

すべきと考えられた。

ダイオキシン類やPCBsを除くPOPsの研究について、国内には分析法について多くの報告があるものの、毒性や有害性に関する系統的な報告や参考となる総説は見当たらなかった。今回の報告書では、POPsのうちDDT/DDE、HCB、Toxophaneについて重点的に文献調査を実施したが、他のPOPsについても先行研究を調査し基礎資料とすることが必要であろう。疫学調査と並行して今後とも文献調査を進め、化学物質としての特徴や有害性評価について情報収集することが必要と考えられた。

E. 結論

母乳を用いたPOPs曝露評価を平成19-20年度に計画している。それに先立ち、母乳中POPsの解析をn=18で実施した。母乳中に多くの化学物質が検出され、絶対濃度としてはDDT/DDEやPCBsの存在が強調されるとともに、国内で使用歴がないToxaphene、Mirexも検出され、その曝露経路等が懸念された。次に、POPsの健康影響に関する先行研究の整理を実施した。特に、小児の発達への影響を解析したコホート調査について検討し、HCBおよびDDT/DDE曝露の健康影響に関する報告に注目した。母乳中POPs分析および周産期におけるPOPs曝露の影響評価を行う上で、今回の知見を活用したい。

F. 研究発表

1. 論文発表

Nakamura T, Nakai K, Suzuki K, Koizumi A, Shamoto H, Yamauchi M, Matsumura T, Saito Y, Kameo S, Satoh H. Concentrations of dioxins and PCBs in cord blood in Japanese children from the Tohoku Study of Child Development. *Organohalogen Compounds* 68:1631-1634, 2006.

2. 学会発表

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G. 知的所有権の取得状況

なし

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IV. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍名	出版社名	出版地	出版年	ページ
Suzuki K. et al.	Tohoku Study of Child Development, a cohort study to examine the effects of perinatal exposure to methylmercury, PCB or dioxins on child development; the association of neonatal neurobehavioral status with maternal hair mercury concentration and fish intake.	Recent Topics of Fetal Methylmercury Exposure and its effects	National Institute for Minamata Disease	Minamata	2006	97-105

雑誌

著者氏名	論文タイトル名	発表雑誌	巻名	ページ	出版年
Suzuki K. et al.	Association of maternal smoking during pregnancy and infant neurobehavioral status	Psychol Reports	99	97-106	2006
Suzuki K. et al.	Effect of perinatal exposure to environmentally persistent organic pollutants and heavy metals on neurobehavioral development in Japanese children: PCBs exposure and neonatal neurobehavioral status	Organohalogen Compounds	68	1201-1204	2006
Arakawa C. et al.	Fish consumption and time to pregnancy in Japanese women	Int J Hyg Environ Health	209	337-344	2006
Nakamura T. et al.	Concentrations of dioxins and PCBs in cord blood in Japanese children from the Tohoku Study of Child Development	Organohalogen Compounds	68	1631-1634	2006
村田勝敬, 坂本峰至	妊婦における魚摂取の考え方	臨床栄養	109	191-194	2006
Sakamoto M. et al.	Correlations between mercury concentrations in umbilical cord tissue and other biomarkers of fetal exposure to methylmercury in the Japanese population	Environ Res	103	106-111	2007

V. 研究成果の刊行物・別刷

Tohoku Study of Child Development, a Cohort Study to Examine the Effects of Perinatal Exposure to Methylmercury, PCBs or Dioxins on Child Development; the Association of Neonatal Neurobehavioral Status with Maternal Hair Mercury Concentration and Fish Intake.

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Abstract

Aims- We have been performing a cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposures to methylmercury (MeHg) and environmentally persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs) on child development. In the present study, we report the protocol of this study and some preliminary results about the association of neonatal neurobehavioral status with maternal hair mercury concentration and fish intake.

Protocol- Healthy pregnant women were registered between January 2001 and September 2003 in urban area of Tohoku district, Japan. Maternal peripheral blood, placenta, cord, cord blood and breast milk were collected for chemical analysis. Maternal hair samples were also taken for MeHg analysis. Maternal diet including the fish intake was assessed with a semi-quantitative food frequency questionnaire (FFQ). For the assessment of neurobehavioral development, Brazelton Neonatal Behavioral Assessment Scale (NBAS) was performed when children were three days old, and other test including Bayley Scales of Infant Development second edition, Fagan Test for Infant Intelligence, Kaufman Assessment Battery for Children were performed with growth of the children.

Results- 599 mother-infant pairs were registered in a cohort study. Among all subjects, analytical subjects were 529 mother-infant pairs whose data on hair mercury concentration, FFQ, NBAS and their characteristics were available. Maternal hair mercury concentration was associated with the decreased score of motor cluster. On the other hand, maternal fish intake was associated with the increased score of the same cluster. These findings suggested that the fish intake had two aspects of a potential risk and a benefit for the neonatal neurobehavioral development.

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Introduction

Negative effects of perinatal exposure to methylmercury (MeHg) and environmentally persistent organic pollutants including polychlorinated biphenyls (PCBs) on child development are great concern worldwide (Nakai and Satoh, 2002). There have been reports on the association of prenatal MeHg exposure with poorer cognitive functions in New Zealand (Kjellstorm et al, 1986), Faroe Islands (Grandjean et al, 1997), and Madeira Islands (Murata et al, 1999), although the cohort study in Seychelles Islands did not observe the negative effect of prenatal MeHg exposure on the development (Davidson et al, 1998). Several epidemiological studies have also reported on the effects of perinatal exposure to PCBs on neurobehavioral development. The cohort studies in North Carolina (Rogan et al, 1986), Michigan (Jacobson et al, 1985, 1990), New York (Darvill et al, 2000; Stewart et al, 2000), The Netherlands (Patandin et al, 1999; Vreugdenhil et al, 2002), Germany (Winneke et al, 1998; Walkowiak et al, 2001) and Faroe Islands (Grandjean et al, 2001) demonstrated negative associations of perinatal exposure to PCBs with cognitive function of children. These chemicals accumulate in humans mostly through the consumption of food, especially fish and shellfish. From the nutritional perspective, fish is usually recommended for pregnant women because it is rich in nutrients such as polyunsaturated fatty acids (PUFA) essential for brain development. Therefore, from the perspective of risk assessment, these health hazard issues are particularly of importance in fish-eating populations.

We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to MeHg and PCBs on child development. In the present study, we report the protocols of study and preliminary results about the association of neonatal neurobehavioral status with maternal hair mercury concentration and fish intakes.

Protocols

The protocols of the cohort study have been described previously (Nakai et al, 2004), but will be briefly outlined. The study had been performing in the urban area of Tohoku district, Japan. Healthy pregnant women were recruited with their informed consent at obstetrical wards of two hospitals. To establish an optimal study population, only infants born at full-term (36 to 42 weeks of gestation) without the congenital anomalies or diseases were included. Pregnancy and delivery should have been completed without overt signs of serious illness or complications. The study protocol has the approval of the ethical committee of Tohoku University Graduate School of Medicine. Fig.1 shows the outline of registration and sample collection. Various samples were collected for the determination of chemicals including the total mercury concentration and PCBs. For example, maternal hair samples were taken at two days after delivery from the back of head near the occipital protuberance, and the proximal three cm from the scalp were used for analysis of total mercury concentration. The total hair mercury concentration (hair Hg) was measured by cold vapor atomic absorption (Akagi, 1991) at National Institute of Minamata Diseases. To

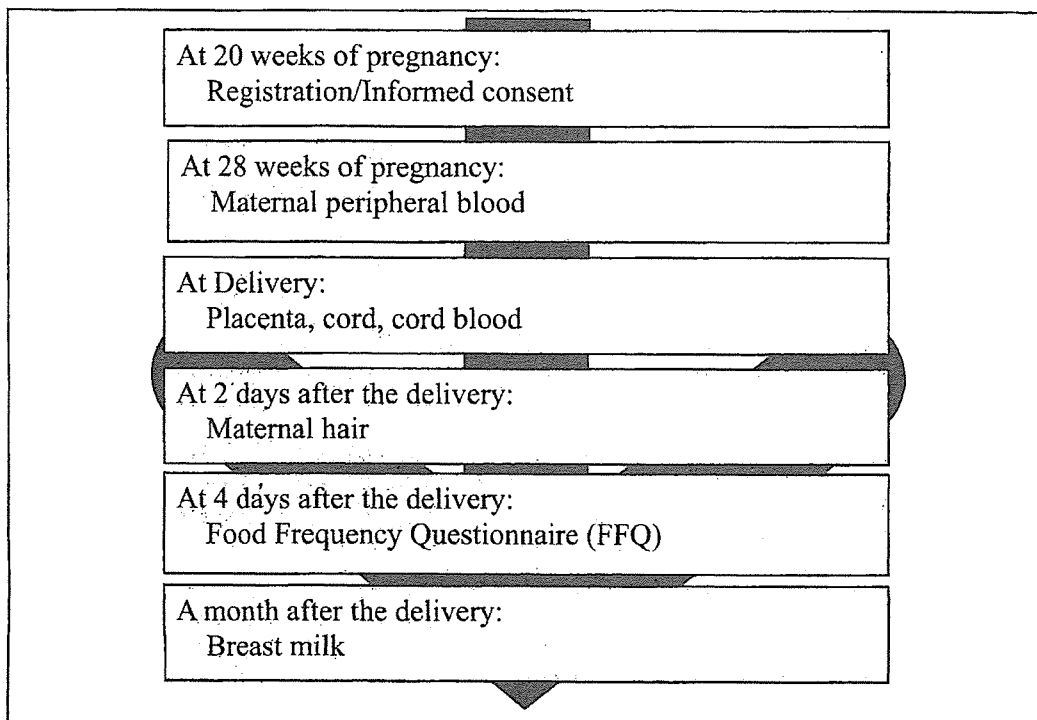


Fig.1 The registration and sample collection

estimate the maternal diet including the fish intake, the semi-quantitative food frequency questionnaire (FFQ) for 122 individual foods and recipes (Date et al., 1996) and 13 additional items regarding fish and shellfish was administered at four days after delivery. Trained investigators showed the real size photograph of each food, then, mothers answered the frequency and the amount of the intake per meal. Fig.2 shows the outline for the assessment of neurobehavioral development. The Neonatal Behavioral Assessment Scale (NBAS) (Brazelton, 1974) was performed when children were three days old. All examiners had been trained in the NBAS training center at Nagasaki University School of Medicine, Japan. When children were seven months old, the Bayley Scales of Infant Development (BSID) (Bayley, 1993), Kyoto Scales of Psychological Development (KSPD) (Ikuzawa, 1985) and Fagan Test for Infant Intelligence (FTII) (Fagan and Shepherd, 19987) were performed. BSID and KSPD also performed when children were 18 months old. When children were 42 months old, Kaufman Assessment Battery for Children (Kaufman et al, 1983) and CATSYS (Despres et al. 2000) were performed.

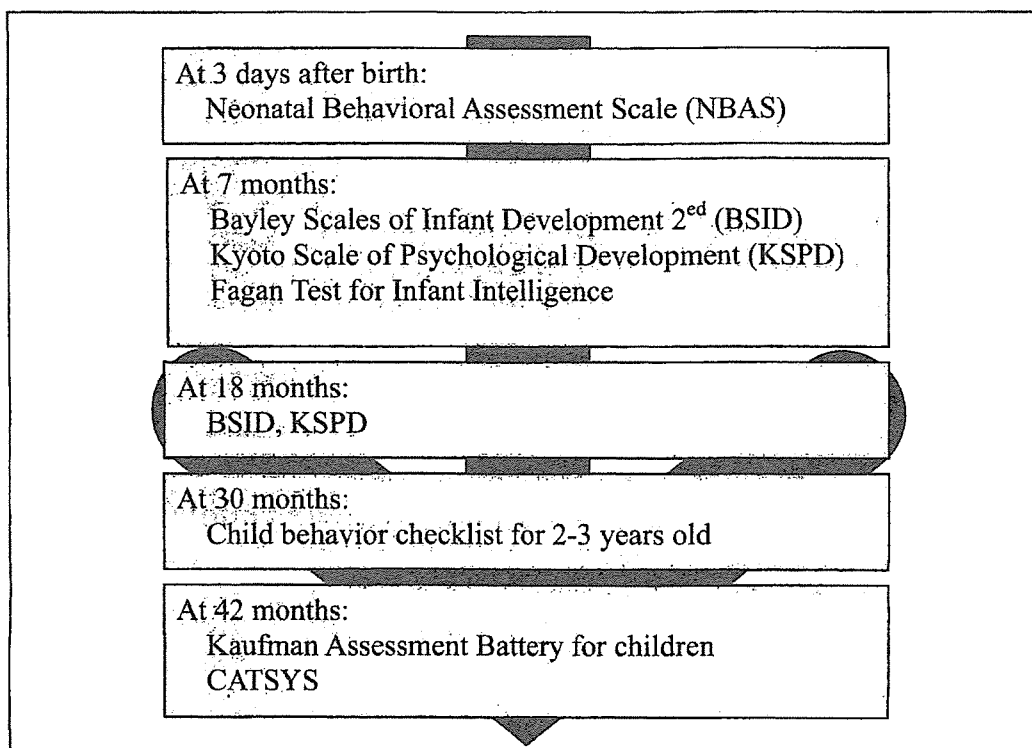


Fig.2 The measurement of neurobehavioral development

Results and Discussion

During the registration period from January 2001 to September 2003, 599 mother-infant pairs were registered in a cohort study. The follow up examination has been administrated when children are three days, seven months, 18 months, 30 months, and 42 months old, respectively, and the percentages of families who participated in each examination were always over 80% (data not shown).

Among all participants, analytical subjects were 529 mother-infant pairs whose data about hair Hg, FFQ, NBAS, and their characteristics are available. In the statistical analysis, the multiple regression analyses were performed for adjustment of covariates. The two multiple regression models were made based on the fish intake. In the model 1, seven clusters of NBAS were set as a dependent variables and total hair mercury concentration, total fish intake and covariates as independent variables. In the model 2, the intake of 13 categorized fish was set as independent variables instead of total fish intake. Potential covariates were selected from maternal and neonatal characteristics (Table 1).

Table 1. Maternal and neonatal characteristics

	Mean (SD)	Min	Max
Maternal characteristics			
# Maternal age at the time of delivery	31.3 (4.3)	20.0	42.0
# Educational status (under 12y/over 13y)	128/396		
# Alcohol drinking during pregnancy (n/y)	416/113		
# Smoking (no/ceased/yes)	427/82/20		
# Delivery type (spontaneous/caesarian)	457/72		
# Parity (first/others)	269/260		
# Total energy intake (kcal/day)	1600 (642)	399	6539
Infant characteristics			
# Gender (male/female)	275/254		
# Gestational age (weeks)	39.6 (1.2)	36.0	42.0
# Birth weight (g)	3074 (330)	2412	4176
Birth length (cm)	49.0 (1.8)	44.0	55.0
Head circumference' (cm)	33.5 (1.3)	28.0	37.0
# Apgar score 1m after birth	8.2 (0.7)	1.0	10.0
# Cord blood T3 (ng/ml)	0.53 (0.15)	0.28	1.82

These items were used as a covariates at a multiple regression analyses.

There were moderate to strong correlations between birth weight and head circumference (pearson's $r = .57$), and birth weight and birth length (pearson's $r = .70$). In consideration of multicollinearity, birth length and head circumference were excluded from the covariates.

Table 2 and 3 shows the distribution of total hair Hg concentration and maternal fish intakes, respectively. The mean hair Hg was 2.2 $\mu\text{g/g}$ (SD1.1), and the mean total fish intake was 25.2 kg/year (SD17.1). Table 4 shows the result of model1. Maternal hair Hg was associated with the decreased score of motor cluster ($\beta = -0.32$, $p < .05$) (Fig. 3). On the other hand, total fish intake was associated with the increased score of the same cluster ($\beta = 0.23$, $p < .01$) (Fig. 4). As results of model2, hair Hg was associated with the decreased score of motor cluster ($\beta = -0.25$, $p < .01$). Silvery blue fish intake was associated with increased score of motor cluster ($\beta = 0.08$, $p < .05$) and range of state cluster ($\beta = 0.09$, $p < .01$), respectively (data not shown). The association of hair Hg with the decreased score of motor cluster was observed. It is suggested that methylmercury affects adversely neonatal neurobehavioral development. On the other hand, we found the positive association of motor cluster with total fish intake and silvery blue fish intake. It is suggested that the beneficial nutritive factors of fish intake contribute to neonatal neurobehavioral development. Indeed, silvery blue fish consisted of pacific saury, mackerel, sardine, and caperine, and it is known as rich in PUFA. These findings suggested that the fish intake had two aspects of a potential risk and a benefit for the neonatal neurobehavioral development. We will readdress this health issue after the completion of PCBs determination and when children become older.

Table 2. Total hair mercury concentration

	Mean	SD	Median	Min	Max
Maternal hair	2.20	1.14	1.95	0.29	9.35

(µg/g)

The hair sample was used 3cm from scalp.

Table 3. Maternal fish intake

	Mean	SD	Median	Min	Max
Total fish intake	25.19	17.06	21.72	0.91	143.78

(Kg/year)

Table 4. Multiple regression model¹

	Habituation	Orientation	Motor	Range of state	Regulation of state	Autonomic stability	Reflex
Hair Hg ¹	0.29	0.31	-0.32*	0.30	-0.12	-0.01	0.47
Total fish intake ¹	-0.26	-0.03	0.23**	-0.01	0.11	-0.07	0.07

(β value)

* p < .05, ** p < .01

¹Log transformations, Log₁₀X, were used on the value of hair Hg and total fish intake.

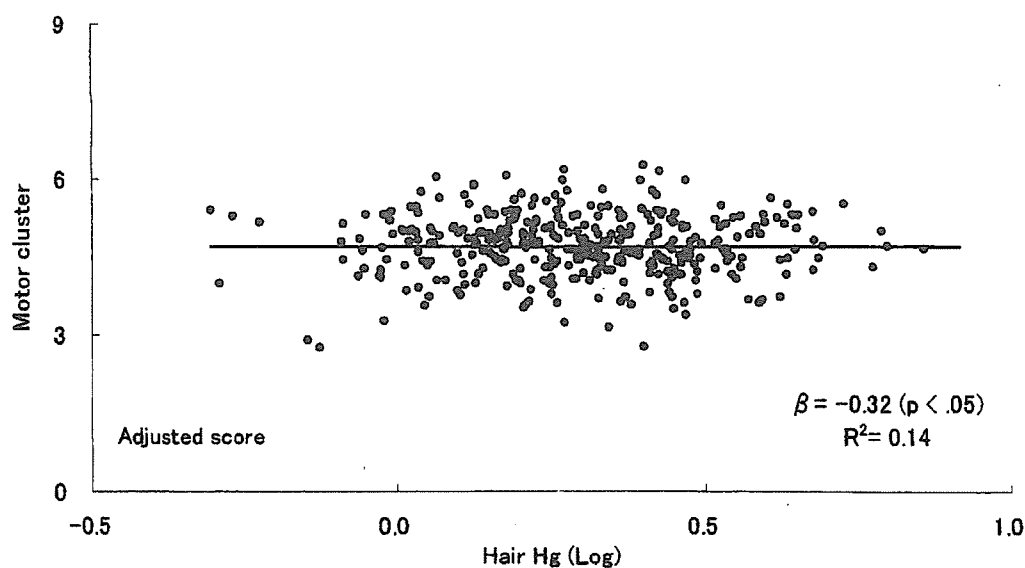


Fig. 3. The association between hair mercury and motor cluster

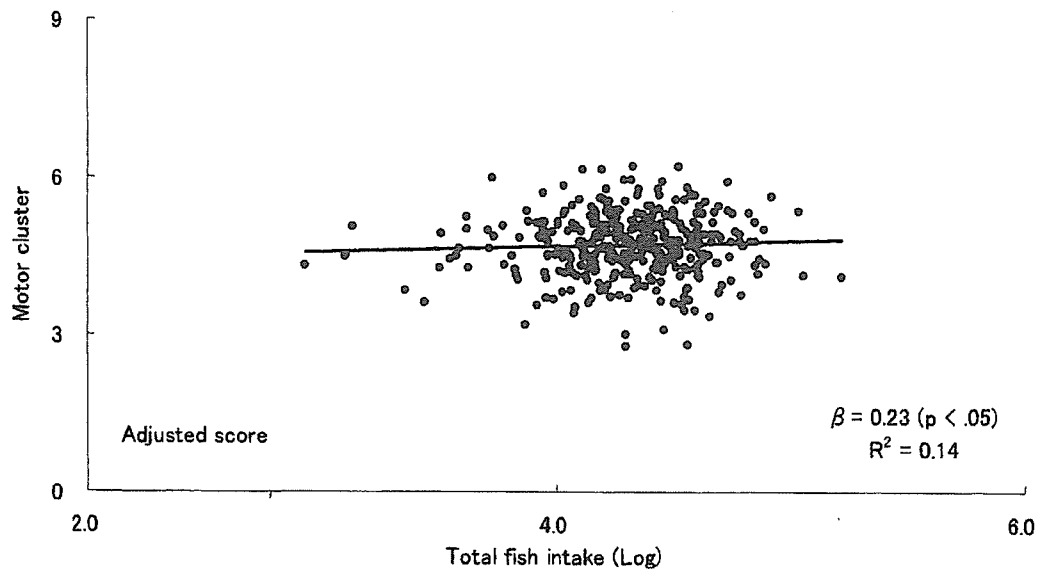


Fig. 4. The association between total fish intake and motor cluster

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ASSOCIATION OF MATERNAL SMOKING DURING PREGNANCY
AND INFANT NEUROBEHAVIORAL STATUS^{1,2}

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Summary.—The association of maternal smoking during pregnancy with neurobehavioral status was examined in 344 Japanese infants. Based on a questionnaire, their mothers were classified into three groups, Nonsmokers, Exsmokers, and Smokers. The Neonatal Behavioral Assessment Scale was administered three days after birth. Among the three groups, on the seven clusters and their 28 behavioral subscales there were no significant differences. The infants of Smokers had lower scores than those of Exsmokers and Nonsmokers on two behavioral items, general tone and peak of excitement. General tone remained significant after adjustment for covariates.

Although the number of cigarette smokers in Japan declined during the 1990s, the numbers of women who smoked increased. The percentage of women who smoked during pregnancy was 5.6% in 1990 and increased to 10.0% in 2000 (Maternal and Child Health Division, Equal Employment,

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Children and Families Bureau, Ministry of Health, Labour and Welfare, 2000). Cigarette smoking of women, especially smoking during pregnancy, continues to be a significant public health concern in Japan.

Maternal smoking during pregnancy is well known to have various effects on the fetus and infant. Children of smoking mothers are often born with lower birth weight or lower gestational age (Matsubara, Kida, Tamakoshi, Wakai, Kawamura, & Ohno, 2000; Weitzman, Byrd, Aligne, & Moss, 2002). For instance, Weitzman, *et al.* (2002) reported that the infants born to smoking mothers weigh an average of 150 to 300 gm less than infants born to nonsmoking mothers. In addition, there is an increase in the risk of premature labour, intrauterine growth retardation, and perinatal mortality as the result of cigarette use in the prenatal period (Mattson, Calarco, Chambers, & Jones, 2002).

Although there are many reports about maternal smoking and infant neurobehavioral status, the effects of maternal smoking on infant neurobehavioral status are poorly understood. Several studies indicated maternal smoking adversely affected infant muscle tone (Fried, Watkinson, Dillon, & Dulberg, 1987; Dempsey, Hajnal, Partridge, Jacobson, Good, Jones, & Ferrero, 2000; Law, Stroud, LaGasse, Niaura, Liu, & Lester, 2003). In a study using the NICU Network Neurobehavioral Scale (NNNS), infants of the smoking group showed more signs of stress or abstinence and were hypertonic and excitable (Law, *et al.*, 2003). A study in which the Prechtl Neurological Examination was used yielded an association between maternal smoking during pregnancy and infant hypertonicity and increased nervous system excitation (Fried, Watkinson, & Gray, 1998). In a study using neurological examination, maternal smoking was significantly associated with infants' muscle-tone abnormalities (Dempsey, *et al.*, 2000). On the other hand, studies in which the Neonatal Behavioral Assessment Scale was used yielded an association of a lower orientation cluster but not muscle tone with maternal smoking (Saxton, 1978; Oyamade, Cole, Johnson, Knight, Westney, Laryea, Hill, Cannon, Fomufod, Westney, Jones, & Edwards, 1994). In other studies using the Neonatal Behavioral Assessment Scale, smoking was not associated with neonatal neurobehavioral status after adjustment for covariates (Richardson, Day, & Taylor, 1989), although effects of alcohol and caffeine intake during pregnancy were found (Jacobson, Fein, Jacobson, Schwartz, & Dowler, 1984). Espy, Riese, and Francis (1997) found associations of negative changes in motor development and vigor cluster with maternal cocaine exposure and alcohol drinking during pregnancy but not an association of neonatal neurobehavioral status with maternal smoking. Although several studies yielded effects of maternal smoking on infants' muscle tone, the results obtained so far are controversial. There have been no reports on the association of infant neurobehavioral status with maternal smoking in Japan.

In an ongoing longitudinal prospective cohort study, the Tohoku Study of Child Development, the effects of perinatal exposure to environmentally persistent organic pollutants or heavy metals on child development are being examined (Nakai, Suzuki, Oka, Murata, Sakamoto, Okamura, Hosokawa, Sakai, Nakamura, Saito, Kurokawa, Kameo, & Satoh, 2004). Since maternal smoking is thought to be an important confounding factor in children's neurobehavioral development, the effects of maternal smoking on child development were independently analyzed. In the present study, the relation of infants' neurobehavioral status with maternal smoking during pregnancy was examined.

METHOD

Subjects

The subjects were 344 singleton babies and their mothers. The healthy pregnant women were registered between January 2001 and August 2002 at obstetrical wards of two hospitals in Sendai, Japan. They were registered with their informed consent for participation in this study according to guidelines established by the ethical committee of Tohoku University Graduate School of Medicine. Mean maternal age at the time of delivery was 30.6 yr. ($SD = 0.8$). The infants were 179 boys and 165 girls. They were full-term (36 to 42 weeks of gestation), with birth weight of over 2500 gm and without congenital anomalies or diseases.

Procedure and Measures

Infant neurobehavioral examination.—Infant neurobehavioral status was examined using the Neonatal Behavioral Assessment Scale (Brazelton, 1973). The scale combines neurological items with an extended behavioral repertoire of the infant in an interactional process (Brazelton & Nugent, 1995). The basic score is composed of 28 behavioral items and 18 reflex items. To compare the evaluations of the infants' behavior, the items that interact in similar ways have been classified into seven clusters describing global functions (Lester, Als, & Brazelton, 1982): (1) Habituation which includes items assessing an infant's reactivity to stimulation from a rattle, bell, light, and mild pin prick, followed by response decrement while in a light sleep state; (2) Orientation which includes attention to visual and auditory stimuli during alert states; (3) Motor which measures the quality of muscle tone and movement; (4) Range of state which includes items related to arousal; (5) Regulation of state which reflects the quality of the infant's responses when aroused and ability to control arousal in response to environmental stimulation; (6) Autonomic stability which includes items assessing physiologic responses to stress; and (7) Reflexes which reflects the number of abnormally elicited reflexes.

Neurobehavioral status was assessed with the Neonatal Behavioral Assessment Scale when the infants were three days old. All examinations took place in a quiet room under predetermined lighting and temperature conditions. Examiners were trained and certified to administer the scale at the Training Center for the Neonatal Behavioral Assessment Scale in Nagasaki University School of Medicine in Japan. Reliability confirmation was conducted throughout data collection to maintain 90% agreement for measurements.

Maternal questionnaire and smoking classification.—Mothers were asked about their characteristics, including smoking and alcohol consumption during pregnancy, on the fourth day after delivery. Maternal smoking was divided into four categories; (1) Never smoked, (2) Ceased to smoke in the past, (3) Ceased to smoke on discovery of pregnancy, and (4) Smoked throughout pregnancy. When the statistical analyses were conducted, mothers were classified into three groups by their smoking habits based on the questionnaire; Nonsmokers including those in (1) and (2), Exsmokers who ceased (3), and Smokers who ceased (4).

Statistical Analysis

For three-group comparisons, homogeneity of variance was assessed by the Levene test. Parametric comparisons used analysis of variance. *Post hoc* analysis was performed with Tukey's *HSD* test. When the Levene test indicated significant differences among groups, a nonparametric test, the Kruskal-Wallis test, was performed. The *post hoc* test employed the Mann-Whitney test. Comparisons of categorical variables were performed by Fisher's exact test. On the Neonatal Behavioral Assessment Scale, seven clusters and their 28 behavioral subscales were used for analysis.

Adjusted mean scores on the Neonatal Behavioral Assessment Scale were compared among groups. To adjust for covariates, analysis of covariance was applied, *post hoc* followed by Tukey's *HSD* test. Potential covariates were selected from maternal and infant characteristics (Table 1 below) by four examiners. There were moderate to strong correlations between birth weight and birth head circumference (Pearson $r = .54$, $p < .01$), and birth weight and birth length (Pearson $r = .71$, $p < .01$). In consideration of multicollinearity, birth head circumference and birth length were excluded from the covariates. All analyses were done using the statistical program JMP, Version 5, for Macintosh computers (SAS Institute Japan, Inc.). Significance was set at 5%.

RESULTS

Status of Maternal Smoking

Twelve of 344 (3.4%) mothers smoked throughout pregnancy. Of the 332 mothers who did not smoke, 44 were Exsmokers and 288 were Non-