Genet. Res., Camb. (1987), **49**, *pp.* 43–49 *Printed in Great Britain*

Susceptibility to phenytoin-induced cleft lip with or without cleft palate: many genes are involved

I. JILL KAROLYI, SHARON LIU AND ROBERT P. ERICKSON*

Departments of Human Genetics and Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, Michigan 48109–0618

(*Received 28 July 1986 and in revised form 16 September 1986*)

Summary

In a search for genetic differences in susceptibility to cleft lip with or without cleft palate [CL(P)], congenic and recombinant inbred strains of mice were treated with phenytoin or control injections. Of six loci tested, five were found to affect susceptibility to phenytoin-induced and/or sporadic CL(P): (1) the major histocompatibility locus, *H*-2; (2) the locus controlling β_2-microglobulin, *B2m*; (3) a locus controlling β-glucuronidase, *Gus*; (4) the locus controlling *N*-acetyl transferase, *Nat*; and (5) the locus for brown pigmentation, *b*. *B2m* and *Gus* only affected the sporadic incidence of CL(P), while the *b* locus only affected phenytoin-induced incidence of CL(P). Three of these loci are also known to affect glucocorticoid-induced isolated cleft palate (CP), but different alleles of the loci are involved. Phenytoin did not affect levels of adenosine 3′,5′-cyclic monophosphate (cAMP) in palates and tongues of day 15 fetuses. A comparison of glucocorticoid receptor parameters with the incidence of phenytoin-induced CL(P) found no correlation.

1. Introduction

Controversies over the role of phenytoin (sodium 5,5-diphenyl-2,4-imidazolidinedione) in human birth defects may be resolvable if there are genetic differences among humans in susceptibility to the teratogenicity of this drug. The fetal hydantoin syndrome (Barr, Poznanski & Schmickel, 1974; Hanson & Smith, 1975; Hanson *et al.* 1976; Monson *et al.* 1973; Shapiro *et al.* 1976) only occurs in a small number of foetuses who are exposed to the drug. Genetically determined differences in metabolism of phenytoin may explain such differences in susceptibility (Strickler *et al.* 1985). Genetic differences in the teratogenic actions of phenytoin are well recognized in mice (Massey, 1966; Sulik *et al.* 1980). The differences in susceptibility between the A/J and C57BL/6J strains have been related to differences at histocompatibility loci (Goldman *et al.* 1982; Goldman, Baker & Gasser, 1983) but not to differences in genes which might affect phenytoin metabolism (Atlas, Zweier & Nebert, 1980). Evidence for an effect of phenytoin on glucocorticoid receptor-influenced prostaglandin levels has been used to suggest that the teratogenic effects of phenytoin and glucocorticoids are interrelated metabolically (Katsumata *et al.* 1982).

Recombinant inbred (RI) strains of mice provide a powerful tool to test such hypotheses. These strains, with their strain distribution patterns of genes marking chromosomal regions, are valuable for identifying genetic associations (Bailey, 1971; Swank & Bailey, 1973; Taylor, 1978). Since the RI strains are homozygous at all loci, large numbers of identical mice are available for testing.

We investigated the effect of six loci on the incidence of CL(P) with and without phenytoin treatment in a highly susceptible strain, A/J; a highly resistant strain, C57BL/6J; relevant *H*-2 congenic strains (strains C57BL/6 and C57BL/10 are related but not genetically identical), and nine recombinant inbred strains. These strains have been typed for the six genetic markers we selected. In phenytoin-treated mice we found a significant effect of the *H*-2 region, but with increased CL(P) associated with the low susceptibility strain's *H*-2 allele. We also found highly significant effects of the chromosome region controlling *Nat* and a locus (i) near *b* on chromosome 4. In non-treated mice we found small effects of two of these three chromosomal regions and, in addition, similar small effects of the *B2m* locus and the *Gus* locus on CL(P). Since an effect of phenytoin on prostaglandin levels was claimed, and since prostaglandin levels affect cAMP levels, we also studied palatal shelf and tongue cAMP levels in phenytoin-treated and control foetuses at the time of palatal rotation. No effect of *H*-2 on cAMP levels in phenytoin-treated palatal shelves was

* To whom correspondence should be addressed.

https://doi.org/10.1017/S0016672300026719 Published online by Cambridge University Press

seen but a small *H*-2–background interaction effect was found. We looked for correlations between incidence of phenytoin-induced CL(P) and glucocorticoid receptor parameters but found none.

2. Materials and Methods

(i) *Mouse maintenance and drug administration*

A/J, C57BL/6J, A.BY/SnJ and B10.A mice were purchased from the Jackson Laboratory. Recombinant inbred strains of A/J and C57BL/6J were supplied by Dr Muriel Nesbitt at the University of California (Nesbitt & Skamene, 1984). The mice were then bred and maintained in our mouse colony. Timed females of the parental strains, A/J (A background, *H-2* a) and C57BL/6J (B background, *H-2* b); related congenic strains, A.BY/SnJ (A background, *H-2* b) and B10.A (B background, *H-2* a); and the recombinant inbred strains were injected intraperitoneally with phenytoin (Dilantin®; Parke Davis, Detroit, MI) on day 10 of pregnancy. The dose was 60 mg per kg. Control injections of 40% propylene glycol in 10% ethanol (the drug solvent) were administered in a similar manner. The foetuses were examined on day 17 and were scored for cleft lip and/or cleft palate.

(ii) *Palatal shelf and tongue cAMP determinations*

Timed females were dissected on day 15 of pregnancy after phenytoin or control injections as described previously. The accumulated pairs of palatal shelves and tongues from pups were placed separately into 200 μl of boiling water for 10 min. The samples were cooled on ice, spun at 1300 *g* at 4 °C and the supernatant removed. The pairs of palates and the tongue were homogenized in 1·0 ml 1 N-NaOH prior to protein determination (Lowry *et al.* 1951). Fifty microlitre aliquots of the supernatant were assayed for cAMP by radioimmunoassay (Frendsen & Krishna, 1977) using rabbit antibody to cyclic AMP-BSA serum from Miles-Yeda, Ltd and adenosine 3′,5′-cyclic phosphoric acid, [^{125}I-2′-0]succinyl (iodotyrosine methyl ester) from New England Nuclear as a ligand. The samples were assayed in duplicate, along with standards, and the results were calculated by a specifically designed computer program (Duddleson, Midgley & Niswender, 1972).

(iii) *Statistical analyses*

The nonparametric Fisher's exact probability test statistic (Siegel, 1956; Dixon & Massey, 1969) was chosen for the incidence data, because it does not presuppose a large sample in contrast to the chi-squared test. For the cAMP data, a natural log transformation was used to reduce the positive skew in the cAMP measurements (pmol cAMP/mg protein) and to correct inequalities of variance. Analysis of variance procedures were then employed on this log transformation. Scheffe's method for computation of multiple contrasts (Bancroft, 1968) was used to obtain tests of hypotheses concerning the effects of strain and injection. Separate analyses were carried out on the ln cAMP levels determined in palates and tongues.

3. Results

Table 1 summarizes the CL(P) response to phenytoin in the mouse strains studied. The percentage of phenytoin-induced CL(P) reported is the percentage after sporadic levels were subtracted, since a moderately high sporadic percentage of CL(P) occurred in the A/J strain, the A.BY/SnJ strain and the AxB6 strain. The sporadic levels of CL(P) presented are combined results from females who had either been injected with this drug solvent, had received no injection, or had been injected intramuscularly with PBS (phosphate-buffered saline) or intraperitoneally with distilled water (two controls used for other teratogens), since there is no significant difference between these four controls ($P = 1·0$) (Liu & Erickson, 1986*a*). Some 56% of the variability in phenytoin-induced CL(P) is explained by variability in sporadic levels of CL(P) ($P = 0·003$), suggesting that some genetic factors influence both of these variables.

The highly susceptible parental A/J strain showed a 53·6% phenytoin-induced CL(P) incidence. The congenic strain which shares the A background (A.BY/SnJ) showed a 43·5% incidence of phenytoin-induced CL(P). Three of the RI strains also showed a somewhat lesser incidence of phenytoin-induced CL(P). These are AXB2, AXB6 and AXB13. The low-susceptibility parental C57BL/6J strain showed an incidence less than 2% of phenytoin-induced CL(P). A similarly low level of incidence was found also in a related congenic line sharing the B background (B10.A), and in 6 of the RI lines.

Table 2 indicates the association of six genetic markers with the incidence of CL(P), and Table 3 shows the distribution of these markers in the strains studied. Alleles a and b refer to the A/J allele (a) or C57BL/6J allele (b). These genetic markers identify chromosomal regions and not only the named locus. *H-2* (the major histocompatibility locus) has previously been implicated as a causal factor in phenytoin-induced CL(P) (Monson *et al.* 1973); *B2m* (β_2 microglobulin) is closely linked to *H*-3 (histocompatibility 3) which has been implicated in the etiology of phenytoin-induced CL(P) (Goldman *et al.* 1982; Goldman, Baker & Gasser, 1983); *Gus* (β-glucuronidase) marks chromosome 5 for which a locus has been implicated in the genesis of glucocorticoid-induced isolated cleft palate (CP) (Vekemans, Taylor & Fraser, 1981); *Nat* (N-acetyl transferase) is not known to affect phenytoin metabolism but is a marker for a gene (or is the gene) strongly influencing susceptibility to glucocorticoid-induced CP (unpublished data); *b* (brown pigementation) has been suggested as one component of susceptibility to 6-aminonicotinamide-

https://doi.org/10.1017/S0016672300026719 Published online by Cambridge University Press

Table 1. *Cleft lip (palate) response in parental, congenic and RI strains*

Strain	Phenytoin-induced CL(P)			Sporadic CL(P)		
	% CL(P) ±S.E.	No. of litters	No. of foetuses	% CL(P) ±S.E.	No. of litters	No. of foetuses
A/J	53·6±10	16	123	5·5±2	66	449
C57BL/6J	1·3±1	22	142	0	39	301
A.BY/SnJ	43·5±8	17	114	17·8±2	73	431
B10.A	0	18	142	0	57	457
AXB2	20·4±6	28	108	0	41	202
AXB5	6·5±4	9	51	0	12	51
AXB6	26·3±7	20	105	11·1±4	27	162
AXB7	3·4±3	17	99	0	12	74
AXB13	14·3±6	9	56	0	16	103
AXB15	0	15	121	0	23	183
AXB17	2·7±3	25	109	0	40	204
BXA8	0	8	57	0	12	93
BXA14	1·4±1	18	130	0	49	366

Table 2. *Phenytoin-induced and sporadic cleft lip (palate) association with a and b alleles of H-2, B2m, Gus, Nat, b and Hlt*

Gene	Fisher's exact probability test statistic		
	Small litters	Large litters	Gene effect
Phenytoin-induced CL(P)			
H-2 (Chromosome 17)	0·06	0·0007	*H-2* b↑
B2m (Chromosome 2)	0·29	0·36	—
Gus (Chromosome 5)	0·007	0·19	—
Nat (Chromosome 8)	0·05	0·0000	*Nat* b↑
b (Chromosome 4)	0·03	0·0002	*b* a↑
Hlt (Chromosome 13)	0·05	0·13	—
Sporadic CL(P)			
H-2	0·004	0·004	*H-2* b↑
B2m	0·10	0·006	*B2m* a↑
Gus	0·22	0·003	*Gus* a↑
Nat	0·50	0·005	*Nat* b↑
b	0·54	0·01	—
Hlt	0·41	0·02	—

Table 3. *Genotypes of lines studied**

Strain	*H-2* allele	*B2m* allele	*Gus* allele	*Nat* allele	*b* allele	*Hlt* allele
A/J	a	a	a	a	a	a
C57BL/6J	b	b	b	b	b	b
A.BY/SnJ	b	a	a	a	a	a
B10.A	a	b	b	b	b	b
AXB2	a	b	a	b	a	b
AXB5	a	a	a	b	a	b
AXB6	b	a	a	b	a	b
AXB7	a	b	b	a	b	N.A.
AXB13	b	b	a	b	a	a
AXB15	a	a	N.A.†	a	b	a
AXB17	a	a	a	b	a	b
BXA8	a	b	N.A.	a	b	b
BXA14	b	b	b	a	b	a

* From Liu and Erickson (1986*a*).
† N.A. = not available.

https://doi.org/10.1017/S0016672300026719 Published online by Cambridge University Press

induced CP (Biddle, 1977), and *H1t* (histone-1 testes) was included as a control marker.

Statistical analyses reported here of the relationship between CL(P) with and without phenytoin treatment and these six genetic markers were carried out on the RI lines only. Litter size had a strong effect on the incidence data, so litters were classified as either small (1–5 foetuses) or large (6–12 foetuses). The results indicate that three of the markers (*H-2* b allele, *Nat* b allele and *b* a allele) significantly increase phenytoin-induced CL(P) when litter size is large. The small *Gus* effect seen in small litters does not hold up when measured in large litters. Four markers slightly increase sporadic levels of CL(P) in large litters (*H-2* b allele, *B2m* a allele, *Gus* a allele and *Nat* b allele) while the *H-2* effect is evident in small litters also. Since 6 statistical tests were performed on each data set, the significance level for an individual contrast must be $< 0{\cdot}008$ ($0{\cdot}05/6$) to achieve an experiment error rate of $< 0{\cdot}05$ (Bancroft, 1968).

The cAMP measurements were performed on the parental strains and congenic strains. The ln cAMP means of palatal shelves and tongues are presented in Table 4. In palates the comparisons of data from phenytoin-injected mice with data from control injected mice shows no significant correlation. Similarly, no correlation is found in *H-2* contrasts or in background contrasts. (Background reflects incidence data as A background showed high incidence and B background showed low incidence). However, the two-way analysis of the effects of background and *H-2* interaction in palates does show a significant effect on the cAMP level ($P = 0{\cdot}003$). cAMP levels in tongues were measured primarily to serve as a control in the study of cAMP in the palatal shelf. No significant correlation was found in injection, *H-2* or background contrasts or in any of the interaction contrasts in tongues.

Table 5 shows the association of phenytoin-induced CL(P) with (*a*) glucocorticoid receptor binding

Table 4. *Levels of cAMP on palatal shelves and tongues*

Strain	Genetic background	*H-2* allele	ln cAMP (pmol/mg protein) Phenytoin injected (no. of animals) mean ± S.E.	ln cAMP (pmol/mg protein) Control injected (no. of animals) mean ± S.E.
Palatal shelves				
A/J	A	a	(11) 3·65 ± 0·15	(12) 3·26 ± 0·13
C57BL/6J	B	b	(13) 3·66 ± 0·14	(12) 3·66 ± 0·14
A.BY/SnJ	A	b	(10) 3·18 ± 0·10	(12) 3·37 ± 0·11
B10.A	B	a	(12) 3·35 ± 0·11	(12) 3·24 ± 0·10
Tongues				
A/J	A	a	(11) 3·28 ± 0·15	(12) 3·32 ± 0·13
C57BL/6J	B	b	(13) 3·22 ± 0·14	(11) 3·56 ± 0·14
A.BY/SnJ	A	b	(12) 3·17 ± 0·09	(11) 3·61 ± 0·14
B10.A	B	a	(11) 3·08 ± 0·10	(9) 3·11 ± 0·06

Table 5. *Relationship of phenytoin-induced CL(P) to glucocorticoid receptor binding measurements**

Strain	Phenytoin induced, % CL(P)	pmol/mg cytosol protein: In Hepes buffer	pmol/mg cytosol protein: In Hepes buffer and DTT	Ratio with DTT: without DTT
A/J	53·6	242	382	1·58
C57BL/6J	1·3	153	212	1·39
AXB2	20·4	176	217	1·23
AXB5	6·5	79	186	2·36
AXB6	23·6	201	199	0·99
AXB7	3·4	413	421	1·02
AXB13	14·3	285	274	0·96
AXB15	0	265	286	1·08
AXB17	2·7	194	227	1·17
BXA8	0	157	157	1·00
BXA14	1·4	52	141	2·69

* From Liu and Erickson (1986*b*).

https://doi.org/10.1017/S0016672300026719 Published online by Cambridge University Press

capacity in Hepes buffer; (*b*) glucocorticoid receptor binding capacity in the presence of DTT (dithiothreitol) and (*c*) the ratio of binding in DTT to binding without DTT. The ratio of binding with DTT to that without DTT expresses the substantial increase in binding seen in the A/J strain compared to the C57BL 6J strain ($P = 0{\cdot}05$) (Liu & Erickson, 1986*b*). None of these three glucocorticoid receptor variables was found to have a significant association with the incidence of phenytoin-induced CL(P). Using least-squares regression, glucocorticoid receptor-binding capacity in the presence of DTT explained only 21% of the variance in the incidence data. The other measures showed no correlation with the incidence of phenytoin-induced CL(P) (glucocorticoid receptor activity in Hepes without DTT $R^2 = 0{\cdot}03$ and ratio: $R^2 = 0{\cdot}00$).

4. Discussion

The *H-2* effect seen in phenytoin-induced and sporadic CL(P) is an intriguing finding (Table 2). The major histocompatibility locus' role in human disease is usually explained in terms of altered immune response, but there is no evidence for a role of the immune system in the etiology of cleft lip and/or cleft palate. However, the major histocompatibility locus also affects a variety of physiological responses, in mice (Meruelo & Edidin, 1975) and in man (Erickson *et al.* 1985). Goldman and collaborators have argued that phenytoin has a shared *H-2* effect with glucocorticoids because it binds to the glucocorticoid receptor (Katsumata *et al.* 1982). This seems an unlikely explanation, since glucocorticoids induce CP and phenytoin induces CL(P) in susceptible strains. In addition, in the congenic strains we find no *H-2* association with phenytoin-induced CL(P) ($P = 0{\cdot}46$) while the *H-2*b allele is associated with a higher incidence of sporadic CL(P) ($P = 0{\cdot}0000$), but this may be due to substrain differences and not to *H-2* variation (Erickson, Butley & Sing, 1979). In RI lines we find the *H-2*b allele associated with a higher incidence of phenytoin-induced CL(P). CP induced by glucocorticoids is associated with the high-susceptibility strains *H-2*a allele in congenics (Erickson, Butley & Sing, 1979) but not in RI lines (Liu & Erickson, 1986a). Thus the degree and cause of *H-2* effects on teratogen-induced CL(P) remain enigmatic. The possible association of alleles at the major histocompatibility locus with sporadic CL(P) was an unexpected outcome of this study. Several, albeit small, studies of HLA and human CL(P) have not found an association (Bonner *et al.* 1978; Rapaport *et al.* 1979; VanDyke *et al.* 1980).

Given the association of *H-2* with phenytoin-induced and sporadic CL(P), there is a ready hypothesis for the smaller association of *B2m* with the susceptibility in control animals. β_2-microglobulin associates with class I antigens on the plasma membrane and they cannot be expressed without it. Thus a particular *B2m* allele (in this case the A/J strain a allele) may interact with a particular *H-2* allele to heighten susceptibility. Alternatively, it may not be the *B2m* locus itself but some linked locus on chromosome 2 of the mouse. As mentioned, *H-3* has been used as a marker for this portion of chromosome 2 (Goldman *et al.* 1982; Goldman, Baker & Grasser, 1983). However, *H-3* and *B2m* have not been separated by crossing-over and may be alternative expressions of the same locus. In addition, we only find an association with sporadic, and not with phenytoin-induced CL(P). In a related study we had found an increase in glucocorticoid-induced CP associated with the b allele of *B2m* (Liu & Erickson, 1986*a*).

The small association of the β-glucuronidase structural locus, *Gus*a allele, with susceptibility to sporadic CL(P) was also unexpected. The marker was chosen because it is on chromosome 5 and phosphoglucomutase-1, which maps 25 cM from *Gus*, had segregated with a component of susceptibility to glucocorticoid-induced CP in another set of RI lines (Vekemans, Taylor & Fraser, 1981). We had found an association of *Gus*b allele with susceptibility to glucocorticoid-induced CP in the RI strains used in this study (Liu & Erickson, 1986a), but do not find an association with phenytoin-induced CL(P). The a and b alleles are electrophoretic alleles which determine enzymes of similar activity (Paigen *et al.* 1975).

It was also of interest that another allele (or chromosomal region) which increases susceptibility to glucocorticoid-induced CP, the *Nat*b allele (unpublished results), influenced phenytoin-induced and sporadic CL(P). However, here we find the *Nat*a allele increasing susceptibility. In general, human families which are at increased risk for CP are not at increased risk for CL(P) (WHO Scientific Group, 1970; Ching & Chung, 1974), although there are dissenting voices (Chabora & Horowitz, 1974; Bonaiti *et al.* 1982). In the set of RI lines studied, we then find a correlation between three loci (or chromosomal regions) that influence glucocorticoid-induced CP and phenytoin-induced and/or sporadic incidence of CL(P). However, in all three cases it is a different allele that is associated with CP in the one case and with CL(P) in the other case. The genetic separateness of the two birth defects is reflected in the association of different alleles with each, even though the loci involved are partially shared.

The *b* (brown) locus provides another example of a single genetic region affecting teratogen-induced CP or CL(P). We searched for possible relationships of the *b* locus with CL(P) because of its previously reported association with 6-aminonicotinamide-induced CP (Biddle, 1977). We found a very strong association with phenytoin-induced CL(P).

cAMP levels were studed with and without phenytoin treatment because altered levels of cAMP

https://doi.org/10.1017/S0016672300026719 Published online by Cambridge University Press

have been implicated in the etiology of glucocorticoid-induced CP (Pratt *et al.* 1980) and because *H-2* affects cAMP levels in a variety of tissues (Meruelo & Edidin, 1975; Erickson *et al.* 1985; Erickson *et al.* 1979). However, no evidence was obtained that phenytoin treatment perturbs palatal or tongue cAMP levels (Table 3). This is in contrast to the effect of glucocorticoids, which altered palatal shelf concentrations of cAMP, interacting with both *H*-2 and other genes in doing so (Erickson *et al.* 1979). Again, it seems unlikely that the effects of phenytoin on the incidence of CL(P) can be explained by the interaction of this drug with glucocorticoid receptors. We looked at the association of phenytoin-induced CL(P) with glucocorticoid-receptor binding parameters because phenytoin has been reported to bind to the glucocorticoid receptor (Katsumata *et al.* 1982), but no significant correlation was found.

Although studies on susceptibility to foetal hydantoin syndrome have mentioned the possible relevance of human variation in phenytoin metabolism, our results indicate that other genes may be important. To our knowledge, the human equivalent of *H-2*, HLA, has not yet been studied in children expressing the foetal hydantoin syndrome. We believe that studies of HLA, and other genetic markers for which we have found positive associations in mice, are warranted in man.

This work was supported by NIH grant DE 05322. We wish to thank Dr Muriel Nesbitt for the breeding stocks of the recombinant inbred lines, Dr William Erickson for statistical advice, and Grace Sundling, Mary Kellogg and Ann Mogan for secretarial assistance.

References

Atlas, S. A., Zweier, J. L. & Nebert, D. W. (1980). Genetic differences in phenytoin pharmacokinetics: *in vivo* clearance and *in vitro* metabolism among inbred strains of mice. *Developmental Pharmacology and Therapeutics* **1**, 281–304.

Bailey, D. W. (1971). Recombinant-inbred strains: an aid to finding identify, linkage, and function of histocompatibility and other genes. *Transplantation* **11**, 325–327.

Bancroft, T. A. (1968). *Topics in Intermediate Statistical Methods*, vol. 1. Ames, Iowa: Iowa State University Press.

Barr, M., Jr. Poznanski, A. K. & Schmickel, R. D. (1974). Digital hypoplasia and anticonvulsants during gestation: a teratogenic syndrome? *Journal of Pediatrics* **84**, 254–256.

Biddle, F. G. (1977). 6-Aminonicotinamide-induced cleft palate in the mouse: the nature of the difference between the A/J and C57BL/6J strains in frequency of response and its genetic basis. *Teratology* **16**, 301–312.

Bonaiti, C., Briard, M. L., Feingold, J., Pavy, B., Psaume, J., Migne-Tufferaud, G. & Kaplan, J. (1982). An epidemiological and genetic study of facial clefting in France. I. Epidemiology and frequency in relatives. *Journal of Medical Genetics* **19**, 8–15.

Bonner, J. J., Terasaki, P. I., Thompson, P., Holve, L. M., Wilson, L., Ebbin, A. J. & Slavkin, H. C. (1978). HLA phenotype frequencies in individuals with cleft lip and/or cleft palate. *Tissue Antigens* **12**, 228–232.

Chabora, H. J. & Horowitz, S. L. (1974). Cleft lip and cleft palate: one genetic system. *Oral Surgery* **38**, 181–186.

Ching, C. H. S. & Chung, C. S. (1974). A genetic study of cleft lip and palate in Hawaii. I. Interracial crosses. *American Journal of Human Genetics* **26**, 162–176.

Dixon, W. J. & Massey, F. J. (1969). *Introduction to Statistical Analysis*, 3rd ed., p. 243. New York: McGraw-Hill.

Duddleson, W. C., Midgley, A. R., Jr & Niswender, G. D. (1972). Computer program sequence for analysis and summary of radioimmunoassay data. *Computers and Biomedical Research* **5**, 205–217.

Erickson, R. P., Butley, M. S. & Sing, C. F. (1979). *H-2* and non-*H-2* determined strain variation in palatal shelf and tongue adenosine 3′:5′ cyclic monophosphate. *Journal of Immunogenetics* **6**, 253–262.

Erickson, R. P., Pairitz, G. L., Karolyi, J. M., Kapur, J. J., Odenheimer, D. J., Schultz, J. S. & Sing, C. F. (1985). HLA-B18 is associated with decreased levels of isoproterenol-stimulated cAMP in lymphocytes. *American Journal of Human Genetics* **37**, 124–132.

Frendsen, E. K. & Krishna, G. (1977). A simple ultrasensitive method for the assay of cyclic AMP and cyclic GMP in tissues. *Life Sciences* **18**, 529–542.

Goldman, A. S., Baker, M. K. & Grasser, D. L. (1983). Susceptibility to phenytoin-induced cleft palate in mice is influenced by genes linked to *H-2* and *H-3*. *Immunogenetics* **18**, 17–22.

Goldman, A. S., Baker, M. K., Tomassini, N. & Hummeler, K. (1982). Occurrence of cleft palate, micrognathia, and agnathia in selected strains of cortisone- and phenytoin-treated mice. *Journal of Craniofacial Genetics and Developmental Biology* **2**, 277–284.

Hanson, J. W., Myrianthopoulos, N. C., Sedgwick Harvey, M. A. & Smith, D. W. (1976). Risks to the offspring of women treated with hydantoin anticonvulsants, with emphasis on the fetal hydantoin syndrome. *Journal of Pediatrics* **89**, 662–668.

Hanson, J. W. & Smith, D. W. (1975). The fetal hydantoin syndrome. *Journal of Pediatrics* **87**, 285–290.

Katsumata, M., Gupta, C., Baker, M. K., Sussdorf, C. E. & Goldman, A. S. (1982). Diphenylhydantoin: an alternative ligand of a glucocorticoid receptor affecting prostaglandin generation in A/J mice. *Science* **218**, 1313–1315.

Liu, S. J. & Erickson, R. P. (1986*a*). Genetic differences among the A/J × C57BL/6J recombinant inbred mouse lines and their degree of association with glucocorticoid induced cleft palate. *Genetics* **113**, 745–754.

Liu, S. J. & Erickson, R. P. (1986*b*). Genetics of glucocorticoid receptor levels in recombinant inbred lines of mice. *Genetics* **113**, 735–744.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Massey, K. M. (1966). Teratogenic effects of diphenylhydantoin sodium. *Journal of Oral Therapeutic Pharmacology* **2**, 380–385.

Meruelo, D. & Edidin, M. (1975). Association of mouse liver adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels with *Histocompatibility-2* genotype. *Proceedings of the National Academy of Sciences, USA* **72**, 2644–2648.

Monson, R. R., Rosenberg, L., Hartz, S. C., Shapiro, S., Heinonen, O. P. & Slone, D. (1973). Diphenylhydantoin and selected congenital malformations. *New England Journal of Medicine* **289**, 1049–1052.

Nesbitt, M. N. & Skamene, E. (1984). Recombinant inbred mouse strains derived from A/J and C57BL/6J: a tool for the study of genetic mechanisms in host resistance to infection and malignancy. *Journal of Leukocyte Biology* **36**, 357–364.

Paigen, K., Swank, R. T., Tomino, S. & Ganschow, R. E.

https://doi.org/10.1017/S0016672300026719 Published online by Cambridge University Press

(1975). The molecular genetics of mammalian glucuronidase. *Journal of Cell Physiology* **85**, 379–392.

Pratt, R. M., Salomon, D. S., Diewert, V. M., Erickson, R. P., Burns, R. & Brown, K. S. (1980). Cortisone-induced cleft palate in the brachymorphic mouse. *Teratogenesis, Carcinogenesis and Mutagenesis* **1**, 15–23.

Rapaport, F. T., Bach, F. H., Bachraroff, R. J., McCarthy, J. G., Raisbeck, A. P., Egelandsdal, B. & Converse, J. M. (1979). The major histocompatibility complex (HLA) as a genetic marker in human craniofacial anomalies. *Tissue Antigens* **14**, 407–421.

Shapiro, S., Slone, D., Hartz, S. C., Rosenberg, L., Siskind, V., Monson, R. Mitchell, A. A. & Heinonen, O. P. (1976). Anticonvulsants and parental epilepsy in the development of birth defects. *Lancet* **i**, 272–275.

Siegel, S. (1956). *Non Parametric Statistics for the Behavioural Sciences*, pp. 96–104, 111–127, 193–194. New York: McGraw-Hill.

Strickler, S. M., Dansky, L. V., Miller, M. A., Seni, M. H., Andermann, E. & Spielberg, S. P. (1985). Genetic predisposition to phenytoin-induced birth defects. *Lancet* ii, 746–749.

Sulik, K. K., Johnson, M. C., Ambrose, L. J. & Dorgan, R. D. (1980). Mechanisms of phenytoin-induced malformations in a mouse model in *Phenytoin-induced Teratology and Gingival Pathology* (ed. T. M. Hassell, M. C. Johnston, and K. H. Dudley), pp. 67–74. New York: Raven Press.

Swank, R. T. & Bailey, D. W. (1973). Recombinant-inbred lines, value in the genetic analysis of biochemical variants. *Science* **181**, 1249–1252.

Taylor, B. A. (1978). Recombinant-inbred strains: use in gene mapping. In *Origins of Inbred Mice* (ed. H. Morse), pp. 423–438. New York: Academic Press.

VanDyke, D., Goldman, A., Spielmann, R., Zmijewski, C. & Oka, S. (1980). Segregation of HLA in sibs with cleft lip or cleft lip and cleft palate: evidence against genetic linkage. *Cleft Palate Journal* **17**, 189–193.

Vekemans, M., Taylor, B. A. & Fraser, F. C. (1981). The susceptibility to cortisone-induced cleft palate of recombinant inbred strains of mice: lack of association with the *H-2* haplotype. *Genetical Research, Cambridge* **38**, 327–331.

WHO Scientific Group (1970). *Genetic Factors in Congenital Malformations: Report of a WHO Scientific Group. World Health Organization Technical Report Series* **438**, 11–17.

https://doi.org/10.1017/S0016672300026719 Published online by Cambridge University Press