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

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Abbreviations: dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; SSLP, simple sequence length polymorphism

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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 μg/kg of 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 B6CBAF_1 intercross and a CBAxB6CBAF_1 backcross were performed. The incidence 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.

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The term "gene-environment interaction" is used to describe the altered risk of disease resulting from the interplay between an individual's genotype and their 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 individuals at higher risk of 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 (Anderson et al., 1993; Poland and Glover, 1973; Poland and Knutson, 1982), cancer (Becher and Flesch-Janys, 1998; Pitot et al., 1980) and terata in experimental animals (Abbott and Birnbaum, 1991; Couture et al., 1990; 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; Dolwick et al., 1993; Harper et al., 1992). In the mouse, the *Ahr* locus exists as four alleles, denoted Ahr^{b1}, Ahr^{b2}, Ahr^{b3} and Ahr^{d}. The

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"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, while 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 mouse strain was relatively resistant to dioxin-induced cleft palate, as compared to other strains that also harbored *Ahr b*-alleles (Poland and Glover, 1980). In this regard, exposure of pregnant CBA dams to 30 μg/kg of dioxin did not induce cleft palate in any of 61 scored offspring. In contrast, other *Ahr b*-allele strains, C57BL6/J 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 B6 and CBA 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).

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MATERIALS AND METHODS

Mice and terata induction: The C57BL/6J (B6) and CBA/J (CBA) inbred mouse strains were purchased from the Jackson Laboratory Mice (Bar Harbor, ME). Mice were housed, bred and maintained in microisolator cages in a specific-pathogen-free facility on corncob bedding (Bed O'Cobs, Maumee, OH) at the McArdle Laboratory for Cancer Research. Animals were provided Purina 5020 diet (9% fat; St. Louis, MO) and water *ad libitum.* Embryos were generated by placing female mice with male mice for 12 hours. Embryonic day 1 (E1) was designated as the day females were separated from males. At E9, females that gained at least 2 grams since cohabitation with males were assumed to be pregnant and injected with a single intraperitoneal dose of 64 μg/kg of dioxin dissolved in dimethylsulfoxide.

Assessment of terata: At E17 the dam was sacrificed by cervical dislocation, and the uterus was dissected from the dam and placed into cold phosphate-buffered saline. A three 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 (Figure 1A). Palatal shelves that failed to fuse were recorded as "cleft palate" (Figure 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 (Figure 1C). If such swelling was observed, it was scored as hydronephrosis (Figure 1D). The sex of each embryo was scored according to its internal anatomy (Figure 1C,D). Comparisons of the incidence of terata between groups were made using the Fisher's exact test.

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Genotype analysis: The DNA was isolated from the tail of the embryo via standard proteinase K-phenol extraction protocol (Sambrook, 1989). The polymerase chain reaction (PCR) was used to amplify informative SSLP microsatellites in the embryo DNA (Bilger et al., 2004). Ninety-six $B6CBAF_2$ embryos from a $B6CBAF_1$ (the dam is listed first and the sire second) intercross were scored and genotyped at 58 SSLP loci, while 100 embryos from a CBA×$B6CBAF_1$ 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 minutes. After denaturation, 30 cycles were performed at 95°C for 1 minute, 54°to C for 45 seconds, and 72°C for 1 minute. A final extension step was carried out at 72°C for 5 minutes. 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 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).

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RESULTS

Linkage analysis of B6CBAF$_2$ and CBAxB6CBAF$_1$ 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 (Figure 1A and 1B) and hydronephrotic or not hydronephrotic (Figure 1C and 1D). 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 in the materials and methods section.

Incidence of dioxin-induced cleft palate: The cleft palate incidence in the progeny exposed to dioxin is presented in Figure 2A. The incidence of dioxin-induced cleft palate was significantly higher in progeny of the B6 parental strain as compared to the CBA parental strain, 97% and 8% respectively ($p < 10^{-6}$). There was also a significantly greater induction of cleft palate in the B6CBAF$_1$ embryos as compared to the CBAB6F$_1$ embryos, 44% and 15%, respectively ($p \leq 0.002$). The progeny from the B6CBAF$_1$×CBA backcross exhibited cleft palate incidence of 72%. The progeny of the B6CBAF$_1$ 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×B6CBAF$_1$ backcross gave rise to progeny with a cleft palate incidence of 38% that was significantly different from the parental strain, CBA (8%, $p < 0.01$), but not different than B6CBAF$_1$ (44%). The cleft palate sensitivities of male and female CBA×B6CBAF$_1$ backcross progeny did not differ significantly and were found to be 21% and 17%, respectively.

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Incidence of dioxin-induced hydronephrosis: The hydronephrosis incidences in the progeny exposed to dioxin are presented in Figure 2B. The incidence of hydronephrosis is nearly 100% for most of the progeny. However, the incidences of hydronephrosis in both CBA and CBA x $B6CBAF_1$ progeny were significantly less than B6 progeny, 69% and 67% respectively ($p < 0.001$). The hydronephrosis sensitivity of male versus female progeny of the CBA×$B6CBAF_1$ 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 $B6CBAF_1$ intercross and CBA×$B6CBAF_1$ 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%, while those for heterozygous or homozygous B6 mice were 67% and 100%, respectively. The LOD score for *D3Mit62* was 4.01 (genome-wide *P*-value = 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*.

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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 were 85%, 78%, and 48%, respectively. Logistic regression analysis indicated that the effects of *Cmt1* and the Chromosome 12 locus were additive.

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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 recently, 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 has 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.

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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 B6CBAF_1 intercross and the CBAxB6CBAF_1 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, in comparison 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 F_1 embryos born to B6 dams (44%) compared to those born to CBA dams (15%; Figure 2A; $P<0.002$). Therefore in our case it was important that we employed both strategies in order to detect genetic modifiers in the progeny that may be masked by the maternal environment.

With respect to phenotype, we scored for two well-characterized terata that are induced by dioxin exposure, cleft palate and hydronephrosis. Given that dioxin-induced hydronephrosis occurs at a dose about ten-fold lower than 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 as *Cmt2*.

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The *Cmt1* locus and candidate genes on chromosome 3: A number of genes map within the *Cmt1* region: *Fgf, Il7, Glrb, 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 (FGF), which is highly expressed in the developing mammalian palate and has been suggested to play an important role in epithelial-mesenchymal interactions that dictate fusion and maturation of the developing palate (Lee et al., 2001). Specifically, the FGFs have been shown to influence the activity of transforming growth factor beta-3 (TGFβ3), 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 *Tgfβ3* 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 TGFβ3 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 TGFβ3 expression (Thomae et al., 2005).

The Cmt 2 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. Interestingly, the B6 and CBA mouse strains are polymorphic at *Ahr*, harboring distinct $Ahr^{b\text{-}1}$ and $Ahr^{b\text{-}2}$ 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, $Ahr^{b\text{-}1}$ (~95 kDa), $Ahr^{b\text{-}2}$ (~104 kDa) and $Ahr^{b\text{-}3}$ (~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

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potency (Ema M, 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. Prior to 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 utilized 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 (6-AN) 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 sixteen susceptibility regions. Interestingly, the region on Chromosome 12 identified in our screen co-maps to a region identified in a screen for modifier loci of 6-AN 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

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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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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Pathology of dioxin-induced terata. Difference in palatogenesis and urogenesis of embryos exposed to 64µg/kg of dioxin via maternal injection at E9. At E17 embryos were dissected from the dam and assessed for normal palate formation (1A), and normal urogenital tract formation (1C; female). Figure 1B is a representation of cleft palate exhibited by an embryo exposed to dioxin. The arrows in 1B 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. Figure 1D (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.

Figure 2. Incidence of cleft palate and hydronephrosis. Pregnant dams were dosed at E9 with 64µg/kg of 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 (2A). The breeding crosses 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 (2B). The numbers located within the bars show the number of progeny assessed at each endpoint.

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Table 1. Simple sequence length polymorphism (SSLP) microsatellite markers used in linkage analysis of the progeny from a B6CBAF_1 intercross and a CBA×B6CBAF_1 backcross Located to the right of each marker are the annealing temperatures that were used in the amplification protocol. The (*) denotes the two additional markers that were used to screen the CBA×B6CBAF_1 progeny. 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	D1Mit3	58	D1Mit5	60	D1Mit30	46	D1Mit33	59	D1Mit113	62	D1Mit150*	54				
2	D2Mit1	59	D2Mit48	58	D2Mit49	59	D2Mit62	59								
3	D3Mit6	59	D3Mit11	59	D3Mit14	58	D3Mit17	59	D3Mit19	58	D3Mit62	58	D3Mit203*	54		
4	D4Mit9	58	D4Mit16	53	D4Mit33	64										
5	D5Nds2	56														
6	D6Mit1	56	D6Mit9	59	D6Mit10	58	D6Mit15	65	D6Mit17	59	D6Mit25	60				
7	D7Nds4	60														
8	D8Mit3	58														
9	D9Mit2	58	D9Mit10	58												
10	D10Mit3	58	D10Mit10	55	D10Mit31	62	D10Mit67	56								
11	D11Nds1	49	D11Mit23	59	D11Mit19	58										
12	D12Mit5	72	D12Mit12	55	D12Mit20	64	D12Mit34	65								
13	D13Mit3	58	D13Mit13	58	D13Mit30	62										
14	D14Mit7	55	D14Mit14	56	D14Mit28	65	D14Mit62	59								
15	D15Mit3	58														
16	D16Mit30	58														
17	D17Mit16	58	D17Mit34	52	D17Mit38	65	D17Mit60	53	D17Mit70	62						
18	D18Mit4	58														
19	D19Mit10	62	D19Mit13	58	D19Mit31	55	D19Mit33	56								

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Table 2. LOD scores of the genome-wide linkage analysis of dioxin-induced cleft palate sensitivity

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

[a]CBA×B6CBAF$_1$ Backcross; B6CBAF$_1$ Intercross.

[b]The values in the table indicate the incidence of cleft palate for each genotype with the number of mice of that genotype in parentheses.

[c]Position based on data at the Mouse Genome Informatics web site (http://www.informatics.jax.org/).

[d]Genome-wide *P*-value, $p < 0.01$, by permutation test.

[e]Genome-wide *P*-value, $p < 0.05$, by permutation test.

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Figure 1

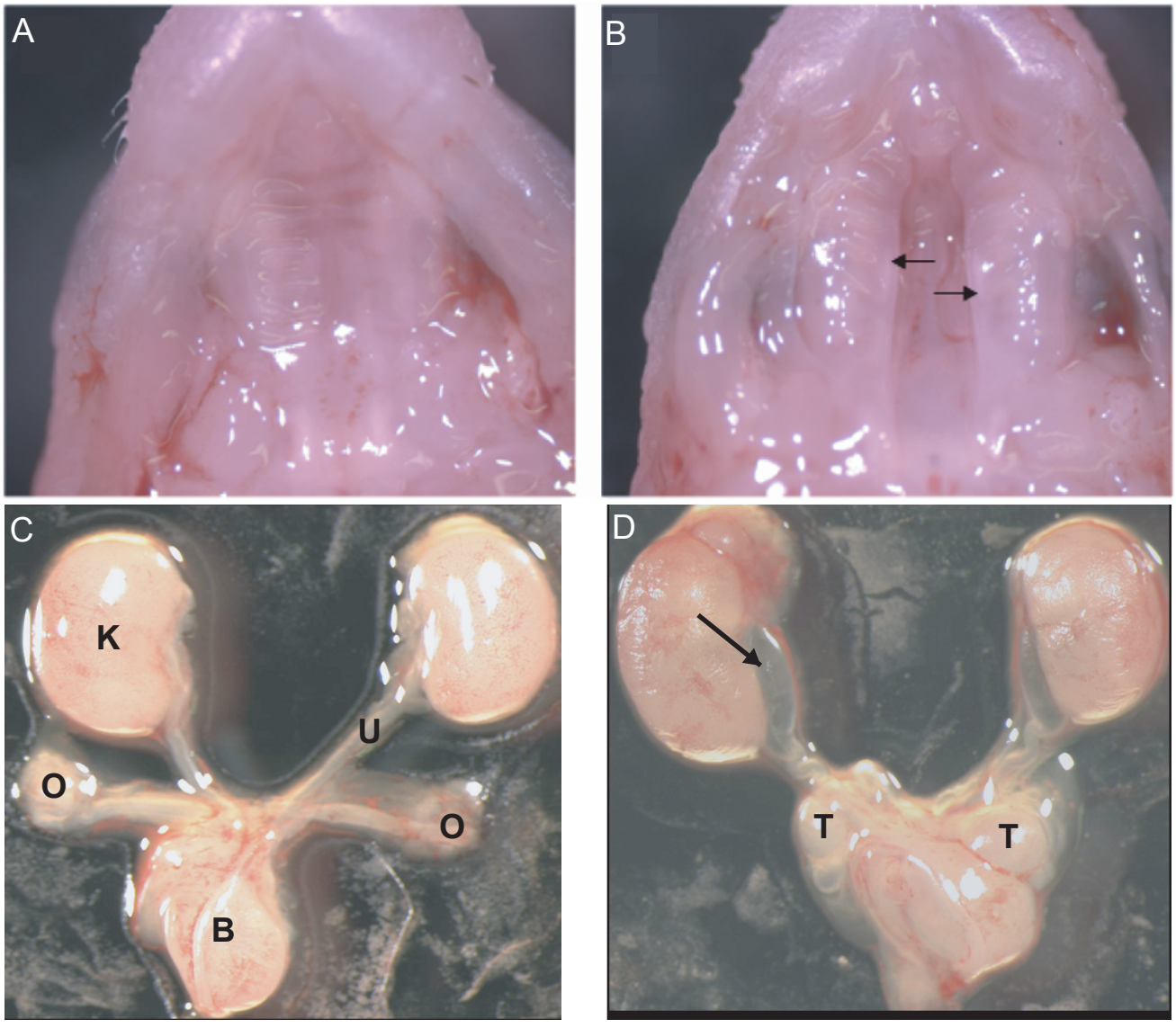

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Figure 2