

The Teratogenic Sensitivity to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Is Modified by a Locus on Mouse Chromosome 3

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ABSTRACT

In an effort to understand how genetics can influence individual sensitivity to environmentally induced disease, we performed a linkage analysis to identify murine loci in addition to the *Ahr* locus that influence the incidence of cleft palate and hydronephrosis in developing mice exposed to the pollutant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin). Administration of 64 $\mu\text{g/kg}$ dioxin to C57BL/6J (B6) dams at embryonic day 9 (E9) led to palatal clefting and hydronephrosis in nearly 100% of embryos by E17. In contrast, similar exposure of CBA/J (CBA) dams led to cleft palate in only 8% and hydronephrosis in 69%

of embryos. To determine the genetic basis for this strain-dependent sensitivity, linkage analyses on the progeny of a B6CBAF₁ intercross and a CBA \times B6CBAF₁ backcross were performed. The incidences of cleft palate and hydronephrosis were assessed and genomic DNA from embryos was analyzed at informative simple sequence length polymorphism (SSLP) markers. One locus segregating with dioxin-induced cleft palate was identified ($p < 0.01$) and designated as chemically mediated teratogenesis number 1 (*Cmt1*). The *Cmt1* locus is located on chromosome 3.

The term “gene-environment interaction” is used to describe the altered risk of disease resulting from the interplay between an individual organism’s genotype and its chemical or physical environment (Botto and Khoury, 2001; Brennan, 2002; Cooper, 2003). It has been proposed that a better understanding of gene-environment interactions could help identify persons at higher risk for environmentally induced disease (Murray, 2002). The benefit to human health can come in two ways. First, the recognition of higher risk combinations of genetic determinants and environmental conditions can be used to develop prevention strategies for sensitive populations. Second, the identification of polymorphisms that influence an adverse response to the environment will help define relevant genetic loci and shed light on the mechanism of action of human toxicants, carcinogens, and teratogens. In turn, this information can lead to the development of antidotes and antagonists when chemical exposures are unavoidable or have already occurred.

The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin) is a prototype for the study of gene-environment interactions. Dioxin is an ubiquitous environmental pollutant that is

known to cause toxicity (Poland and Glover, 1973; Poland and Knutson, 1982; Anderson et al., 1993), cancer (Pitot et al., 1980; Becher and Flesch-Janys, 1998), and terata in experimental animals (Couture et al., 1990; Abbott and Birnbaum, 1991; Hamm et al., 2000; Loertscher et al., 2002). Moreover, this compound has been associated with a spectrum of adverse health outcomes in human populations (Mastroiacovo et al., 1988; Rogan et al., 1988; Stockbauer et al., 1988; Wolfe et al., 1995). The most well characterized genetic modifier of dioxin signaling is the *Ahr* locus. The *Ahr* locus encodes the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor that transduces the dioxin signal to the nucleus (Bank et al., 1992; Harper et al., 1992; Dolwick et al., 1993). In the mouse, the *Ahr* locus exists as four alleles, denoted *Ahr*^{b1}, *Ahr*^{b2}, *Ahr*^{b3}, and *Ahr*^d. The *b* alleles encode receptors with a high binding affinity for dioxin, and the *d* allele encodes a receptor with a lower affinity (Poland et al., 1994). C57BL/6 mice that express the *Ahr*^{b1} allele are highly sensitive to the toxic and teratogenic effects of dioxin, whereas DBA/2 mice with the *Ahr*^d allele are less sensitive (Poland and Glover, 1980).

Genetic analyses in the mouse have provided evidence that polymorphisms at loci other than *Ahr* may have a significant impact on dioxin sensitivity. In an early screen of mouse strains for their sensitivity to dioxin-induced cleft palate, it was observed that the CBA/J mouse strain was relatively

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ABBREVIATIONS: dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; B6, C57BL/6J; CBA, CBA/J; PCR, polymerase chain reaction; SSLP, simple sequence length polymorphism; LOD, logarithm of the odds (to the base 10); TGF, transforming growth factor.

resistant to dioxin-induced cleft palate compared with other strains that also harbored *Ahr b* alleles (Poland and Glover, 1980). In this regard, exposure of pregnant CBA/J dams to 30 $\mu\text{g/kg}$ dioxin did not induce cleft palate in any of 61 scored offspring. In contrast, other *Ahr b* allele strains, C57BL/6J and BALB/c, displayed a cleft palate incidence ranging from 54 to 95%. These data support the idea that the mammalian genome harbors additional genetic loci, other than *Ahr*, that significantly influence the teratogenic sensitivity to dioxin.

Given the importance of identifying genetic modifiers of toxicity, we have performed linkage analyses to identify and enumerate loci that influence the teratogenic sensitivity to

dioxin. To this end, we are taking advantage of the polymorphic response to dioxin-induced cleft palate exhibited by the C57BL/6J and CBA/J strains and have begun a search for the loci responsible for this differential sensitivity (Poland and Glover, 1980). In an effort to gain maximal information from this study, we expanded our genotype-phenotype screen to include dioxin-induced hydronephrosis (Couture et al., 1990).

Materials and Methods

Mice and Terata Induction. The C57BL/6J (B6) and CBA/J (CBA) inbred mouse strains were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed, bred, and maintained in microisolator cages in a specific-pathogen-free facility on corn cob bedding (Bed O'Cobs, Maumee, OH) at the McArdle Laboratory for Cancer Research. Animals were provided Purina 5020 diet (9% fat; Purina, St. Louis, MO) and water ad libitum. Embryos were generated by placing female mice with male mice for 12 h. Embryonic day 1 (E1) was designated as the day female mice were separated from male mice. At E9, female mice that had gained at least 2 g since cohabitation with male mice were assumed to be pregnant and injected with a single intraperitoneal dose of 64 $\mu\text{g/kg}$ dioxin dissolved in dimethyl sulfoxide.

Assessment of Terata. At E17, the dam was sacrificed by cervical dislocation, and the uterus was dissected from the dam and placed into ice-cold phosphate-buffered saline. A 3-mm section of each embryo tail was taken for DNA isolation and later genotyping. Fusion of the hard palate was scored by gross examination of the oral cavity (Fig. 1A). Palatal shelves that failed to fuse were recorded as "cleft palate" (Fig. 1B). The cleft palate phenotype occurred as a binary event, in that all of the shelves that failed to fuse exhibited an identical cleft of the secondary palate. The urogenital tract was dissected and macroscopically assessed for swelling of the kidneys and ureters (Fig. 1C). If such swelling was observed, it was scored as "hydronephrosis" (Fig. 1D). The sex of each embryo was scored according to its internal anatomy (Fig. 1, C and D). Comparisons of the incidence of terata between groups were made using the Fisher's exact test.

Genotype Analysis. The DNA was isolated from the tail of the embryo via standard proteinase K-phenol extraction protocol (Sambrook et al., 1989). The polymerase chain reaction (PCR) was used to amplify informative SSLP microsatellites in the embryo DNA (Bilger et al., 2004). Ninety-six B6CBAF₂ embryos from a B6CBAF₁ (the dam is

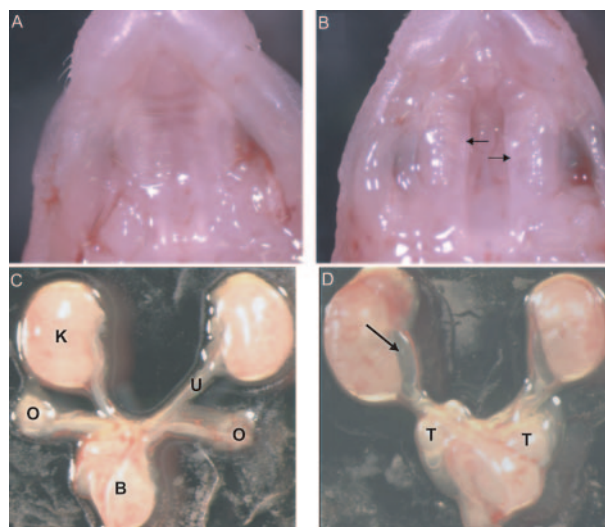


Fig. 1. Pathology of dioxin-induced terata. Difference in palatogenesis and urogenesis of embryos exposed to 64 $\mu\text{g/kg}$ dioxin via maternal injection at E9. At E17 embryos were dissected from the dam and assessed for normal palate formation (A) and normal urogenital tract formation (C; female). B is a representation of cleft palate exhibited by an embryo exposed to dioxin. The arrows in B indicate the individual palatal shelves which have not fused, resulting in cleft palate. The urogenital tract is composed of the kidney (K), ovary (O), testis (T), bladder (B), and ureter (U). D (male) is a representation of a hydronephrotic urogenital tract. The arrow points to the ureter that is clearly distended by fluid that backs up into the kidney.

TABLE 1

SSLP microsatellite markers used in linkage analysis of the progeny from a B6CBAF₁ intercross and a CBA×B6CBAF₁ backcross

Located to the right of each marker are the annealing temperatures (°C) that were used in the amplification protocol. The marker positions and oligonucleotide sequences on the genetic map were retrieved from the Mouse Genome Database (http://mgd.wehi.edu.au:8080/searches/polymorphism_form.shtml).

Chromosome				SSLP Markers										
1	<i>D1Mit3</i>	58	<i>D1Mit5</i>	60	<i>D1Mit30</i>	46	<i>D1Mit33</i>	59	<i>D1Mit13</i>	62	<i>D1Mit150*</i>	54		
2	<i>D2Mit1</i>	59	<i>D2Mit48</i>	58	<i>D2Mit49</i>	59	<i>D2Mit62</i>	59						
3	<i>D3Mit6</i>	59	<i>D3Mit11</i>	59	<i>D3Mit14</i>	58	<i>D3Mit17</i>	59	<i>D3Mit9</i>	58	<i>D3Mit62</i>	58	<i>D3Mit203*</i>	54
4	<i>D4Mit9</i>	58	<i>D4Mit16</i>	53	<i>D4Mit33</i>	64								
5	<i>D5Nds2</i>	56												
6	<i>D6Mit1</i>	56	<i>D6Mit9</i>	59	<i>D6Mit10</i>	58	<i>D6Mit15</i>	65	<i>D6Mit17</i>	59	<i>D6Mit25</i>	60		
7	<i>D7Nds4</i>	60												
8	<i>D8Mit3</i>	58												
9	<i>D9Mit2</i>	58	<i>D9Mit10</i>	58										
10	<i>D10Mit3</i>	58	<i>D10Mit10</i>	55	<i>D10Mit31</i>	62	<i>D10Mit67</i>	56						
11	<i>D11Nds1</i>	49	<i>D11Mit23</i>	59	<i>D11Mit19</i>	58								
12	<i>D12Mit5</i>	72	<i>D12Mit12</i>	55	<i>D12Mit20</i>	64	<i>D12Mit34</i>	65						
13	<i>D13Mit3</i>	58	<i>D13Mit13</i>	58	<i>D13Mit30</i>	62								
14	<i>D14Mit7</i>	55	<i>D14Mit14</i>	56	<i>D14Mit28</i>	65	<i>D14Mit62</i>	59						
15	<i>D15Mit3</i>	58												
16	<i>D16Mit30</i>	58												
17	<i>D17Mit16</i>	58	<i>D17Mit34</i>	52	<i>D17Mit38</i>	65	<i>D17Mit60</i>	53	<i>D17Mit70</i>	62				
18	<i>D18Mit4</i>	58												
19	<i>D19Mit10</i>	62	<i>D19Mit13</i>	58	<i>D19Mit31</i>	55	<i>D19Mit33</i>	56						

* Additional markers that were used to screen the CBA×B6CBAF₁ progeny.

listed first and the sire second) intercross were scored and genotyped at 58 SSLP loci, and 100 embryos from a CBA×B6CBAF₁ backcross were scored and genotyped by PCR at 60 SSLP loci (Table 1). The PCR was performed using 100 ng of genomic DNA at an initial denaturing step of 95°C for 5 min. After denaturation, 30 cycles were performed at 95°C for 1 min, 54°C for 45 s, and 72°C for 1 min. A final extension step was carried out at 72°C for 5 min. The specific annealing temperatures were optimized for each primer set (Table 1). The PCR products were resolved on 4% (w/v) NuSieve 3:1 agarose gels (Cambrex Bio Science Rockland, Inc., Rockland ME).

Linkage Analysis. For each SSLP microsatellite marker, a likelihood ratio test was used to test for linkage to a quantitative trait

locus influencing the incidence of cleft palate. The LOD score for each marker was computed by dividing the value for the likelihood ratio test statistic by $2 \ln(10)$. The genome-wide significance level (P value) at each marker within a cross was determined by a permutation test (Churchill and Doerge, 1994) in which 100,000 random permutations of the phenotypes (cleft palate or normal) were performed. For each permutation, the largest likelihood ratio statistic across the set of markers was recorded, and this null distribution was used to determine the genome-wide significance (two-sided) for linkage to each marker (Lystig, 2003). This approach has been used previously by our group (Lee et al., 1997; software is available at <http://www.mcardle.wisc.edu/qlink>).

Results

Linkage Analysis of B6CBAF₂ and CBA×B6CBAF₁ Backcross Progeny. Terata for these experiments were scored as a binary event, and linkage analysis was performed accordingly. That is, the cleft palate and hydronephrosis phenotypes were scored as one of two outcomes, either fused or not fused (Fig. 1, A and B) and hydronephrotic or not hydronephrotic (Fig. 1, C and D). All of the autosomes in the progeny were scanned for quantitative trait loci that influence susceptibility to dioxin-induced cleft palate and hydronephrosis. The genome-wide analysis was performed using the 58 SSLP markers in 96 intercross progeny and 60 SSLP markers in 100 backcross progeny as described under *Materials and Methods*.

Incidence of Dioxin-Induced Cleft Palate. The cleft palate incidence in the progeny exposed to dioxin is presented in Fig. 2A. The incidence of dioxin-induced cleft palate was significantly higher in progeny of the B6 parental strain (97%) compared with the CBA parental strain (8%) ($p < 10^{-6}$). There was also a significantly greater induction of cleft palate in the B6CBAF₁ embryos (44%) compared with the CBAB6F₁ embryos (15%) ($p \leq 0.002$). The progeny from the B6CBAF₁ × CBA backcross exhibited a cleft palate incidence of 72%. The progeny of the B6CBAF₁ intercross had a cleft palate incidence of 70%, which was significantly different from both of the parental strains, B6 (97%) and CBA (8%) ($p < 0.001$). The CBA×B6CBAF₁ backcross gave rise to progeny with a cleft palate incidence of 38%, which was significantly different from the parental strain, CBA (8%; $p < 0.01$),

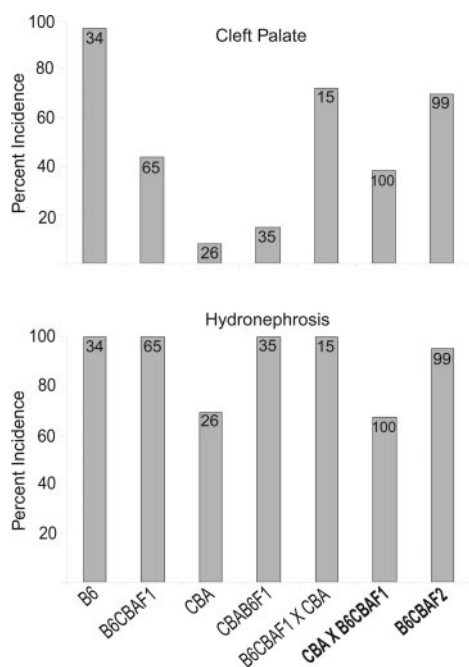


Fig. 2. Incidence of cleft palate and hydronephrosis. Pregnant dams were dosed at E9 with 64 $\mu\text{g/kg}$ dioxin. Embryos were harvested at E17 and assessed for sex, cleft palate and hydronephrosis. The top graph represents the cleft palate incidence found in the progeny (A). The breeding crosses are labeled along the x-axis, and bold-faced font represents the progeny genotyped for the linkage analysis. The bottom graph depicts the hydronephrosis incidence found in the same progeny (B). The numbers located within the bars show the number of progeny assessed at each endpoint.

TABLE 2

LOD scores of the genome-wide linkage analysis of dioxin-induced cleft palate sensitivity

The values indicate the incidence of cleft palate for each genotype; the number of mice of that genotype is shown in parentheses. Position based on data at the Mouse Genome Informatics web site (<http://www.informatics.jax.org>).

DNA Marker	Position	CBA×B6CBAF1 Backcross			B6CBAF1 Intercross				Combined LOD
		Incidence (n)			Incidence (n)				
		CBA/CBA	CBA/B6	LOD	CBA/CBA	CBA/B6	B6/B6	LOD	
	<i>cM</i>								
<i>D3Mit62</i>	4.6	0.27 (45)	0.48 (54)	1.06	0.57 (28)	0.67 (39)	1.0 (23)	4.01 ^a	5.07 ^b
<i>D3Mit6</i>	23.3	0.32 (59)	0.48 (40)	0.51	0.63 (19)	0.76 (53)	0.77 (22)	0.27	0.78
<i>D3Mit11</i>	49.0	0.38 (52)	0.38 (47)	<0.01	0.63 (19)	0.73 (52)	0.79 (24)	0.30	0.30
<i>D3Mit14</i>	64.1	0.43 (47)	0.35 (52)	0.14	0.61 (23)	0.70 (47)	0.88 (24)	1.03	1.17
<i>D3Mit17</i>	71.8	0.42 (50)	0.34 (47)	0.14	0.75 (20)	0.74 (46)	0.59 (17)	0.32	0.46
<i>D3Mit19</i>	87.6	0.44 (54)	0.33 (45)	0.19	0.71 (24)	0.72 (43)	0.80 (20)	0.13	0.32
<i>D12Mit12</i>	6.0	0.51 (47)	0.28 (50)	1.19	0.85 (20)	0.78 (45)	0.48 (25)	1.96	3.15
<i>D12Mit34</i>	29.0	0.50 (50)	0.26 (49)	1.27	0.83 (18)	0.74 (49)	0.63 (27)	0.51	1.78
<i>D12Mit5</i>	37.0	0.48 (46)	0.28 (50)	0.88	0.87 (15)	0.67 (52)	0.83 (27)	0.77	1.65
<i>D12Mit20</i>	58.0	0.36 (56)	0.40 (40)	0.04	0.72 (43)	0.57 (21)	0.80 (25)	0.63	0.67

^a Genome-wide $p < 0.01$ by permutation test.

^b Genome-wide $p < 0.05$ by permutation test.

but not different from B6CBAF₁ (44%). The cleft palate sensitivities of male and female CBA×B6CBAF₁ backcross progeny did not differ significantly and were found to be 21 and 17%, respectively.

Incidence of Dioxin-Induced Hydronephrosis. The hydronephrosis incidences in the progeny exposed to dioxin are presented in Fig. 2B. The incidence of hydronephrosis is nearly 100% for most of the progeny. However, the incidences of hydronephrosis in both CBA and CBA×B6CBAF₁ progeny were significantly less than in B6 progeny (69 and 67%, respectively; $p < 0.001$). The hydronephrosis sensitivity of male versus female progeny of the CBA×B6CBAF₁ backcross was found to be 72% versus 62%, respectively, which was not statistically significant. Given the high incidence of hydronephrosis in the backcross and intercross progeny under these treatment conditions, we were unable to identify loci that influenced this phenotype by linkage analysis in our screen.

Linkage Analysis of Cleft Palate in B6CBAF₁ Intercross and CBA×B6CBAF₁ Backcross Mice. We observed that the incidence of cleft palate in mice exposed to dioxin in utero was highly dependent on the genotype of the mice for the marker *D3Mit62* on proximal chromosome 3 (Table 2). In the intercross, the incidence of cleft palate in mice homozygous for the CBA allele at *D3Mit62* was 57%, whereas those for heterozygous or homozygous B6 mice were 67% and 100%, respectively. The LOD score for *D3Mit62* was 4.01 (genome-wide $P = 0.009$). The same trend was observed in the backcross, with a 27% incidence of cleft palate in the animals homozygous for the CBA allele at *D3Mit62* and a 48% incidence in the heterozygotes. Although significant linkage was not observed for the backcross (LOD = 1.06), combining the results of the two crosses (LOD = 5.07) demonstrated significant linkage between dioxin-induced cleft palate and *D3Mit62* (genome-wide $P = 0.046$, by permutation test). We have designated the locus on proximal chromosome 3 that modulates cleft palate incidence as *Cmt1*, indicating its role in chemically mediated teratogenesis.

The marker *D12Mit12* on proximal chromosome 12 had the second highest LOD score (3.15) for the combined crosses. Although linkage of cleft palate to *D12Mit12* was not statistically significant, this result is of interest in that *D12Mit12* is within 12 cM of *Ahr*. In contrast to *Cmt1*, the CBA allele conferred an increased sensitivity of cleft palate. In the intercross, the incidence of cleft palate in *D12Mit12* homozygous CBA, heterozygous, and homozygous B6 mice was 85, 78, and 48%, respectively. Logistic regression analysis indicated that the effects of *Cmt1* and the chromosome 12 locus were additive.

Discussion

The role of the *Ahr* locus in dioxin-induced teratogenesis has been clearly demonstrated in a number of previous studies. The earliest genetic support for its role came from the demonstration that sensitivity to dioxin-induced cleft palate segregates with the known *Ahr*^b/*Ahr*^d polymorphism in mice (Poland and Glover, 1980). More recent supporting evidence has come from experiments that use mice with induced mutations at *Ahr*. For example, mice harboring a null allele at *Ahr* are resistant to dioxin-induced cleft palate (Mimura et al., 1997), and mice with an induced mutation in exon-2 of the *Ahr* locus are also resistant to dioxin-induced terata

(Bunger et al., 2003). These genetic proofs, along with considerable pharmacological analysis, have provided compelling evidence that the *Ahr* locus is an essential player in mediating the toxic and teratogenic effects of dioxin (Abbott and Birnbaum, 1990; Poland et al., 1994; Jain et al., 1998).

Given the multiple steps in AHR signal transduction and the complexities of mammalian development, we predicted that additional genetic modifiers would exist for dioxin-induced teratogenesis. To gain support for the idea that genes other than the *Ahr* might influence the teratogenic response to dioxin, we first examined the literature and found that there was evidence for unidentified loci that have a significant impact on AHR biology (Poland and Glover, 1980; Robinson et al., 2002). In particular, data from a screen of inbred mouse strains demonstrated significant variation in their sensitivity to dioxin-induced cleft palate. The observation that two *Ahr*^b allele strains, B6 and CBA, displayed significantly different sensitivities led us to use crosses of these inbred strains to identify loci that modify the teratogenic sensitivity to dioxin.

To identify modifier loci, we employed a classic linkage-analysis approach, where polymorphic SSLP microsatellite markers were analyzed for their segregation with sensitivity to dioxin-induced terata in crosses between B6 and CBA strains. To this end, we genotyped the progeny from the B6CBAF₁ intercross and the CBA×B6CBAF₁ backcross and followed cosegregation of the markers with sensitivity to dioxin-induced cleft palate. A backcross analysis is more efficient than an intercross because each backcross animal will have one of only two genotypes at each locus compared with three genotypes in intercross progeny. This allows for the analysis of fewer mice. On the other hand, the intercross is able to map recessive alleles. We also uncovered a potential maternal effect in our initial crosses. That is, there was an increased incidence of cleft palate among F₁ embryos born to B6 dams (44%) compared with those born to CBA dams (15%; Fig. 2A; $P < 0.002$). Therefore, in our case, it was important to employ both strategies to detect genetic modifiers in the progeny that might be masked by the maternal environment.

With respect to phenotype, we scored for cleft palate and hydronephrosis, two well characterized terata that are induced by dioxin exposure. Given that dioxin-induced hydronephrosis occurs at a dose approximately 10-fold lower than the dose that produces cleft palate, the screen was less sensitive for detecting loci influencing dioxin-induced hydronephrosis (Thomae et al., 2004). Although our linkage analysis suggested the potential for numerous modifiers of dioxin-induced hydronephrosis, the relatively low LOD scores led us to focus on modifiers of dioxin-induced cleft palate where our screen was more sensitive. We identified two loci that modify sensitivity to dioxin-induced cleft palate. The most potent modifier maps to chromosome 3 and has been designated *Cmt1*, for "chemically mediated teratogenesis." A weaker modifier maps to chromosome 12 and has been tentatively designated *Cmt2*.

The *Cmt1* Locus and Candidate Genes on Chromosome 3. A number of genes map within the *Cmt1* region: *Fgf*, *Il7*, *Glrh*, *Fgg*, *Tshb*, *Amy1*, *Egf*, *Adh7*, *Car1*, and *Ptger3*. The *Fgf* locus is the closest to *Cmt1* and has been implicated in normal palatogenesis. The *Fgf* locus encodes the fibroblast growth factor which is highly expressed in the developing mammalian palate and has been suggested to play an impor-

tant role in epithelial-mesenchymal interactions that dictate fusion and maturation of the developing palate (Lee et al., 2001). In particular, the fibroblast growth factors have been shown to influence the activity of transforming growth factor β -3 (TGF β 3), a regulator of apoptosis and epithelial-to-mesenchymal transdifferentiation at the medial edge of the developing palatal epithelium (Britto et al., 2002). An interaction between *Fgf* and *Tgf β 3* has been suggested to explain a unique pathological mechanism for the cleft palate phenotype exhibited in human Apert syndrome (Britto et al., 2002). We find the link to the TGF β 3 signaling to be interesting, given our recent observation that dioxin's effects on the medial epithelial edge of the palate are remarkably similar to those observed in mice null for TGF β 3 expression (Thomae et al., 2005).

The *Cmt2* Locus on Chromosome 12. Although the identification of the *Cmt2* locus is only tentative, it is worth noting that it is near the *Ahr* locus. It is also noteworthy that the B6 and CBA mouse strains are polymorphic at *Ahr*, harboring distinct *Ahr*^{b1} and *Ahr*^{b2} alleles, respectively (Poland et al., 1994). Cloning studies have revealed three *b* alleles of the murine *Ahr*, differing by eight point mutations in the common open reading frame and by additional sequences at the carboxyl end (Ema et al., 1994; Poland et al., 1994). The alleles, *Ahr*^{b1} (~95 kDa), *Ahr*^{b2} (~104 kDa), and *Ahr*^{b3} (~105 kDa), encode proteins of slightly different size (Poland et al., 1994). Thus, although the receptors have been reported to bind dioxin with similar affinity, these proteins are of slightly different molecular mass and may display differential expression levels, stability, or transcriptional potency (Ema et al., 1994; Poland and Glover, 1994). Although very preliminary, these data suggest the possibility that subtle differences in *Ahr* alleles could play a role in differential sensitivity to cleft palate.

Comparison with Other Genome-Scans for Clefting Modifiers. Before this work, two genome-wide scans have been performed using mouse models to identify genes and loci that play a role in cleft lip and cleft palate formation. One screen used a spontaneous cleft lip phenotype in the sensitive A/WySnJ and resistant B6 mouse strains (Juriloff et al., 2001). This screen identified two loci on chromosome 13, designated *Clf1* and *Clf2*, that segregated with spontaneous cleft lip susceptibility. A second genome scan used phenytoin and 6-aminonicotinamide to induce cleft lip and cleft palate, respectively, in the AXB and BXA set of recombinant inbred mouse strains (Diehl and Erickson, 1997). This screen identified 16 susceptibility regions. It is noteworthy that the region on chromosome 12 identified in our screen comaps to a region identified in a screen for modifier loci of 6-aminonicotinamide induced cleft palate. This result may implicate the locus on chromosome 12 as an important modifier of sensitivity to a broad spectrum of teratogenic chemicals.

Conclusion

Mammalian development is a complicated process that can be disrupted by both genetic aberrations and chemical insults. The loci that we have identified on chromosomes 3 and 12 modify the sensitivity to dioxin-induced cleft palate and contain several interesting gene candidates. Plans to characterize these loci include fine mapping to identify the gene(s) responsible. The identification of a gene conclusively linked

to teratogen-induced cleft palate sensitivity could add to the understanding in the field of birth defects for which there are few identified causative genes and many unanswered questions.

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