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Identification of a Murine Locus Conveying Susceptibility to Cadmium-Induced Forelimb Malformations

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The heavy metal cadmium (Cd), an environmentally ubiquitous contaminant, is a potent teratogen in mice. When administered parenterally, it induces an array of malformations that vary in scope and severity with the route, dose, time of administration, and the strain of the animal. When administered intraperitoneally on day 9.0 of gestation, 4 mg/kg cadmium chloride produces forelimb defects (predominantly ectrodactyly) in over 80% of fetuses of the C57BL/6 mouse strain, while no limb defects are observed in the identically treated SWV strain. Like other examples of strain-specific teratogenic activity, the underlying nature of the differential susceptibility remains unknown. The present study investigates the segregation of sensitivity to Cd-induced forelimb defects in crosses between C57BL/6 and SWV mice and provides evidence for the involvement of both maternal and fetal factors in the determination of defect expression. In addition, quantitative trait loci (QTL) analysis of the fetal genetic component was performed among 198 backcross progeny, utilizing a genomic linkage map of 149 informative microsatellite markers. One QTL demonstrating significant linkage to expression of the defect, designated *Cadfar* (cadmium-induced forelimb autopod reduction), was mapped to the distal end of chromosome 6 with a lod score of 3.1.

INTRODUCTION

Each year in the United States, approximately 150,000 babies (4% of live births) are born with a major congenital malformation (Petrini *et al.*, 1997). While 30% of these defects can be causally attributed to chromosomal aberrations, prenatal disease, or specific environmental exposures, the vast majority are of unknown etiology (Nelson and Holmes, 1989; Petrini *et al.*, 1997; Stevenson, 1993). In addition to gross genomic alterations, other more subtle genetic factors contribute to the occurrence of human congenital malformations, as evidenced by the differing incidence figures among racial and ethnic groups (Lary and Edmonds, 1997; Leck, 1993; Warkany, 1971). The identities of these contributory genetic factors have yet to be established. This may be due in part to small sample sizes, heterogeneity of genetic background, and the presence of confounding environmental variables, all of which render human populations difficult to study.

Congenital malformations can be modeled in laboratory mammals with teratogenic treatments, allowing the study of large sample sizes with a uniform genetic background and a highly controllable environment (Lander and Schork, 1994; Silver, 1995). Furthermore, the incidence of teratogen-induced defects varies dramatically among inbred strains of mice (Kalter, 1965). This permits investigation of the effects of genetic background in determining sensitivity. By identifying factors contributing to murine congenital defects, candidate pathways and systems conveying sensitivity in human populations can be suggested.

The heavy metal cadmium (Cd) is a potent developmental toxicant in laboratory mammals (Domingo, 1994; Webster, 1990). Cadmium causes embryo/fetal lethality and a wide range of malformations that vary in scope and severity according to species, route, dose, and gestational time of administration (Barr, 1973; Ferm and Carpenter, 1967; Ishizu *et al.*, 1973). Furthermore, sensitivity to cadmium-induced birth defects varies dramatically among inbred strains of mice (Layton and Layton, 1979; Pierro and Haines, 1978; Wolkowski, 1974). While numerous studies have described this differential sensitivity, studies attempting to associate candidate genes with cadmium-induced teratogenicity have been largely unsuccessful (Feuston and Scott, 1985; Layton and Layton, 1979; Wolkowski, 1974; Wolkowski-Tyl, 1978). Therefore, the mechanism

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of cadmium-induced teratogenesis remains unknown, as do the reasons for its strain-specific activity.

Quantitative trait loci (QTL) analysis allows for the identification of loci underlying strain differences in the expression of genetically complex traits (Lander and Botstein, 1989). The technique utilizes a statistical method that compares trait segregation in backcross or F2 animals to that of markers in a polymorphic linkage map constructed between the two strains (Lander and Botstein, 1989). This method has facilitated the mapping of loci underlying many murine traits, including cardiovascular disease (Ivandic *et al.*, 1996), cancer (Jacoby *et al.*, 1994), and obesity (Taylor and Phillips, 1996). Recently, QTL analysis has been applied to toxicant-induced traits, such as ozone-induced lung damage (Kleeberger *et al.*, 1997; Prows *et al.*, 1997) and hexachlorobenzene-induced porphyria (Akhtar and Smith, 1998). Two published studies have focused on genome scans for teratogenic end-points, namely phenytoin- and 6-aminonicotinamide-induced cleft lip and palate in recombinant inbred strains (Diehl and Erickson, 1997) and radiation-induced gastroschisis (Hillebrandt *et al.*, 1998).

One of the most striking malformations induced in rats and mice by midgestational cadmium treatment is a reduction defect of the forelimb autopod. The most common manifestation of the defect is a thin, short, or missing fifth digit, the absence of which is termed ectrodactyly (Barr, 1973; Kuczuk and Scott, 1984; Layton and Layton, 1979; Messerle and Webster, 1982). When administered by intraperitoneal injection to C57BL/6 mice on day 9.0 of gestation, cadmium induces this forelimb reduction defect in greater than 80% of fetuses, while the same regimen produces no fetuses with limb defects in the SWV strain (Hovland *et al.*, 1999). The basis for this strain-specific sensitivity is unknown. The present study utilizes standard crosses, and the first application of QTL analysis in an experimental backcross of a chemically induced teratogenic end-point, to investigate the genetic factors determining susceptibility to cadmium-induced forelimb defects in the C57BL/6 and SWV mouse strains.

MATERIALS AND METHODS

Teratology. The mice used in this study were the inbred C57BL/6NCrlBR, C57BL/6J, and SWV/Fnn strains. A breeding colony of SWV/Fnn (SWV) mice was established from mice provided by Dr. Richard Finnell (Texas A&M University, College Station, TX). C57BL/6NCrlBR (C57BL/6) mice were obtained from Charles River Laboratories (Portage, MI). C57BL/6J mice were obtained from The Jackson Laboratories (Bar Harbor, ME). Animals were housed in transparent plastic cages with stainless steel lids and microisolator-filter covers with wood shavings for bedding. Water and a commercial diet (Purina Formulab 5008) were available *ad libitum*. Animals were maintained in climate-controlled rooms under an alternating 12-h light/dark cycle. Timed matings were produced by placing individual male mice into cages containing multiple females for the last 2 h of the dark cycle, 7:00 to 9:00 AM. Detection of a vaginal plug was used to designate gestational day 0.

Cadmium chloride (Sigma Chemical Co.) was dissolved in deionized water at 0.4 mg/ml and administered to pregnant mice by intraperitoneal (ip) injection (10 ml/kg body weight) on gestational day 9.0 (9:00 AM) at a dose of 4.0 mg/kg body weight. Pregnant mice were sacrificed on gestational day 18 by inhalation of ethyl ether and subsequent cervical dislocation. The uterus of each animal was removed and the numbers of implantation sites, resorptions, and viable fetuses were recorded. Individual fetuses were removed from the uterus, and body weight, sex, and gross malformations were recorded. Standardized nomenclature for malformations was obtained from Wise *et al.* (1997).

The fetuses were cleared and stained with alizarin red and alcian blue for skeletal examination employing the methods of Inouye (1976) and Kimmel and Trammel (1981), as modified by Kuczuk and Scott (1984). Forelimb malformations observed at the time of C-section were confirmed by subsequent analysis of the fetal skeletal preparations. For those fetuses to be genotyped, visceral tissue normally discarded during the skeletal preparation protocol was collected with sterile technique, snap-frozen in liquid nitrogen, and stored at −70°C. Genomic DNA from murine tissue was subsequently isolated by phenol/chloroform extraction as described by Couse *et al.* (1994). The concentration and purity of the genomic DNA preparations were estimated by A_{260}/A_{280} absorption spectrophotometry (Sambrook *et al.*, 1989).

Identification of fetal and maternal effects. To assess the penetrance and observe the inheritance of sensitivity to cadmium-induced forelimb malformations, a number of crosses between C57BL/6 and SWV mice were performed. The expression of the limb defect was analyzed in C57BL/6, SWV, their F1 hybrid, backcrosses to both parental strains, and an F2 intercross. Maternal animals were treated on day 9.0 of gestation with an intraperitoneal injection of 4 mg/kg cadmium chloride.

To investigate the contribution of fetal genotype to the expression of the malformation, defect incidence was compared among crosses in which the maternal animal was identical while the male animal in the cross was varied, e.g., C57BL/6 × C57BL/6, C57BL/6 × SWV, and C57BL/6 × F1 (female listed first in crosses). Likewise, to evaluate the contribution of maternal genotype, crosses were compared in which the average fetal genotype was identical, and only the maternal environment was varied, e.g., C57BL/6 × SWV versus SWV × C57BL/6, and C57BL/6 × F1 versus F1 × C57BL/6.

Statistical analyses. Tests for the effects of maternal and fetal genotype on fetal incidence of forelimb defects were performed using 2 × 2 or 3 × 2 contingency table analyses of counts of fetal forelimb defects, using Yates' correction for continuity for small samples (Fisher and Yates, 1963). Differences between crosses in average litter percentages of forelimb malformations were assessed using the Student *t*-test following an arcsine transformation to stabilize the variance, and a correction for extreme percentage values (Dagg *et al.*, 1966). Statistical significance was inferred at a p value of 0.05.

Constructing the genome spanning array and genotyping the backcross panel. The C57BL/6J strain has been widely utilized in linkage analysis studies, and accordingly, the microsatellite allele sizes for this strain are well characterized and readily available from a public database (MGD, 1999). However, no previous studies have utilized the SWV strain in linkage analysis. Therefore, to construct a linkage map for use in QTL analysis, identification of microsatellites that are polymorphic between C57BL/6 and SWV was first required.

Polymorphism testing of genomic DNA isolated (see above) from the two strains was accomplished using polymerase chain reaction (PCR) with 5′-end-labeled forward primers according to standard methods. Products were separated on 5% polyacrylamide sequencing gels and visualized with autoradiography. The details of the method are available upon request from the authors. To allow the successful amplification of the majority of markers, PCR was performed under rather nonstringent conditions (53°C annealing temperature, 1.5 mM Mg^{2+}). A large stock of commercially available microsatellite markers, provided as PCR primer pairs, were kindly made available for polymorphism testing by the laboratory of A. J. Lusis (UCLA). Additional markers were ordered from Research Genetics (Hunts-

ville, AL). A total of 877 markers were assessed for polymorphism between the strains.

Markers producing amplification products of the size reported for C57BL/6J by Research Genetics or by the Mouse Genome Database (MGD, 1999) were observed for polymorphism between C57BL/6N and SWV. One hundred forty-nine polymorphic markers were assembled into an array spanning the 19 autosomes at an average spacing of less than 10 cM (Table 1). The genotyping of each animal in the backcross panel (see below) at each marker in the array was performed using a method similar to that described for polymorphism testing.

Generating and phenotyping the backcross mapping panel. Because F1 fetuses differed only slightly from inbred SWV fetuses, in their expression of limb defects whole genome linkage analysis was performed on mice generated from a backcross, as opposed to an F2 intercross. To control for maternal effects and isolate the fetal factors contributing to expression of the trait, only inbred C57BL/6 mice were used as maternal animals. Matings were performed with F1 male mice, generated from both directions of the outcross between C57BL/6 and SWV mice. A panel of 198 backcross fetuses was assembled from 42 litters of Cd-treated C57BL/6 mice. Only litters containing fetuses with limb defects were utilized for panel assembly, and thus all unaffected mice were from litters in which affected fetuses were found. While this method introduced some degree of selection bias by eliminating a small number of litters from the total pool of backcross animals, this avoided the possibility that an errant ip injection caused a litter without congenital malformations.

Binary and semiquantitative traits. Fetuses in the panel were phenotyped for the presence or absence and severity of forelimb malformations. Two traits were analyzed, one of which was qualitative, giving affected mice a score of "1" and nonaffected mice a "0," and the other a semiquantitative score-based estimate of the severity of the defect. The second trait was created to approximate a continuous distribution among the backcross fetuses. Each fetus was assigned a score reflecting the status of its forelimbs, with higher scores given for more severe defects ("1" for normal, "2" for rudimentary fifth digit, "3" for ectrodactyly of the fifth digit, "4" for ectrodactyly of the fifth digit with a rudimentary fourth digit, "5" for ectrodactyly of the fourth and fifth digits, "6" for ectrodactyly of the fourth and fifth digits with a rudimentary third digit, "7" for ectrodactyly of the third, fourth, and fifth digits). The scores for both autopods were added to attain the final, semiquantitative trait value for each fetus. This scoring system assumed a linear, stepwise progression of the defect's severity, which is likely a gross simplification of its true nature. To improve normality, a square-root transformation was applied to this semiquantitative trait prior to QTL analysis.

QTL linkage analyses. For data management and preliminary linkage map construction, the genotype of each fetus at each marker was entered into the Macintosh-based MapManager QT 2.6 software package (Manly, 1993). Linkage map construction was performed by the Unix-based MAPMAKER/EXP (Lander *et al.,* 1987; Lincoln *et al.,* 1992a).

Multipoint linkage analysis was performed with MAPMAKER/QTL (Beta version 2.0) (Lincoln *et al.,* 1992b; Paterson *et al.,* 1988). For the binary trait, a QTL *penetrance scan* (Gorham *et al.,* 1996), newly developed for MAPMAKER/QTL, was performed. This mapping function optimizes the penetrance parameters (probabilities of affectation given the genotype of the class) for each genotypic class (homozygous or heterozygous with a backcross) at each locus. The resulting lod score reflects the likelihood of the optimal penetrance values compared with the likelihood under the null hypothesis of equal penetrances.

Mapping was also performed on the semiquantitative trait data using both nonparametric and parametric linkage methods. The *nonparametric scan* function (performed by the Beta version of MAPMAKER/QTL) does not assume that the trait under analysis is normally distributed. It calculates a nonparametric statistic, Z_w, which analyzes the ranks of the trait values, rather than the trait values themselves (Kruglyak and Lander, 1995; Manly and Olson, 1999). The *scan* function of MAPMAKER/QTL conducts parametric linkage analysis, where a model of the inheritance pattern is assumed. This approach to linkage is more powerful than the binary and nonparametric analyses when the proposed model is correct. The locations of QTL peaks from all analyses were inferred from scan results and extrapolated to distances described in the Mouse Genome Database (MGD, 1999).

RESULTS

Primer Testing Results and Assembly of Genome-Spanning Array

A total of 877 markers were tested for polymorphism between the C57BL/6 and the SWV mouse strains; 833 (95.0%) of the markers produced visible PCR products, and 451 (54.1%) were polymorphic between the two strains. The percentage of polymorphism between C57BL/6 and SWV for the individual chromosomes ranged from 26.7 to 73.3%. As only a single set of PCR conditions was utilized, it is possible that the number of markers producing visible products would increase with titration of the cycling parameters. Consequently, the number of markers found to be polymorphic in this study is likely to underestimate the number truly polymorphic between the strains.

In addition to assessing polymorphisms between the C57BL/6 and the SWV strains, 867 of the microsatellite markers were tested for polymorphism between C57BL/6 mice obtained from two different providers: C57BL/6J from The Jackson Laboratories and C57BL/6N from Charles River Laboratories. Of 867 markers tested, 823 (95.0%) produced visible products using a uniform PCR protocol, and of these, 13 (1.6%) were polymorphic between C57BL/6J and C57BL/6N. The markers that were polymorphic between The Jackson Laboratories and Charles River Laboratories C57BL/6 mice were D1Mit81, D1Mit43, D1Mit241, D2Mit1, D7Mit66, D7Mit207, D9Mit90, D10Mit107, D10Mit 145, D11Mit70, D12Mit95, D17Mit221, and D19Mit100. All other experiments were performed with C57BL/6N mice.

Table 1 lists the polymorphic markers selected for a genome spanning array of chromosomes 1 to 19. The array was utilized for the construction of a linkage map among the animals in the backcross panel for QTL analysis in the present study.

Cadmium-Induced Forelimb Malformations: Inheritance of Sensitivity

The most common forelimb defect resulting from day 9.0 cadmium treatment of C57BL/6 mice was ectrodactyly of the fifth digit of the autopod and is shown in Fig. 1. The defect occurred predominantly on the right limb, but was frequently seen on both forelimbs and was almost exclusively a deficit of the postaxial side of the autopod. A range of severity was observed, even among fetuses from inbred C57BL/6 crosses with identical genotypes. The most benign of the malformations was

TABLE 1

Markers Selected for Genome Spanning Array

Microsatellite	cM Position[a]	Microsatellite	cM Position[a]	Microsatellite	cM Position[a]
Chromosome 1		D6Mit67	41.5	D12Mit98	48.0
D1Mit296	8.3	D6Mit105	45.5	D12Mit263	58.0
D1Mit276	12.5	D6Mit374	63.9	*Chromosome 13*	
D1Mit76	32.8	D6Mit291	66.0	D13Mit55	6.0
D1Mit83	52.0	D6Mit390	73.0	D13Mit115	11.0
D1Nds2	59.0	*Chromosome 7*		D13Mit64	30.0
D1Mit43	76.0	D7Mit227	16.0	D13Mit145	52.0
D1Mit207	100.0	D7Mit228	18.0	D13Mit230	62.0
D1Mit154	112.0	D7Mit230	24.5	D13Mit32	71.0
Chromosome 2		D7Mit82	25.0	D13Mit204	72.0
D2Mit79	10.0	D7Mit212	37.0	D13Mit78	75.0
D2Mit269	30.0	D7Mit321	48.5	*Chromosome 14*	
D2Mit61	34.0	D7Mit237	52.8	D14Mit109	3.0
D2Mit12	50.3	D7Mit105	63.5	D14Mit133	10.0
D2Mit224	74.0	D7Mit362	72.4	D14Mit129	14.5
D2Mit57	82.0	*Chromosome 8*		D14Mit5	22.5
D2Mit48	87.0	D8Mit3	10.0	D14Mit165	52.0
D2Mit52	99.0	D8Mit281	11.0	D14Mit178	60.0
D2Mit213	105.0	D8Mit4	14.0	*Chromosome 15*	
D2Mit74	107.0	D8Mit190	21.0	D15Mit174	4.3
Chromosome 3		D8Mit134	37.0	D15Nds2	11.4
D3Mit60	0.0	D8Mit113	52.0	D15Mit86	22.2
D3Mit166	4.6	D8Mit120	61.0	D15Mit101	30.2
D3Mit46	13.8	*Chromosome 9*		D15Mit33	48.6
D3Mit73	39.7	D9Mit89	8.0	D15Mit159	49.6
D3Mit106	55.0	D9Mit2	17.0	D15Mit108	55.6
D3Mit288	58.8	D9Mit254	25.0	D15Mit40	65.0
D3Mit14	64.1	D9Mit21	31.0	*Chromosome 16*	
D3Mit291	66.2	D9Mit35	52.0	D16Mit32	1.7
D3Mit194	67.6	D9Mit24	56.0	D16Mit59	27.8
D3Mit127	70.3	D9Mit116	61.0	D16Mit64	38.0
D3Mit19	87.6	*Chromosome 10*		D16Mit105	44.0
Chromosome 4		D10Mit1	6.0	D16Mit19	54.0
D4Mit19	5.0	D10Mit183	17.0	D16Mit86	66.0
D4Mit151	28.6	D10Mit15	35.0	*Chromosome 17*	
D4Mit15	42.6	D10Mit42	44.0	D17Mit223	3.0
D4Mit31	51.3	D10Mit10	51.0	D17Mit80	12.9
D4Mit12	57.6	D10Mit162	59.0	D17Mit7	32.3
D4Mit148	66.0	D10Mit233	62.0	D17Mit3	41.5
D4Mit59	78.9	*Chromosome 11*		D17Mit121	47.4
Chromosome 5		D11Mit16	0.25	*Chromosome 18*	
D5Mit145	0.0	D11Mit151	13.0	D18Mit64	2.0
D5Mit73	11.0	D11Mit20	20.0	D18Mit59	16.0
D5Mit148	18.0	D11Mit24	27.85	D18Mit24	25.0
D5Mit11	26.0	D11Mit177	36.0	D18Mit181	32.0
D5Mit197	36.0	D11Mit39	49.0	D18Mit141	45.0
D5Mit10	54.0	D11Mit59	58.0	D18Mit16	58.0
D5Mit188	64.0	D11Mit100	68.0	*Chromosome 19*	
D5Mit174	70.0	D11Mit48	77.0	D19Mit32	0.0
D5Mit287	94.0	*Chromosome 12*		D19Mit31	7.0
Chromosome 6		D12Mit85	13.0	D19Mit41	16.0
D6Mit86	0.5	D12Mit2	19.0	D19Mit30	20.0
D6Mit314	20.5	D12Mit31	25.0	D19Mit5	34.0
D6Mit19	33.5	D12Mit202	30.0	D19Mit66	41.0
D6Mit6	35.3	D12Mit128	32.0	D19Mit38	47.0
D6Mit31	38.5	D12Mit14	37.0	D19Mit71	54.0

[a] Mouse Genome Database (1999).

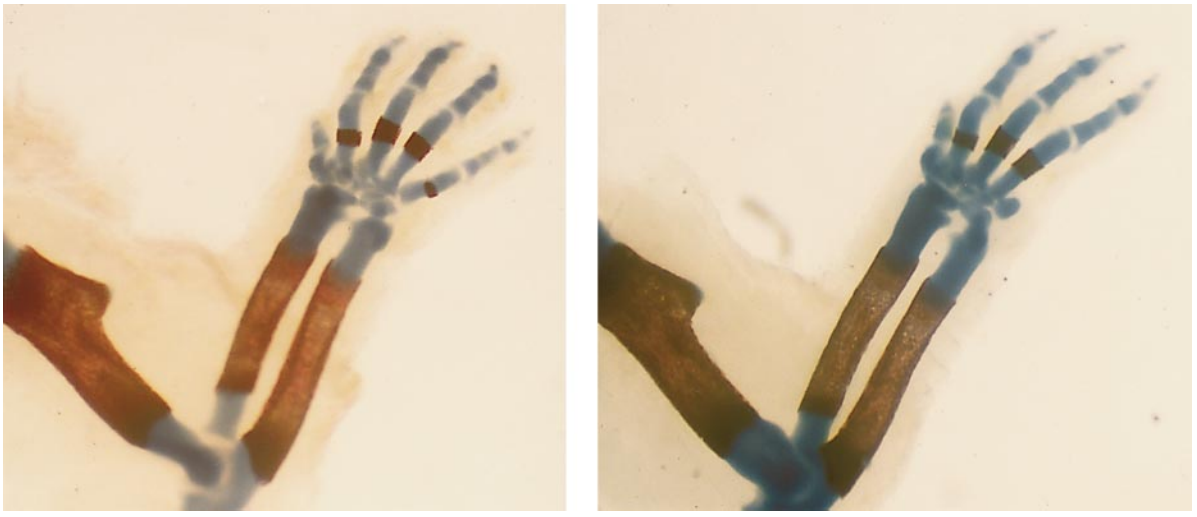

FIG. 1. Typical manifestation of the forelimb defect induced by day 9.0 Cd-treatment in C57BL/6 mice. (**Left**) A double-stained skeletal preparation of a normal right forelimb from a day 18 C57BL/6 mouse fetus, palm down. (**Right**) A right forelimb with ectrodactyly of digit 5.

a reduction in the girth or length of the metacarpals and phalanges of digit 5, while the more common defect was a lack of all structures distal to the carpals on the fifth digit. More severe presentations included the lack of multiple digits, progressing in a postaxial to preaxial direction. In combination with the most severe digital reductions, the ulna was frequently shortened, malformed, or missing.

The results of various crosses between C57BL/6 and SWV show that the incidence of defects induced by day 9.0 cadmium treatment is influenced by both fetal and maternal genetic factors. When one compares the percentage of limb malformations among the three crosses where the C57BL/6 strain is the maternal animal, the contribution of fetal genotype to expression of the trait can be observed. The incidence of defects differs significantly between the C57BL/6 inbred cross, the outcross in C57BL/6 maternal mice, and the backcross to C57BL/6, despite the fact that the maternal animal is identical (Table 2).

The contribution of maternal genotype to trait expression can be observed in the crosses in which fetuses with the same genotype are developing in different maternal environments. This condition occurs in the two directions of the outcross between the parental strains and in the backcrosses. Significant differences in the percentage of limb defects were observed between F1 outcross fetuses from C57BL/6 maternal animals (5.6%) and SWV maternal animals (0%). In the backcross to the sensitive strain, 37% of fetuses were observed with forelimb defects when C57BL/6 was the maternal animal, but when an F1 animal was the mother, a significantly smaller percentage of fetuses had defects (7%).

Identification of QTLs

To identify potential fetal loci influencing differential sensitivity to cadmium-induced forelimb malformations, a series of QTL analyses was performed among 198 backcross mice utilizing a linkage map of 149 microsatellite markers. The results of these analyses are shown in Table 3.

A penetrance scan of the binary trait (Gorham *et al.*, 1996) detected one locus with a lod score of 2.67 ($P = 0.00045$), located on the distal end of chromosome 6 near D6Mit390 (Table 3). Three additional loci were also identified with lesser lod scores, located on chromosomes 14, 11, and 3. For the QTL on chromosome 6, the penetrance was estimated to be 0.67. Of the 198 fetuses that were genotyped, 102 were homozygous for the C57BL/6 genotype at the chromosome 6 QTL, and 68 of the 102 were affected with the limb defect (67%). It is presumed that 67% of fetuses homozygous for the C57 allele at this locus will develop the limb defect when treated with the same dose of cadmium.

The binary data were converted to a semiquantitative trait based on the severity of the defect and analyzed by the nonparametric and parametric scan functions of MAPMAKER/QTL (Beta version 2.0). The results of these analyses are also shown in Table 3. As expected, both analyses identified the same QTL detected by the penetrance scan on chromosome 6, just centromeric of D6Mit390. The peak lod score for the locus was 2.32 when analyzed with the nonparametric approach. Three additional loci with suggestive linkage on chromosomes 14, 11, and 1 were also identified with this method. The parametric scan located the chromosome 6 QTL with a lod score peaking at 3.08, along with two suggestive loci on chromosomes 13 and 11.

The QTL located on chromosome 6 has been designated *Cadfar* (cadmium-induced forelimb autopod reduction defect). The MAPMAKER/QTL-determined lod score plot identifying *Cadfar* is shown in Fig. 2.

TABLE 2

Resorption and Forelimb Defect Percentages for Various Crosses between C57BL/6 and SWV Treated on Day 9.0 of Gestation with 4 mg/kg Cadmium Chloride

Cross[a]	Total number of litters	Total number of implants	Total number of live fetuses	Fetal % of resorbed implants	Mean litter % of resorptions (±SEM)	Fetal % of forelimb defects	Mean litter % of forelimb defects (±SEM)
Inbred cross							
C57BL/6 × C57BL/6	14	111	86	22.5	23.7 (6.50)	80.2[b,c]	67.5 (10.4)[d,e]
SWV × SWV	11	144	132	8.3	8.6 (3.32)	0.0[b]	0.0[d]
Outcross (F1)							
C57BL/6 × SWV	14	118	108	8.5	9.4 (3.70)	5.6[c]	5.3 (2.3)[e,f]
SWV × C57BL/6	8	83	81	2.4	2.8 (1.86)	0.0	0.0[f]
Backcross to C57BL/6							
C57BL/6 × Pooled F1	42	370	290	21.6	21.0 (3.05)	37.2[c,g]	36.1 (4.6)[e,h]
Pooled F1 × C57BL/6	8	82	56	31.7	32.3 (11.68)	7.1[g]	5.5 (3.2)[e,i]
Backcross to SWV							
SWV × Pooled F1	11	131	79	39.7	41.2 (8.78)	0.0	0.0
Pooled F1 × SWV	10	103	72	30.1	38.9 (13.73)	0.0	0.0[i]
F2 Intercross							
F1 × F1	14	177	112	36.7	36.4 (7.22)	3.6	3.8 (2.0)

[a] Maternal animal listed first.
[d,e,f,h,i] Percentage significantly different from cross with same superscript, *t* test on transformed and corrected data, $p < 0.05$.
[b,g] Significantly different from cross with same superscript, 2×2 χ^2 test using Yate's Correction for continuity, $p < 0.05$.
[c] Significantly different from crosses with same superscript, 3×2 χ^2 test using Yate's Correction for continuity, $p < 0.05$.

DISCUSSION

The varying sensitivities of inbred mouse strains to teratogen-induced malformations have been described for almost 50 years (Kalter, 1965; Vekemans and Biddle, 1984), but little progress has been made toward revealing the underlying nature of this differential sensitivity. One approach for identifying the specific genetic factors causing strains to differ in response to chemical exposures is to perform a whole genome scan to evaluate linkage between the malformation phenotype and specific chromosomal loci. Diehl and Erickson (1997) used this approach to link genomic regions with the expression of cleft lip and palate induced by phenytoin and 6-aminonicotinamide. They used recombinant inbred strains generated from the A/J and C57BL/6J inbred mouse strains. The present study has taken the same general approach in the study of a strain difference in sensitivity to cadmium-induced forelimb ectrodactyly. However, in this case a whole genome scan was performed among a panel of backcross animals generated between the C57BL/6 and the SWV mouse strains using a genome-spanning array of polymorphic microsatellite markers, in a method that is similar to that utilized by Hillebrandt *et al.* (1998) for radiation-induced gastroschisis.

Previous studies observing the segregation of sensitivity in test crosses between inbred strains have found that both maternal and fetal genetic factors influence the expression of cadmium-induced fetal lethality and teratogenicity (Pierro and Haines, 1978; Wolkowski, 1974). The results of the present study support these findings, since the incidence of forelimb malformations in offspring of crosses between C57BL/6 and SWV were observed to vary with both the strain of the maternal animal and the genetic composition of the offspring (Table 2). The second aspect of the current investigation utilizes QTL analysis (Lander and Botstein, 1989; Lander and Schork, 1994) to identify a fetal chromosomal locus that may harbor a gene contributing to the differential incidence of Cd-induced forelimb malformations in the C57BL/6 and SWV mouse strains.

The whole-genome parametric scan of the semiquantitative trait revealed evidence for linkage of a single locus, designated *Cadfar*, found on the telomeric end of chromosome 6 with a lod score of 3.08. While only this single locus demonstrated significant linkage, the standard crosses between C57BL/6 and SWV mice suggest that it is unlikely that a single gene controls sensitivity. The penetrance scan data showed that 68 of the 102 fetuses (67%) that possessed the susceptibility allele at *Cadfar* were affected. However, there were 42 fetuses, of 110 total fetuses with the limb defect, that had a heterozygous marker at the *Cadfar* locus, suggesting that other factors contribute to the expression of this trait.

The sample size of 198 animals permits us to detect loci, each contributing as little as 10% to the total trait variance, with 90% statistical power (Lynch and Walsh, 1998). Thus, we do not have sufficient power to detect *all* QTLs having a lesser effect. Additionally, if *Cadfar* alone was controlling expression, the variance explained by the locus would be expected to be greater than 7.2%. Taken together, these findings suggest that susceptibility to cadmium-induced forelimb birth defects is a complex, multifactorial, and potentially oligogenic phenotype.

The threshold for significant linkage utilized in the present study allowed the detection of only a single

TABLE 3

Parametric Scan Results of Forelimb Defect, Semiquantitative

Microsatellites flanking QTL peaks	Location of QTL peak[a]	Penetrance scan: binary trait		Lod score	*P* value[b]
		Penetrance of C/C[c]	Penetrance of C/S[c]		
D6Mit291–D6Mit390	70.6	0.67	0.43	2.67	0.00045
D14Mit5–D14Mit165	41.4	0.68	0.45	2.27	0.00122
D11Mit20–D11Mit24	26.0	0.65	0.45	1.91	0.00302
D3Mit46–D3Mit73	25.1	0.46	0.66	1.60[d]	0.00664
		Nonparametric scan: Semiquantitative trait			
		Z_w Score			
D6Mit291–D6Mit390	71.5	3.27		2.32[e]	0.00108
D14Mit5–D14Mit165	52.0	3.16		2.17[e]	0.00157
D11Mit24–D11Mit177	27.9	2.90		1.83[e]	0.00370
D1Mit296–D1Mit276	8.3	2.74		1.63[e]	0.00615
		Parametric scan: Semiquantitative trait			
		% Variance explained[f]			
D6Mit390	72.4	7.2		3.08	0.00016
D13Mit78	74.1	4.4		1.89	0.00318
D11Mit24	27.9	4.2		1.82	0.00379

[a] Approximate distance from centromere in centimorgans; Mouse Genome Database (1999).
[b] *P* value calculated from a χ^2 distribution with 1 df.
[c] Probability of affectation for a given genotype at the QTL location; C, C57BL/6 allele; S, SWV allele.
[d] The presence of the SWV allele (in heterozygotes) at this locus increased the probability of being affected.
[e] Nonparametric scan linkage statistic, Z_w, converted to equivalent lod score by $(0.5)\ln(Z_w)^2$.
[f] Total trait variance estimated for the QTL at this location by MAPMAKER/QTL.

locus influencing expression of the malformation. However, it is possible that additional QTLs could be detected under different experimental conditions. Identification of linkage to a particular locus can be complicated by experiment-specific factors, including incomplete penetrance of a trait, maternal influences,

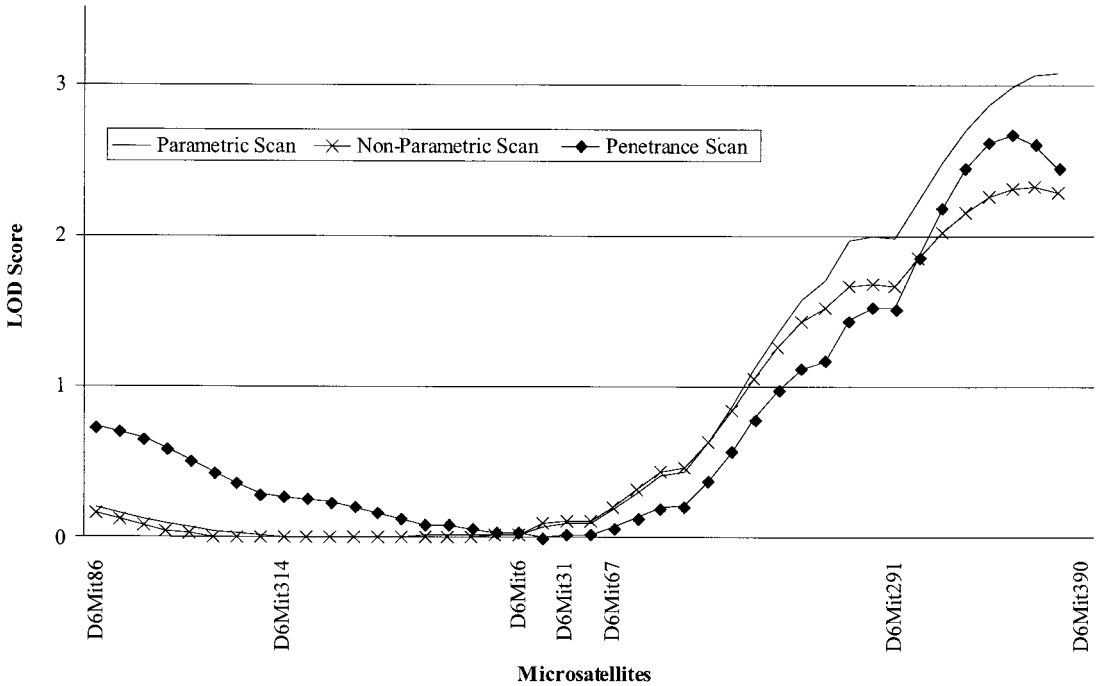

FIG. 2. Lod score plot of chromosome 6 QTL (*Cadfar*).

or an inheritance pattern requiring the simultaneous action of multiple genes (Lander and Schork, 1994), all of which are potential confounding variables in the present study.

The QTL putatively identified in this study, *Cadfar*, potentially marks the location of a gene or genes involved in the differential sensitivity of the C57BL/6 and SWV mouse strains to cadmium-induced forelimb reduction defects. Further analysis, possibly utilizing fine-mapping techniques and congenic strains to increase resolution (Darvasi, 1998; Lander and Schork, 1994; Rikke and Johnson, 1998; Talbot *et al.*, 1999), may refine the location of this QTL and in turn reveal candidate genes conveying sensitivity or resistance to cadmium-induced forelimb reduction defects in the two strains. In addition, several other compounds have been evaluated for their ability to induce forelimb ectrodactyly in the C57BL/6 and SWV mouse strains, including acetazolamide (Biddle, 1988), ethanol (Zimmerman *et al.*, 1990), carbon dioxide (Weaver and Scott, 1984, hyperthermia (Bennett *et al.*, 1989), and all-*trans*-retinoic acid (Collins *et al.*, 1988). Interestingly, the C57BL/6 strain is the more sensitive strain for all of these compounds. Work is currently under way to assess whether loci identified in the present study will be conserved for other teratogens producing the same malformation.

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