PEDIATRIC DENTAL JOURNAL 16(1): 84–90, 2006

Effect of the maternal environment on cortisone-induced cleft palate in mice

Juan Han, Michiko Maeda and Takahide Maeda

Department of Pediatric Dentistry, Nihon University School of Dentistry at Matsudo
2-870-1 Sakaecho-Nishi, Matsudo, Chiba 271-8587, JAPAN

Abstract OBJECTIVE: To investigate the maternal environment influence on cortisone-induced cleft palate in mice. METHODS: The A/WySn and the C3H/He strains of mice were used. Pregnant mice were injected on days 11 through 14 of gestation with 2.5 mg/mouse/day of cortisone. The A/WySn, C3H/He, F_1 hybrids and N_2 backcross fetuses that attained at least day 18 of development were checked for the presence of cleft palate. RESULTS: The frequency of fetuses with cleft palate and the ratio of dams bearing fetuses with cleft palate in the A/WySn strain (40.6% and 67.7%) were both significantly higher than those in the C3H/He (16.7% and 44.4%), F_1 (12.4% and 47.1%) and N_2 (24.0% and 55.3%) mice. The highest frequency of cleft palate was observed when the litter size was 8 in A/WySn, 7 in C3H/He, 8 and 9 in F_1, and 9 in N_2, respectively. CONCLUSION: The results suggested that the reaction to cortisone-induced cleft palate is different from the A/WySn and C3H/He strains of mice. The A/WySn strain of mice was more susceptible than the C3H/He strain of mice. The data suggest that litter size might play a role in defining cleft palate frequency.

Key words
Cleft palate,
Cortisone,
Maternal environment effect,
Mouse

Introduction

Oral clefts, including cleft lip (CL), cleft lip with palate (CLP) and cleft palate (CP), are seen in all races in the world, and are among the most common birth defects, but the cause and mechanism of their formation have not yet been clarified. In many epidemiological studies, the cleft type was CP in all patients who were the 1st degree relatives of the proband with CP, and none of the 1st degree relatives had CL or CLP. In contrast, when the proband had CL or CLP, all patients who were the 1st degree relatives of the proband had CL or CLP, and none of the 1st degree relatives had CP[1]. Thus, it was suggested that the genetic factor of CP are different from that of CL and CLP. However, there are many sporadic cases of CP in patients without familial history. Environmental teratogens such as systemic[2] or topical[3] administration of corticosteroid, exposure to organic solvents[4,5], cigarette smoking[6], and alcohol consumption[4,5] during the first trimester of pregnancy have been implicated in CP etiology. On the other hand, factors related to the maternal physiological environment, such as maternal race[7], age[7] and folate deficient[8], and birth weight of newborn[9], with CP have also been attracting attention.

Mice have been utilized extensively as models for the study of human hereditary diseases because of the high genetic homology between mice and humans. In mice, the CL and CLP occur spontaneously at higher frequencies in inbred mouse "A" strains, but spontaneous CP is very rare[10].

Since Baxter *et al.*[11] reported that production of CP in offspring of pregnant mice treated with cortisone, studies have been performed to elucidate the cause and mechanism of cortisone-induced CP formation in mice[12–18]. Cortisone-induced widening of the maxilla[12], decreased size of the palatal

Received on October 3, 2005
Accepted on January 5, 2006

Table 1 Frequency of CP in fetuses of pregnant mice given doses of cortisone

Dam	Sire	Number of dams	Number of fetuses	Number of fetuses with CP (%)
A	A	31	160	65 (40.6)
C3H	C3H	9	48	8 (16.7)
A	C3H	9	58	5 (8.6)
C3H	A	8	63	10 (15.9)
Pooled F_1		17	121	15 (12.4)
A	F_1	71	412	84 (20.4)
F_1	A	81	619	163 (26.3)
Pooled N_2		152	1,031	247 (24.0)

*: $P<0.05$, **: $P<0.005$, ***: $P<0.0001$

process[13], and delayed the time of palatal shelf elevation[14] in fetuses with CP have been reported to be factors contributing to CP. A quantitative variation of fetal palatal glucocorticoid receptor levels correlates with susceptibility to cortisone-induced CP has been reported[15]. Hyaluronic acid and collagen may be involved in different susceptibility to cortisone-induced CP[16]. Administration of vitamin B6 reduces the incidence of cortisone-induced CP by altering the binding of glucocorticoids to their cytoplasmic receptors and subsequently nuclear acceptors[17] have also been suggested. Furthermore, it was suggested that maternal genotype and environment affected the frequency of cortisone-induced CP by transfer of blastocysts experiment[18]. The purpose of this study was to investigate maternal environmental factors involved in cortisone-induced CP in mice. The frequency of CP, the ratio of dams bearing fetuses with CP, the numbers of implantations, resorbed fetuses, and survive fetuses, litter size, and the frequency of cleft palate in each litter size were investigated in inbred mouse strains A/WySn (A mice) and C3H/He (C3H mice), and F_1 hybrid (F_1 mice) from reciprocal cross between A and C3H and N_2 backcross (N_2 mice) from reciprocal cross between A and F_1.

Materials and Methods

Mice and maintenance conditions

A mice provided by the National Institute of Genetics and C3H mice provided by Sankyo Labo Service Corporation were bred at the Animal Study Center of our University. A and C3H mice were mated, and F_1 (A♀×C3H♂) and F_1 (C3H♀×A♂) were prepared. These A, C3H, and F_1 mice were used for the study.

The experimental mice were maintained in a mouse room in our Animal Study Center under controlled conditions of 24 ± 1°C room temperature, 65 ± 5% humidity, and 12-hour lighting cycle. The mice were given free access to MF pellets (Oriental Inc.) and tap water for drinking in a water bottle.

Methods

A and C3H mice were mated within the same strain. A and C3H mice were also mated between the strains in alternate combinations of male and female to raise F_1 hybrids. F_1 mice were mated with A in alternate combinations of male and female to raise N_2 backcross mice. Female was kept with the male overnight and vaginal plug was examined on the following morning. When this was found, the female was placed in a separate cage; this day was designated as day 0 of gestation. The pregnant mice were injected daily with 2.5 mg/mouse/day of cortisone from the 11th to the 14th days of gestation. On day 18 of gestation, the pregnant mice were sacrificed by cervical dislocation, and fetuses were removed from the uterus. Implantations, resorbed fetuses, and survive fetuses were counted, and examined under a dissection microscope for oral clefts.

The observation was performed in 6 groups of mouse fetuses as described above; A×A, C3H×C3H, F_1 (A×C3H), F_1 (C3H×A), N_2 (A×F_1) and

Table 2 Ratio of dams bearing fetus with CP

Dam	Sire	Number of dams	Number of dams bearing fetus with CP (%)	Mean number of fetuses with CP ± S.D.
A	A	31	21 (67.7)	3.1 ± 1.6
C3H	C3H	9	4 (44.4)	2.0 ± 0.0
A	C3H	9	3 (33.3)	1.7 ± 1.2
C3H	A	8	5 (62.5)	2.0 ± 0.7
Pooled F_1		17	8 (47.1)	1.9 ± 0.8
A	F_1	71	35 (49.3)	2.4 ± 1.7
F_1	A	81	49 (60.5)	3.3 ± 2.2
Pooled N_2		152	84 (55.3)	2.9 ± 2.1

***: $P<0.05$, **: $P<0.005$, ***: $P<0.0001$

N_2 ($F_1 \times A$).

This study was approved by the Institutional Animal Care Committee of Nippon University School of Dentistry at Matsudo (approval number: ECA-02-0001).

Statistical analysis

Differences in the frequency of CP and ratio of dams bearing fetuses with CP among the mouse groups were analyzed by the χ^2 test, and differences in the mean number of fetuses with CP per litter for dams bearing fetuses with CP, the mean numbers of implantations, resorbed fetuses and survive fetuses were analyzed by the *t*-test. A significance level of 5% was regarded as significant.

Results

Frequency of CP

CP was noted in all 6 groups of mice. The frequencies were 40.6%, 16.7%, 8.6%, 15.9%, 20.4%, and 26.3% in A × A, C3H × C3H, F_1 (A × C3H), F_1 (C3H × A), N_2 (A × F_1), and N_2 (F_1 × A) mice, respectively. The frequency in A × A mice was significantly higher than that in the other 5 groups ($P<0.0001$, $P<0.0001$, $P<0.0001$, $P<0.0001$, and $P<0.005$). The frequency in F_1 (A × C3H) mice was significantly lower than that in F_1 (C3H × A) mice ($P<0.05$) (Table 1).

Ratio of dams bearing fetuses with CP

When cortisone was administered to 31 dams of A × A mice, fetal with CP was noted in 21 dams, and the ratio of dams bearing fetuses with CP was 67.7%. When cortisone was administered to 9 dams of C3H × C3H mice, fetal with CP was noted in 4 dams, and the ratio of dams bearing fetuses with CP was 44.4%. When cortisone was administered to 17 dams of F_1 mice, fetal with CP was noted in 8 dams, and the ratio of dams bearing fetuses with CP was 47.1%. When cortisone was administered to 152 dams of N_2 mice, fetal with CP was noted in 84 dams, and the ratio of dams bearing fetuses with CP was 55.3%. The ratio of dams bearing fetuses with CP in A × A was significantly higher than that in C3H × C3H, F_1, and N_2 ($P<0.0001$, $P<0.0001$, and $P<0.05$). In F_1, the ratio of dams bearing fetuses with CP in C3H × A was 62.5%, significantly higher than that in A × C3H (33.3%) ($P<0.0001$). In N_2, the ratio of dams bearing fetuses with CP in F_1 × A was 60.5%, significantly higher than that in A × F_1 (49.3%) ($P<0.05$). In dams bearing fetuses with CP, the mean number of fetuses with CP per dam was 1.7 ± 1.2–3.3 ± 2.2, showing no significant difference among the 6 groups (Table 2).

Numbers of implantations, resorbed fetuses and litter size in dams bearing fetuses with CP and dams bearing fetuses with normal lip and palate

In C3H × C3H, the mean litter size for dams bearing fetuses with CP was 6.3 ± 1.0, significantly higher than that (3.8 ± 1.3) for dams bearing fetuses with normal lip and palate ($P<0.05$). In N_2 (F_1 × A), the mean litter size for dams bearing fetuses with CP was 8.3 ± 2.8, significantly higher than that (6.6 ± 3.1) for dams bearing fetuses with normal lip

Table 3 Differences in the implantations, resorptions and survivals observed both dams bearing fetuses with CP and dams bearing fetuses with normal

Dam	Sire	Dams bearing fetuses with CP				Dams bearing fetuses with normal			
		Dams	Implantations (Mean ± S.D.)	Resorptions (Mean ± S.D.)	Survivals (Mean ± S.D.)	Dams	Implantations (Mean ± S.D.)	Resorptions (Mean ± S.D.)	Survivals (Mean ± S.D.)
A	A	21	190 (9.0 ± 1.9)	74 (3.5 ± 1.7)	116 (5.5 ± 2.1)	7	59 (8.4 ± 2.1)	26 (3.7 ± 1.0)	33 (4.7 ± 2.1)
C3H	C3H	4	34 (8.5 ± 1.7)	9 (2.3 ± 1.3)	25 (6.3 ± 1.0)	5	48 (9.6 ± 2.5) *	25 (5.0 ± 2.8)	19 (3.8 ± 1.3) *
A	C3H	3	33 (11.0 ± 2.6)	13 (4.3 ± 3.8)	20 (6.7 ± 3.2)	6	54 (9.0 ± 2.7)	16 (2.7 ± 1.6)	38 (6.3 ± 2.4)
C3H	A	5	61 (12.2 ± 6.1)	19 (3.8 ± 3.6)	42 (8.4 ± 3.4)	3	30 (10.0 ± 1.7)	9 (3.0 ± 2.6)	21 (7.0 ± 1.0)
Pooled F_1		8	94 (11.8 ± 4.8)	32 (4.0 ± 3.4)	62 (7.8 ± 3.2)	9	84 (9.3 ± 2.3)	25 (2.8 ± 1.9)	59 (6.6 ± 2.0)
A	F_1	35	382 (10.9 ± 3.4)	164 (4.7 ± 3.0)	218 (6.2 ± 2.4)	30	260 (8.7 ± 3.1)	94 (3.1 ± 1.5)	166 (5.5 ± 2.7)
F_1	A	49	683 (13.9 ± 4.2)	277 (5.7 ± 3.0)	406 (8.3 ± 2.8)	22	200 (9.1 ± 4.2) **	55 (2.5 ± 2.7)	145 (6.6 ± 3.1) **
Pooled N_2		84	1,065 (12.7 ± 4.1)	441 (5.3 ± 3.4)	624 (7.4 ± 2.8)	52	460 (8.8 ± 3.5)	149 (2.9 ± 2.1)	311 (6.0 ± 2.9)

*: $P<0.05$, **: $P<0.005$

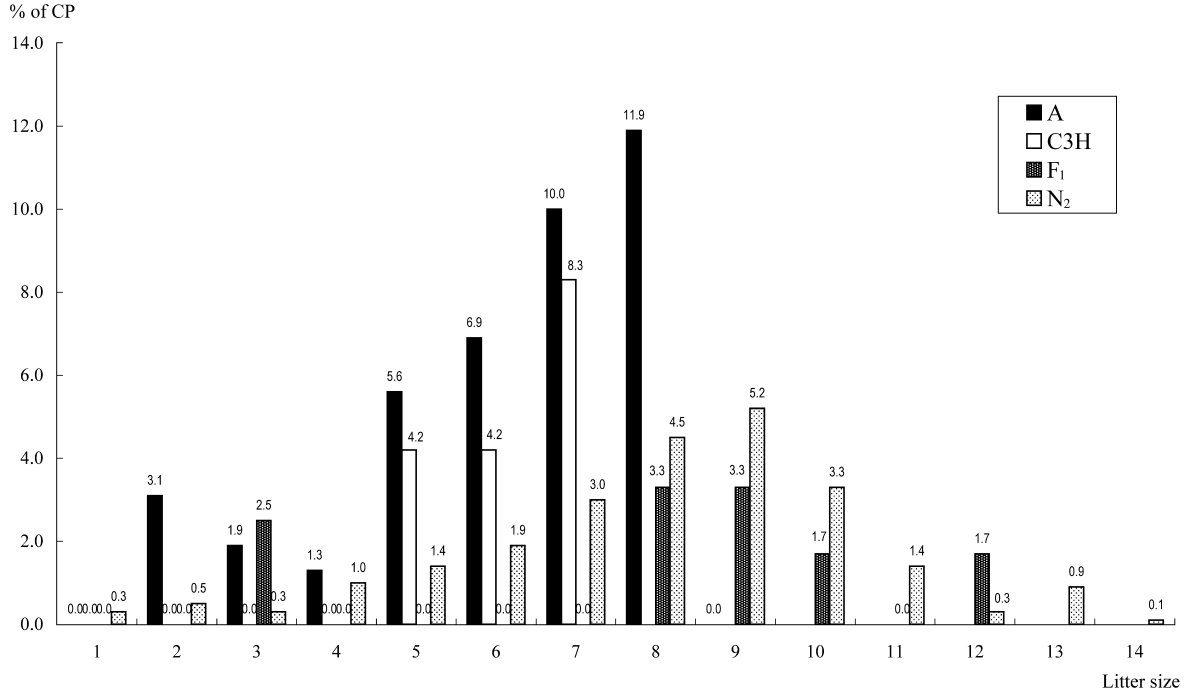

Fig. 1 Frequency of CP among different litter sizes in 4 groups of mice

and palate ($P<0.005$). In A × A, F_1 (A × C3H), F_1 (C3H × A), and N_2 (A × F_1), although no significant difference was noted, the mean litter size for dams bearing fetuses with CP was higher than that for dams bearing fetuses with normal lip and palate (Table 3).

Frequency of CP in each litter size

The frequencies of CP in each litter size among A, C3H, F_1 and N_2 mice are shown in Fig. 1.

In A × A, litter size was 1–9. The frequency of CP was 11.9% when the litter size was 8, and this

was the highest. In C3H×C3H, litter size was 1–7. A frequency of 8.3% in a litter size of 7 was the highest. In F_1 mice, litter size was 1–12. The highest frequency was 3.3% for litter sizes of 8 and 9. And In N_2, litter size was 1–14. A frequency of 5.2% in a litter size of 9 was the highest (Fig. 1).

Discussion

Cortisone treatment to pregnant mice at anywhere from the 9th to the 17th days of gestation, during which palate formation completes, induces CP in the offspring almost all strains of mice[19,20]. The administration of cortisone was used as a single dose on the 12th day of gestation[13] when the fissures of the medial and lateral nasal processes-maxillary process is said to have closed, and before the proliferative peak is reached, severely inhibits proliferation of the palatal processes. The single dose of cortisone was used also on day 11 at 8 a.m. of gestation, which the precocious development of the palatal processes in the closing fissure at various mouse strains had been taken into account[18]. However, the frequency has been reported to be the highest when cortisone was administered for 4 days that began on gestation days 10 or 11[19]. In this study, cortisone was administered to pregnant mice from the 11th to the 14th days of gestation; CP was observed in all 6 groups of mice. The control as non-treatment was not performed in this study, because of the frequency of spontaneous CP is only 0.25% approximately in mice[10], and none spontaneous CP was obtained in our previous study[21].

It has been reported that the frequency of cortisone-induced CP decreased with increasing the age of dams[22] and advancing the parity of dams[23]. To eliminate the influences of age and parity, we used virgin female mice aged 8–10 weeks for dams.

In addition there is a clearcut relationship between the dosage of cortisone and the frequency of CP in the offspring, doses of 5 mg/mouse/day or more usually cause intrauterine death and resorption of the fetuses in the susceptible strains, 2.5 mg/mouse/day cortisone induced CP at the highest frequency in A strain mice[19]. In our study, 2.5 mg/mouse/day cortisone was administered to pregnant mice, and the frequency of CP was 40.6% in A and 16.7% in C3H. Morphological study has shown that different inbred strains of mice have different timing of palatal shelf elevation, A mice are later than C3H, and strains with normally late shelf elevation have been shown to be more susceptible to induction of CP[24]. Our result was in accord with the observations of many studies, which A mice have a high susceptibility[25,26].

Although it was demonstrated that the differences in the frequency of cortisone-induced CP are matroclinous by some studies, such as reciprocal crosses between the susceptible A/J mice and the resistant CBA mice showed that fetuses growing in an A/J dam; F_1 (A/J×CBA) developed a higher percentage of cortisone-induced CP than those in a CBA dam; F_1 (CBA×A/J)[26]. Reciprocal crosses between susceptible Strong *a* strain and resistant C3H strain showed that fetuses growing in a Strong *a* dam; F_1 (Strong *a*×C3H) developed a lower percentage of cortisone-induced CP than those in a C3H dam; F_1 (C3H×Strong *a*)[27]. Our results showed that the frequency was significantly lower when the dams were A; F_1 (A×C3H) than when the dams were C3H; F_1 (C3H×A). Furthermore, the frequency of CP was also investigated in N_2. The frequency was lower when the dams were A; N_2 (A×F_1) than when the dams were F_1; N_2 (F_1×A). These results gave further evidence of the extreme differences in the various strains of mice under the influence of the same teratogen. However, the influence of the paternal genotype cannot be ignored.

On the other hand, the ratio of dams bearing fetuses with CP was significantly higher when the dams were C3H; F_1 (C3H×A) than when the dams were A; F_1 (A×C3H). In N_2, the ratio of dams bearing fetuses with CP was significantly higher in F_1 dams; N_2 (F_1×A) than in A dams; N_2 (A×F_1). These suggested a hypothesis that the frequency of resorbed fetuses with CP is higher when the maternal environment was A, and the frequency of survive fetuses with CP is higher when the maternal environment was C3H or F_1 according to the survival of spontaneous CL and CLP fetuses may be influenced by maternal trait[28].

The mean litter size was significantly higher in dams bearing fetuses with CP than in dams bearing fetuses with normal lip and palate in C3H×C3H and F_1×A. Although no significant difference was noted in the other groups, the mean litter size was higher in dams bearing fetuses with CP than in dams bearing normal fetuses. It was described that litter size may be of possible significance for abnormal development because of the restrictions it imposes on prenatal growth by other study[29]. Our study also

suggested that the growth environment for fetuses with many litter sizes is worse than for fetuses with fewer litter sizes, and is more likely to induce CP.

Kalter[22] reported that the frequency of CP was highest when the litter size was 8, although he did not elucidate the relationship between cortisone-induced CP and the litter size. In our study, the frequency of CP in each litter was investigated, and the frequency was highest when the litter size was 8 in A, 7 in C3H, 8 and 9 in F_1, and 9 in N_2. Although the cause was unclear, these litter sizes at the highest frequency of CP were higher than the mean litter size among all groups[30], suggesting that the poor growth environment due to a large litter size contribute to cortisone-induced CP. However, the frequency of CP decreased when the litter size was 9 in A, 10–12 in F_1, and 10–14 in N_2, suggesting that when the litter size was more than 9, the maternal environment necessary for survival of fetuses worsened, and it may have become more likely to resorb anomalous fetuses with CP.

References

1) Takahashi, S.: Embryogenesis and etiology of cleft lip and cleft palate. *J Jpn Cleft Palate Assoc* **15**: 49–61, 1990. (in Japanese, English abstract)
2) Park-Wyllie, L., Mazzotta, P., Pastuszak, A., Moretti, M.E., Beique, L., Hummisett, L., Friesen, H.M., Jacobson, S., Kasapinovic, S., Chang, D., Diav-Citrin, O., Chitayat, D., Nulman, I., Einarson, T.R. and Koren, G.: Birth defects after maternal exposure to corticosteroids: prospective cohort study and meta-analysis of epidemiological studies. *Teratology* **62**: 385–392, 2000.
3) Edwards, M.J., Agho, K., Attia, J., Diaz, P., Hayes, T., Illingworth, A. and Roddick, L.G.: Case-control study of cleft lip or palate after maternal use of topical corticosteroids during pregnancy. *Am J Med Genet* **120**(A): 459–463, 2003.
4) Wyszynski, D.F. and Beaty, T.H.: Review of the role of potential teratogens in the origin of human nonsyndromic oral clefts. *Teratology* **53**: 309–317, 1996.
5) Leite, I.C., Paumgartten, F.J. and Koifman, S.: Chemical exposure during pregnancy and oral clefts in newborns. *Cad Saude Publica* **18**: 17–31, 2002.
6) Zeiger, J.S., Beaty, T.H. and Liang, K.Y.: Oral clefts, maternal smoking, and TGFA: a meta-analysis of gene-environment interaction. *Cleft Palate Craniofac J* **42**: 58–63, 2005.
7) Forrester, M.B. and Merz, R.D.: Descriptive epidemiology of oral clefts in a multiethnic population, Hawaii, 1986–2000. *Cleft Palate Craniofac J* **41**: 622–628, 2004.
8) Prescott, N.J. and Malcolm, S.: Folate and the face: evaluating the evidence for the influence of folate genes on craniofacial development. *Cleft Palate Craniofac J* **39**: 327–331, 2002.
9) Wyszynski, D.F., Sarkozi, A., Vargha, P. and Czeizel, A.E.: Birth weight and gestational age of newborns with cleft lip with or without cleft palate and with isolated cleft palate. *J Clin Pediatr Dent* **27**: 185–190, 2003.
10) Kalter, H.: Sporadic congenital malformations of newborn inbred mice. *Teratology* **1**: 193–199, 1968.
11) Baxter, H. and Fraser, F.C.: Production of congenital defects in offspring of female mice treated with cortisone. *McGill Med J* **9**: 245, 1950.
12) Peterka, M. and Dostal, M.: Influence of cleft palate on growth of the maxilla in mouse embryos. *Cleft Palate J* **14**: 206–210, 1977.
13) Peterka, M. and Jelinek, R.: Differences in the size of the palatal processes in mouse embryos with cleft palate induced in two critical periods. *Cleft Palate J* **15**: 13–19, 1978.
14) Diewert, V.M. and Pratt, R.M.: Cortisone-induced cleft palate in A/J mice: failure of palatal shelf contact. *Teratology* **24**: 149–162, 1981.
15) Goldman, A.S. and Katsumata, M.: Quantitative variation in hormonal receptors and clefting in the mouse. *Prog Clin Biol Res* **46**: 91–120, 1980.
16) Montenegro, M.A., Rojas, M., Dominguez, S. and Rosales, C.J.: Differences in extracellular matrix components and cell density during normal and dexamethasone-treated secondary palate development in two strains of mice with different susceptibility to glucocorticoid induced-clefting. *J Craniofac Genet Dev Biol* **18**: 100–106, 1998.
17) Yoneda, T. and Pratt, R.M.: Vitamin B6 reduces cortisone-induced cleft palate in the mouse. *Teratology* **26**: 255–258, 1982.
18) Larsson, K.S., Marsk, L., Svanberg-Larsson, A. and Tesh, J.M.: Agent-determined type of maternal influence on induced cleft palate in mice. *Biol Neonate* **31**: 51–59, 1977.
19) Fraser, F.C. and Fainstat, T.D.: Production of congenital defects in the offspring of pregnant mice treated with cortisone. *Pediatrics* **8**: 527–533, 1951.
20) Jirsakova, A. and Holusa, R.: The induction of cleft palate by cortisone in several inbred strains of mice. *Acta Chir Plast* **11**: 85–95, 1969.
21) Han, J., Shimizu, T. and Maeda, T.: The frequency of spontaneous and cortisone-induced cleft lip, cleft lip with palate and cleft palate between the A/WySn and C3H/He strains of mice. *Jpn J Ped Dent* **41**: 887–892, 2003. (in Japanese, English abstract)
22) Kalter, H.: Modification of teratogenic action of cortisone in mice by maternal age, maternal weight and litter size. *Am J Physiol* **185**: 65–68, 1956.
23) Kalter, H. and Fraser, F.C.: The modification of the teratogenic action of cortisone by parity. *Science* **118**: 625–626, 1953.
24) Diewert, V.M.: A comparative study of craniofacial growth during secondary palate development in four strains of mice. *J Craniofac Genet Dev Biol* **2**: 247–

263, 1982.
25) Hackney, J.F.: A glucocorticoid receptor in fetal mouse: its relationship to cleft palate formation. *Teratology* **21**: 39–51, 1980.
26) Marsk, L., Theorell, M. and Larsson, K.S.: Transfer of blastocysts as applied in experimental teratology. *Nature* **234**: 358–359, 1971.
27) Loevy, H.: Genetic influences on induced cleft palate in different strains of mice. *Anat Rec* **145**: 117–122, 1963.
28) Juriloff, D.M. and Fraser, F.C.: Genetic maternal effects on cleft lip frequency in A/J and CL/Fr mice. *Teratology* **21**: 167–175, 1980.
29) Kalter, H.: Prenatal epidemiology of spontaneous cleft lip and palate, open eyelid, and embryonic death in A/J mice. *Teratology* **12**: 245–258, 1975.
30) Han, J., Maeda, M., Matsunaga, R., Shimizu, T., Shimizu, K. and Maeda, T.: Effect of maternal environment on occurrence of cleft lip with palate in mice. *Jpn J Ped Dent* **43**: 624–630, 2005. (in Japanese, English abstract)