen *et al.*, 1995; McMahon *et al.*, 1995; Melo *et al.*, 1996). Genomic imprinting is an epigenetic phenomenon that involves the differential expression of parental alleles in the offspring and is suspected in certain disorders that show a bias in the inheritance of the trait through either the male or female parent (Peterson and Sapienza, 1993; Efstratiadis, 1994; Sapienza, 1994). To date, 13 imprinted genes have been identified in mice, 7 of which map to two separate imprinting domains on chromosome 7 (Cattanach *et al.*, 1992; Barlow, 1995). All of the known imprinted genes appear necessary for normal embryonic growth and development (Barlow, 1995). Neumann and Collins (1991, 1992) suggested previously that genomic imprinting could influence the expression of *Asp3* in mice, but the effect was not seen in a follow-up study. In the present study, we demonstrate a parent-of-origin effect on *Asp3* expression in crosses between the EP and the D2 strains, suggesting an influence of genomic imprinting.

MATERIALS AND METHODS

Mice

DBA/2J (D2) mice (The Jackson Laboratory, Bar Harbor, ME) and epilepsy-prone (EP) mice were maintained by brother–sister inbreeding and were propagated in the animal room of the Biology Department at Boston College. The mice were housed in plastic cages, with Sani-chips as bedding. Food (Agway Prolab Rat, Mouse, Hamster 3000) and water were provided *ad libitum*. The mice were kept on a 12-h light/12-h dark cycle (from 1900 to 0700).

D2 and EP mice were bred to produce reciprocal EPD2F1 hybrids. Backcross mice were generated by reciprocal crosses of EPD2F1 and EP mice. F_1 hybrids were tested for audiogenic responses and mated. The backcross mice were typed for coat color and audiogenic responses at 30 ± 2 days of age. In each cross, the female mouse is presented first, e.g., EP × EPD2F1 designates a cross between an EP female and an EPD2F1 male.

Audiogenic Seizure Typing

All mice were exposed to a loud, high-frequency pure tone sound (120 dB at 12 kHz) for 30 s as described previously (Seyfried *et al.*, 1980). No mouse was exposed to the sound stimulation more than once. The AGS phenotype manifests itself as a series of progressive phases (Seyfried *et al.*, 1980; Schreiber, 1986). After initial sound stimulation, a susceptible mouse will burst into a wild, frenzied run. The wild run is followed by the clonic phase where the mouse falls on its side and kicks its limbs violently. If the seizure continues, the mouse enters a tonic phase, characterized by rigidly extended limbs, clenched forepaws, and splayed hind paws. The mice were tested for AGS between 1000 and 1700.

The seizure response of each mouse was recorded according to the most severe phenotype as described previously (Seyfried *et al.*, 1980), with NR = no response, WR = wild running, CS = clonic seizure, and TS = tonic seizure. Computation of an animals' seizure severity score was based on the following scale, NR/WR = 0, CS = 1, and TS = 2. The incidence of generalized seizures (percentage of mice expressing clonic or tonic seizures) was calculated for each population and was pooled across the sexes since no significant differences were found between males and females.

DNA Isolation

Mouse genomic DNA was isolated from mouse spleens by a modified method of yeast DNA isolation (Hoffman and Winston, 1987). Mouse spleen cells were lysed in 450 ml of a solution containing 2% Triton X-100, 1% SDS, 100 m*M* NaCl, 10 m*M* Tris pH 8, and 1.0 m*M* EDTA. One extraction with phenol:chloroform:isoamyl alcohol (25:24:1) was performed to purify the DNA. Each DNA sample was precipitated in ethanol and

spooled with an autoclaved toothpick. The DNA was resuspended in TE (10 m*M* Tris–Cl, 1.0 m*M* EDTA, pH 8). Optical densities (260 and 280 nm) of the samples were measured to determine the purity and concentration of the DNA. The 260/280 ratios were calculated, and the samples were diluted to working stock solutions of 5 ng/ml.

Polymerase Chain Reaction (PCR)

Oligonucleotide primers for microsatellite loci were purchased from Research Genetics (Huntsville, Alabama) and were end labeled with [γ^{32}P] dATP (New England Nuclear) using T4 Kinase (Promega) according to the manufacturer's protocol. The PCR reagents were obtained from Promega (Madison, WI) and the PCR analysis was the same as described previously (Dietrich *et al.*, 1992; Allen and Seyfried, 1994). Briefly, mouse genomic DNA was used as a template in a 10-ml PCR reaction containing 25 ng DNA, 0.25 U Taq Polymerase (Promega), 20% (v/v) 10 × thermophilic buffer (Promega), 4 m*M* $MgCl_2$ (Promega), 200 n*M* unlabeled reverse primer, 180 n*M* unlabeled forward primer, 20 n*M* labeled forward primer, 2.0 m*M* dNTPs, and 0.2% bovine serum albumin (Sigma). Amplification conditions were initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min.

Linkage Analysis

Backcross mice were scored as either "E" (E/E homozygotes) or "D" (D/E heterozygotes) at the albino (*c*) locus and at 57 microsatellite loci that displayed polymorphisms between the EP and the D2 mice. The percentage of E/E and D/E mice having clonic or tonic seizure was calculated at each marker locus. The chi-square test of association (two-tailed) was used to determine if the number of seizing E/E mice differed significantly from the number of seizing D/E mice. When chi-square analysis detected linkage between AGS susceptibility and a marker locus, linear regression was utilized to map the locus influencing audiogenic seizure susceptibility relative to the marker loci, as previously reported (Neumann and Collins, 1991, 1992; Neumann *et al.*, 1994). In brief, linear regression equations are calculated that express the difference in seizure frequency between homozygotes and heterozygotes as a function of the distance between the marker locus and the QTL. Thus the linear regression provides a map position for the QTL relative to the marker loci and an estimate of the size of the phenotypic effect of allelic substitution at the QTL.

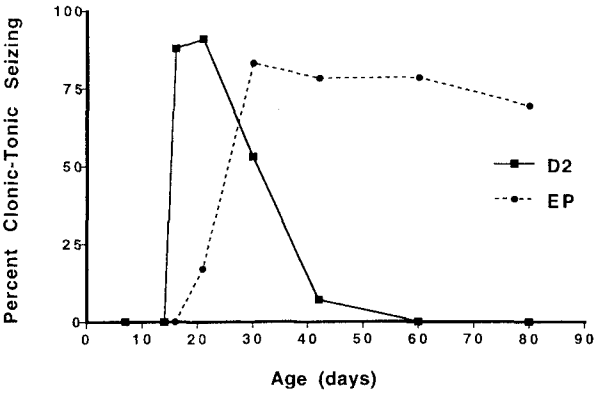

Fig. 1. Development profiles of audiogenic seizure susceptibility in EP and D2 mice. The percentage clonic–tonic seizing for D2 mice was described previously (Seyfried, 1982b).

RESULTS

The AGS phenotype in the EP mice was similar to that described previously in D2 mice (Seyfried, 1982b). The developmental profile of AGS susceptibility in EP mice is shown in Fig. 1. The developmental profile for D2 mice was obtained from data presented previously (Seyfried, 1982b). The onset of AGS occurred later in the EP mice than in the D2 mice. EP mice were unresponsive to sound stimulation at 7, 14, and 16 days of age. The frequency of generalized clonic–tonic seizures at 21 days was 92% in D2 mice but was only 17% in EP mice. By 30 days, AGS frequency was maximal in EP mice (83%) but declined to 53% in D2 mice. In sharp contrast to D2 mice, EP mice remained AGS susceptible into adulthood.

To examine the inheritance of AGS susceptibility in EP mice, we compared the frequency (%) of clonic–tonic seizures in 30-day-old D2 and EP mice, their reciprocal EPD2F1 hybrids, and the reciprocal backcrosses of EPD2F1 hybrids to the EP mice (Table 1). Thirty days of age was chosen for these genetic studies because the frequency of AGS

Table I. Audiogenic Seizure Responses in 30-Day-Old EP, D2, F_1, and Backcross Mice

Generation	Number of mice	Number Responding[a] NR	WR	CS	TS	% clonic–tonic seizures[b]
EP	84	3	11	19	51	83
D2	40	5	14	0	21	53
EPD2F1	98	36	48	4	10	14
D2EPF1	79	20	44	8	7	19
EP × F_1	316	13	131	55	117	54
F_1 × EP	276	20	112	51	93	52

[a] NR, no response; WR, wild run; CS, clonic seizure; TS, tonic seizure.

[b] Values are expressed to the nearest whole percentage and represent the percentage of mice having generalized clonic or tonic seizures.

was greatest in the EP mice at this age. Despite the high AGS susceptibility in the EP and D2 parents, the frequency of AGS was low in both the EPD2F1 and the D2EPF1 hybrids (14 and 19%, respectively) (Table I). Crosses of EP mice with AGS resistant C57BL/6J and epilepsy-resistant (ER) mice also produced F_1 hybrids that were mostly non susceptible (22 and 0%, respectively). The frequency of AGS was similar in the reciprocal EP × EPD2F1 and EPD2F1 × EP backcross populations (54 and 52%, respectively) and was intermediate to that of the EP and EPD2F1 parents (Table I).

To determine if previously mapped *Asp* genes influenced AGS susceptibility in EP mice, we analyzed the association between AGS susceptibility and the segregation of genetic markers linked to *Asp* loci on chromosomes 12, 4, and 7. No significant differences were found for the frequency of AGS between mice that were homozygous (E/E) or heterozygous (D/E) at *D12Mit46* and *D4Mit17*, i.e., microsatellite markers linked to the *Asp1* and *Asp2* loci, respectively (Table II). In contrast, an association was found between AGS frequency and segregation at the *c* locus, which is linked to *Asp3* on chromosome 7. These findings suggest that *Asp3* or another *Asp* in this region may influence AGS susceptibility in EP mice. The frequency of AGS was higher in E/E mice (59%) than in E/D mice (49%) in the total backcross population and a significant difference was found between these respective mouse groups in the EP × EPD2F1 backcross (65 and 46%, respectively; $\chi^2 = 6.61$, 1 df, $p < .02$). The association between AGS susceptibility and the *c* locus was backcross dependent, since no significant difference in AGS frequency was seen between the E/E and the D/E mice in the EPD2F1 × EP backcross (49 and 55%, respectively; $\chi^2 = 0.75$) (Table II).

Several microsatellite markers were used to define better the region on chromosome 7 that influenced AGS susceptibility in the EP mice. Significant associations were found between AGS susceptibility and microsatellite markers for an approximately 30 cM region on proximal chromosome 7 in the total backcross and in the EP × EPD2F1 backcross populations (Tables III and IV, Fig. 2). For each marker in this region, the percentage of clonic–tonic seizures was higher in the E/E mice than in the D/E mice. In the total backcross population, the strongest association was seen at *D7Mit178*, located approximately 2 cM from the centromere ($\chi^2 = 9.89$, 1 df, $p < .002$) (Table III). In the EP × EPD2F1 backcross population, the strongest association was seen at *D7Mit228*, located approximately 17 cM distal to *D7Mit178* ($\chi^2 = 10.44$, 1 df, $p < .001$) (Table IV). A subtle gender effect on recombination frequency was observed, as the marker recombination frequencies (distance in centimorgans from the centromere) were lower in the male F_1 hybrids (Table IV) than in the female F_1 hybrids (Table V).

In marked contrast to the situation in the EP × EPD2F1 backcross, no significant associations were found between AGS susceptibility and these chromosome 7 markers in the EPD2F1 × EP backcross (Table V, Fig. 2). The backcross-dependent association between chromosome 7 and AGS susceptibility was also observed when the data were expressed as mean seizure severity scores. This is illustrated for *D7Mit228* in Fig. 3. A highly significant difference in AGS susceptibility ($p < .00006$) was seen between the E/E and the D/E mice in the EP × EPD2F1 backcross but not in the EPD2F1 × EP backcross.

Linear regression was used to map the *Asp* gene on chromosome 7 that influences AGS susceptibility. First, using the EP × EPD2F1 backcross data, the *Asp* locus was placed 14.5 cM distal to *D7Mit178* (between *D7Mit77* and *D7Mit247*). The regression line was $y = 0.251 - 0.502r$, where y is the difference in clonic–tonic seizure frequency between homozygotes and heterozygotes at a marker locus and r is the recombination frequency between the marker and *Asp* loci. Second, using the

Table II. Tests of Audiogenic Seizure Responses in Backcross Populations with Markers Linked to the Three Known *Asp* Loci[a]

Marker locus	Cross	Allele[b]	Number of mice	Number responding[b]				% clonic–tonic seizures	χ^2 (1 df)
				NR	WR	CS	TS		
D12Mit46	Total backcross	E	150	7	67	19	57	51	1.07
		D	141	11	51	24	55	56	
	EP × EPD2F1	E	105	3	50	13	39	50	0.30
		D	101	5	35	20	41	60	
	EPD2F1 × EP	E	45	4	17	6	18	53	2.92
		D	40	6	16	4	14	45	
D4Mit17	Total backcross	E	128	10	53	20	45	51	0.33
		D	131	6	51	19	55	56	
	EP × EPD2F1	E	81	2	35	15	29	54	1.32
		D	87	3	34	13	37	57	
	EPD2F1 × EP	E	47	8	18	5	16	45	0.32
c		D	44	3	17	6	18	55	
	Total backcross	E	153	8	54	20	71	59	2.95
		D	151	11	66	26	48	49	
	EP × EPD2F1	E	100	3	32	11	54	65	6.61*
		D	108	5	53	23	27	46	
	EPD2F1 × EP	E	53	5	22	9	17	49	0.75
		D	43	6	13	3	21	55	

[a] Abbreviations as described in Table I, footnote *a*.
[b] E, homozygous (E/E) mice; D, heterozygous (D/E) mice.
* The number of seizing E/E mice differed significantly from the number of seizing D/E mice at $p < .02$.

total backcross data set, the *Asp* locus was placed 6.5 cM distal to *D7Mit178* and proximal to *D7Mit114*. The regression line was $y = 0.212 - 0.424r$. Chi-square tests of goodness of fit displayed no significant deviations of the observed frequencies in these populations from the frequencies "expected" from the regression lines. Linear regressions in both cases could be fitted that did not result in rejection at 0.05 significance level with the *Asp* locus placed anywhere from the proximal end of the map (2 cM proximal to *D7Mit178*) to positions 41 or 45 cM, respectively, from the proximal end of the map.

A preliminary genome scan, involving 57 microsatellites with at least two markers on each of the 19 autosomes, was conducted to locate other chromosomal regions that might participate in AGS susceptibility. A couple of suggestive associations were found (chromosomes 1 and 10), but the sample sizes were too small to make a strong case for additional factors. More extensive studies will be needed to assess the significance of these regions. The strongest associations were those seen with markers on chromosome 7. Although the EP mice have been described as inbred in the epilepsy literature, we found that 3 of 57 (5.3%) of the microsatellite loci (*D2Mit48, D4Mit13*, and *D7Mit223*) differed within and between the EP and BALB/cJ mice. None of these polymorphisms occurred, however, in the proximal region of chromosome 7 near *Asp3*.

DISCUSSION

AGS susceptibility is a multifactorial behavioral trait whose inheritance is confounded by genetic heterogeneity and nongenetic factors. These nongenetic factors include both internal environmental factors (age, hormones, and circadian rhythms) and external environmental factors (nutrition, audiogenic priming, and intensity and frequency of sound exposure) (Seyfried *et al.*, 1980; Seyfried, 1982a, 1983). Although monogenic inheritance was originally proposed to account for AGS susceptibility in D2 mice (Fuller *et al.*, 1950; Collins, 1970), more recent studies indicate that susceptibility is influenced by several *Asp* genes (Seyfried *et al.*, 1980; Seyfried and Glaser, 1981; Seyfried, 1983; Neumann and Seyfried, 1990; Neumann and Collins, 1991).

In this study, we used F_1 hybrid and backcross populations to dissect the genetic architecture of AGS susceptibility in the EP mouse, an epilepsy model selected from a line of BALB/c-derived

Table III. Tests of Associations of Audiogenic Seizure Responses in the Total Backcross Population with Chromosome 7 Markers[a]

Marker locus	cM[b]	Allele	Number of mice	Number responding				% clonic–tonic seizures	χ^2 (1 df)[c]
				NR	WR	CS	TS		
D7Mit178	2.0	E	138	6	43	22	67	64	9.89**
		D	166	13	77	24	52	46	
D7Mit114	12.7	E	146	6	47	25	68	64	9.33**
		D	158	13	73	21	51	46	
D7Mit228	20.0	E	144	5	48	22	69	63	8.10**
		D	160	14	72	24	50	46	
D7Mit26	24.3	E	147	5	51	21	70	62	6.09*
		D	157	14	69	25	49	47	
c	42.1	E	153	8	54	20	71	59	2.95
		D	151	11	66	26	48	49	
D7Mit223	90.0	E	138	8	56	22	52	54	0.00
		D	150	9	61	22	58	53	

[a] Abbreviations as described in the footnotes to Tables I and II.
[b] Estimated distance from the centromere in centimorgans (cM).
[c] The number of seizing E/E mice differed significantly from the number of seizing D/E mice, at $*p < .05$ and $**p < .01$.

Table IV. Tests of Associations of Audiogenic Seizure Responses in the EP × EPD2F1 Backcross Population with Chromosome 7 Markers[a]

Marker locus	cM	Allele	Number of mice	Number responding				% clonic–tonic seizures	χ^2 (1 df)[b]
				NR	WR	CS	TS		
D7Mit178	2.0	E	94	3	28	15	48	67	8.70**
		D	114	5	57	19	33	46	
D7Mit191	6.8	E	90	3	27	15	45	67	7.52**
		D	118	5	58	19	36	47	
D7Mit114	11.1	E	91	3	26	15	47	68	9.89**
		D	117	5	59	19	34	45	
D7Mit77	13.0	E	93	3	28	14	48	67	8.00**
		D	115	5	57	20	33	46	
D7Mit247	18.8	E	95	3	28	12	52	67	9.44**
		D	113	5	57	22	29	45	
D7Mit228	19.3	E	94	3	27	12	52	68	10.44**
		D	114	5	58	22	29	45	
D7Mit26	23.6	E	95	3	29	11	52	66	7.80**
		D	113	5	56	23	29	46	
D7Mit199	27.5	E	97	3	30	11	53	66	7.61**
		D	111	5	55	23	28	46	
D7Mit90	34.8	E	102	3	34	9	56	64	5.11*
		D	106	5	51	25	25	47	
c	37.7	E	100	3	32	11	54	65	6.61*
		D	108	5	53	23	27	46	
D7Mit206	49.5	E	94	3	33	13	45	62	2.40
		D	114	5	52	21	36	50	
D7Mit223	84.9	E	86	2	36	16	32	56	0.01
		D	111	4	47	17	43	54	

[a] Abbreviations as described in the footnotes to Tables I–III.
[b] The number of seizing E/E mice differed significantly from the number of seizing D/E mice, at $*p < .05$ and $**p < .01$.

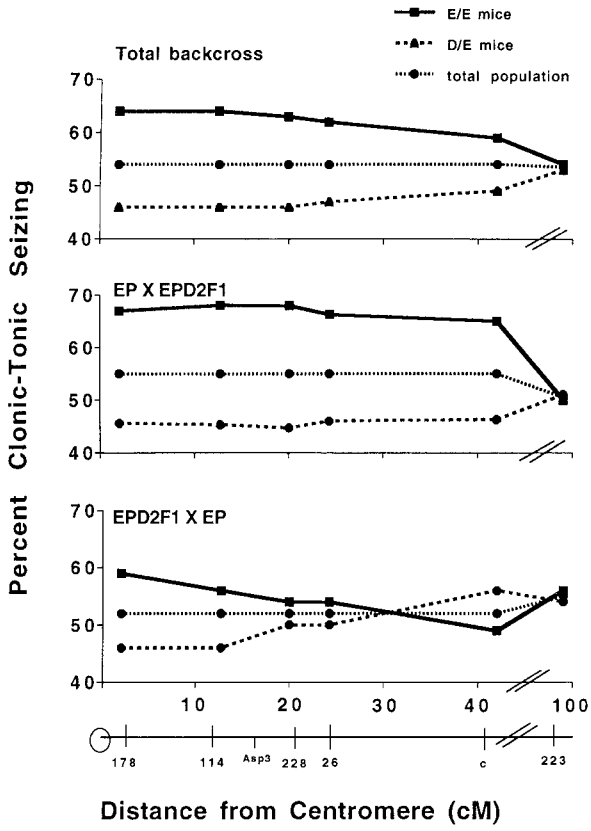

Fig. 2. Association between AGS susceptibility and chromosome 7 markers in backcross mice. The percentage clonic–tonic seizing was calculated at each marker for the E/E and D/E genotypes in the total backcross and in each reciprocal backcross. The schematic chromosome below the *x*-axis shows the distance of the genetic markers from the centromere estimated from the total backcross population. The location of *Asp3* was estimated from linear regression analysis (see Materials and Methods).

mice. Although the AGS phenotype is similar in 30-day-old D2 and EP mice, AGS susceptibility was low in the reciprocal F_1 hybrids. These findings, together with the differences in AGS developmental profile, suggest that a common genetic mechanism is unlikely responsible for the AGS susceptibility of the EP and D2 mice. We recognize that complementation analysis in multifactorial systems is risky and cannot therefore exclude the possibility that reduced AGS susceptibility in the F_1 hybrids results from nonallelic interactions.

We found significant associations between AGS susceptibility and several genetic markers that encompassed approximately 30 cM in the proximal region of chromosome 7. In the EP × EPD2F1 backcross, significant associations were seen between AGS susceptibility and several microsatellite markers in the region containing *Asp3*. As our study involved replicating a prior established hypothesis, that *Asp3* is on chromosome 7, *p* values of .01 are considered adequate for declaring linkage at the 5% level (Lander and Kruglyak, 1995). When the AGS phenotype was expressed as a mean seizure severity score, the association between AGS susceptibility and *D7Mit228* surpassed the linkage threshold required for a whole genome scan (Fig. 3).

In contrast to the EP × EPD2F1 backcross, no associations were seen between AGS susceptibility and the same markers in the EPD2F1 × EP backcross. Although we cannot rule out any position in the proximal half of chromosome 7, we think the most likely position for *Asp3* is between *D7Mit77* and *D7Mit247* (Table IV, Fig. 2). This placement is based on the linear regression of the EP × EPD2F1 backcross data, because the reciprocal cross showed no significant association between the chromosome 7 markers and AGS frequency. The coinheritance of AGS susceptibility and the chromosome 7 markers in the EP × EPD2F1 backcross, but not in the EPD2F1 × EP backcross, suggests that *Asp3* is influenced by genomic imprinting.

Although *Asp3* is likely responsible for most of the association between AGS susceptibility and the chromosome 7 markers, we do not exclude the possibility that another *Asp* locus, which is not imprinted and is located closer to the centromere than *Asp3*, may also influence AGS susceptibility in the EP mice. This comes from finding an association with *D7Mit178* that was greater in the total backcross than in either of the reciprocal backcrosses. A second AGS gene near the centromere could also account for the significant association with markers that encompassed more than 30 cM of chromosome 7 in the total backcross population. Further studies using a larger backcross population will be necessary to establish the validity of the two linked-loci hypothesis.

It is unlikely that a general maternal effect can account for the association between AGS and the chromosome 7 markers. If a maternal effect were contributing to the AGS susceptibility in EP mice, we would expect differences in the seizure frequencies between the reciprocal backcrosses. Instead, we observed that the overall frequency of

Table V. Tests of Associations of Audiogenic Seizure Responses in the EPD2F1 × EP Backcross Population with Chromosome 7 Markers[a]

Marker locus	cM	Allele	Number of mice	Number responding				% clonic–tonic seizures	χ^2 (1 df)
				NR	WR	CS	TS		
D7Mit178	2.0	E	44	3	15	7	19	59	1.12
		D	52	8	20	5	19	46	
D7Mit114	15.9	E	55	3	21	10	21	56	0.59
		D	41	8	14	2	17	49	
D7Mit228	21.1	E	50	2	21	10	17	54	0.04
		D	46	9	14	2	21	50	
D7Mit26	25.3	E	52	2	22	10	18	54	0.03
		D	44	9	13	2	20	50	
c	51.4	E	53	5	22	9	17	49	0.75
		D	43	6	13	3	21	56	
D7Mit223	87.3	E	52	6	20	6	20	50	0.11
		D	39	5	14	5	15	51	

[a] Abbreviations are as described in the footnotes to Tables I–III.

AGS was similar in the EP × EPD2F1 and the EPD2F1 × EP backcrosses (54 and 52%, respectively). The mean seizure severity scores were also similar in both backcrosses (Fig. 3). Rather than a general maternal effect on AGS susceptibility, we observed a parent-of-origin effect at particular linked loci on chromosome 7, indicative of genomic imprinting. The expression of *Asp3* depends upon the parent transmitting the gene.

We suggest a genetic model where the *Asp3* maternal allele is imprinted and therefore repressed (Fig. 3). This would result in expression of only the paternal *Asp3* allele. In the production of the EP × EPD2F1 backcross, for example, the F_1 male would be heterozygous (D/E) at *Asp3*. Thus, he could transmit either the *Asp3* D allele or the E allele to his offspring, generating two populations of backcross mice that would differ in AGS susceptibility. Expression of the paternal E allele would enhance AGS susceptibility compared to the seizure severity in the total EP × EPD2F1 backcross (0.92; Fig. 3). In contrast, the expression of the paternal D allele would decrease AGS susceptibility compared to that in the backcross. This is consistent with our observations in this backcross (Table IV, Fig. 3). In the production of the EPD2F1 × EP backcross, on the other hand, the parental EP male would be homozygous (E/E) for *Asp3*. Consequently, all of his offspring would express only the paternal E allele. In the absence of maternal allele expression, no difference in AGS susceptibility would be expected between the E/E and the D/E mice. This is also consistent with our observations in this backcross (Table V, and Fig. 3).

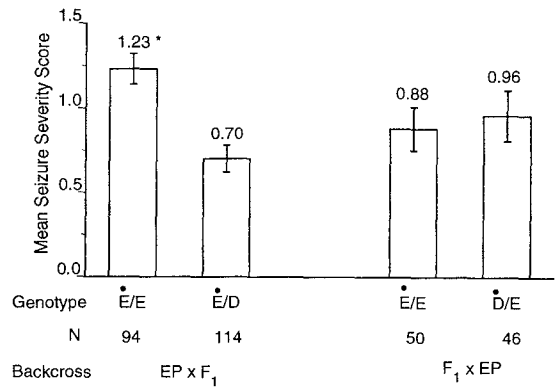

Fig. 3. Association between *D7Mit228* and AGS susceptibility in backcross mice expressed as mean seizure severity scores (± SE). The scores were calculated from the data in Tables IV and V, where NR/WR = 0, CS = 1, and TS = 2. The E/E and D/E genotypes represent mice homozygous and heterozygous, respectively, for the EP and D2 alleles at *D7Mit228*. The maternal allele is given on the left and the paternal allele on the right. The black dot above the maternal allele suggests repression through imprinting. N = the number of mice tested for each genotype and F_1 represents the EPD2F1 hybrids. An asterisk indicates that the mean seizure severity score of E/E mice is significantly higher than that of D/E mice at $p < .00006$ (2×3 χ^2 test = 19.44, with 2 df). The mean seizure severity scores in the total EP × F_1 and F_1 × EP backcross populations were 0.92 ± 0.05 (n = 316) and 0.86 ± 0.05 (n = 276) and were determined from the data in Table I.

Although our model can account for most of our findings, there are some difficulties. It is not

clear, for example, why the percentage of seizures and the mean seizure severity score in the E/E mice in the EPD2F1 × EP backcross (about 54 and 0.88, respectively) were not closer to those seen in the E/E mice in the EP × EPD2F1 backcross (about 66 and 1.23, respectively). We also do not rule out a possible imprinting effect on the expression of the *Asp3* D allele since the seizure severity was slightly higher in the D/E mice in the F_1 × EP backcross (0.96; Fig. 3) than in the EP × F1 backcross (0.70). An influence of imprinting on inherited aspects of auditory system development is also possible, as D2 mice suffer from age-dependent hearing loss (Ralls, 1967; Erway *et al.*, 1993; Willott *et al.*, 1995). The difficulties with the model may be associated with both the complexities of the imprinting phenomenon and the multifactorial basis of AGS susceptibility.

Most known imprinted genes influence embryonic viability and development, and some of these may also affect the developing brain and behavior (Durcan and Goldman, 1993; Allen *et al.*, 1995; Barlow, 1995). Besides AGS susceptibility, EP mice also express neuroanatomical abnormalities including absent or partial corpus callosum and abnormal neuronal fiber projections in the basal forebrain and subcortical structures (Morin *et al.*, 1994). Studies are in progress to determine whether an association exists between the neuroanatomical abnormalities and AGS susceptibility in EP mice.

The imprinting of *Asp3* may result from its location in the more proximal imprinted domain on chromosome 7 (Cattanach *et al.*, 1992; Barlow, 1995) Previous studies suggest that *cis*-acting and *trans*-acting elements can confer an imprint on a gene (Efstratiadis, 1994; Villar *et al.*, 1995). In the case of *H19* and *lgf2r*, competition for an endoderm specific enhancer element results in reciprocal imprinting of these two linked genes (Leighton *et al.*, 1995). Likewise, *Asp3* could compete with a local gene(s) for regulatory elements which subsequently confer an imprint. Also, genotypic specific imprinting modifiers, located on other chromosomes, could influence *Asp3* expression (Allen *et al.*, 1990; Forejt and Gregorova, 1992; Latham *et al.*, 1995).

There are several interesting candidate genes that map in the *Asp3* region. *Grik5*, a kainate-preferring non-NMDA-type glutamate receptor subunit, maps at the proximal end of chromosome 7 (Kirschner *et al.*, 1994; Szpirer *et al.*, 1994). Glutamate is an excitatory amino acid that is implicated in neuronal excitability in the central nervous system (Walker, 1983). Furthermore, recent studies suggest that alterations in glutamate receptors could cause a predisposition to epilepsy (Rogers *et al.*, 1994; Twyman *et al.*, 1995; Obrenovitch *et al.*, 1996). The nerve growth factor (NGF) loci, *Ngfα* and *Ngfγ*, and the $GABA_A$ receptor subunit genes, *b3*, *g3*, and *a5*, also map near our predicted location for *Asp3* (Evans and Richards, 1985; Nakatsu *et al.*, 1993). NGF is a neurotrophic factor that can influence neuronal projections in the basal forebrain, whereas the $GABA_A$ receptor subunit genes may influence the neurophysiological effects of GABA, a major inhibitory neurotransmitter. This is interesting in light of the reported abnormalities in basal forebrain projections and GABA-ergic neurotransmission in EP mice (Dolina *et al.*, 1992, 1993; Morin *et al.*, 1994). It will be interesting to determine if any of these candidate genes is imprinted in EP mice.

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