

(別紙) 研究成果の概要 (英文)

Title of research project	Historical control data from rat reproductive and developmental toxicity testing, and research into toxicologically significant skeletal variations, such as thoracolumbar supernumerary ribs
Research project number	1607
Research period	FY 2016 – 2018
Name of principal research investigator (PI)	Makiko Kuwagata, DVM, PhD.

Abstract/Summary

In this research, the toxicological significance of fetal skeletal variations, such as thoracolumbar supernumerary ribs (TSR), which are often spontaneously observed in rat reproductive and developmental toxicity tests, was examined. Historical control data from rat reproductive and developmental toxicity testing between 2011 and 2015 using Sprague-Dawley (SD) rats and two sub-strains of Wistar Hannover rats were collected from 24 Japanese laboratories, including 15 pharmaceutical and chemical companies and 9 contract research organizations. The data included maternal reproductive findings at terminal cesarean section and fetal findings, including incidences of spontaneous external, visceral, and skeletal anomalies. In this survey, TSR was observed to have a relatively high incidence and showed strain differences. TSR was observed at around 0.07-12.98% in SD rats and 4.98-58.10% in Wistar Hannover rats, respectively. Fetuses showing TSR have also shown accompanying skeletal anomalies, such as incomplete ossification of thoracic vertebrae and an increase in the number of presacral vertebrae, suggesting that spontaneous TSR may be induced by altered homeobox gene (HOX) expression. As a chemically induced TSR animal model, 75 mg/kg of 5-fluorocytosine (5-FC) was administered orally to rat dams on gestational day (GD) 9 or 13, and fetuses were assessed on GD13 in the afternoon. The effects of 5-FC on Hox genes, which control the anterior-posterior axis, were investigated. As a result, administration of 5-FC on GD9 induced a posterior shift of Hoxa10 expression, resulting in induction of TSR formation. Administration of 5-FC on GD13 resulted in abnormal limb morphology, along with altered Hox11-13 gene expression. Taken together, these findings indicate that the timing of 5-FC administration is associated with particular types of abnormalities, along with disruption of particular Hox genes. TSR and digital morphological changes may represent a continuum of axis and appendicular skeletal anomalies. To examine morphological changes of TSR after birth, rat offspring induced by prenatal 5-FC treatment were monitored in the same animal for up to 9 weeks of age using CT scanning. Although the lengths of the 13th and 14th ribs (TSR) extended with

growth, the ratio of the 14th to the 13th rib did not change during the examination period, indicating that the extension of TSR did not exceed the normal range after birth. The incidences of each type of TSR, such as rudimentary, short and full, during fetal skeletal observation were similar to those during postnatal CT scanning observation. This result indicates that TSR can be evaluated at the fetal stage. Our results also show that both, spontaneous and 5-FC induced TSR, may be caused by altered Hox gene expression. Further studies and research into the mechanisms involved in this altering of Hox gene expression are needed to distinguish spontaneous from chemically induced TSR.

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1 . List of papers published on the basis of this research

1-1. M. Kuwagata, Y. Sakai, S. Tanaka et al., Historical control data on developmental toxicity studies in rats. © Congenital Anomalies, *Accepted on Aug 7. 2018.* doi: 10.1111/cga.12305.

1-2. M.Kuwagata, M. Senuma, M.Todoroki, F. Kumagai, T Kumamoto, T Ogawa Induction of a thoracolumbar supernumerary rib in rat developmental toxicity studies: A short discussion on the critical window. © Congenital Anomalies, *Accepted on Nov 29. 2018.* doi: 10.1111/cga.12320.

1-3. T.Kumamoto, M. Senuma, M. Todoroki, F. Kumagai, H. Imai, R. Suzuki, T. Ogawa, M. Kuwagata., 5-Fluorocytosine induces fetal skeletal malformations in rats by altering expression of Homeobox genes. ©Reproductive Toxicology. *Revised ongoing* (returned revised decision on Apr.16. 2019)

2 . List of presentations based on this research

1) Progress report of historical control data from rat developmental and toxicity tests for past 5 years in Japan

Makiko Kuwagata

Hatano Research Institute, Food and Drug Safety Center.

The 57<sup>th</sup> annual meeting of Japanese Teratology Society. Aug.26-28. 2017 (Tokyo, Waseda)

2) Overview of historical control data from rat developmental and toxicity tests between 2011 and 2015 in Japan

M. Kuwagata<sup>1)</sup>, M. Ema<sup>2)</sup> S. Nishizawa<sup>3)</sup>, M. Fujiwara<sup>4)</sup>, M. Horimoto<sup>5)</sup>, H. Mineshima<sup>6)</sup>

1) Hatano Research Institute, FDSC, 2) AIST, 3) TEIJIN Pharma Inc., 4) Astellas Pharma Inc., 5)

Chiba University of Science、 6) Eisai,

The 57<sup>th</sup> annual meeting of Japanese Teratology Society. Aug.26-28. 2017 (Tokyo, Waseda)

3) Historical control data on Crl:CD(SD) rat developmental and toxicity tests between 2011 and 2015 in Japan

T.Tateishi, Y. Sakai, S. Tanaka, H. Takashima, R. Katagiri, T. Matsuoka, K.Noritake, M. Senuma, T. Shimizu, H. Hojo, K. Ibi, S. Kudo, T. Ohta, M. Ube, Y. Miwa, S. Kajita, T. Uesugi, K. Yabe, T. Tateishi, N. Nakano, T. Taniguchi, A. Yamashita, T. Hirano, Y. Kirihata, Y. Sakai, S. Nishizawa, M. Fujiwara, H. Mineshima, M. Horimoto, M. Ema, M. Kuwagata.

(Core-members and representative researchers of 24 Japanese Laboratories)

The 57<sup>th</sup> annual meeting of Japanese Teratology Society. Aug.26-28. 2017 (Tokyo, Waseda)

4) Historical control data on Wistar Hannover rat developmental and toxicity tests between 2011 and 2015 in Japan

T. Taniguchi, Y. Sakai, S. Tanaka, H. Takashima, R. Katagiri, T. Matsuoka, K.Noritake, M. Senuma, T. Shimizu, H. Hojo, K. Ibi, S. Kudo, T. Ohta, M. Ube, Y. Miwa, S. Kajita, T. Uesugi, K. Yabe, T. Tateishi, N. Nakano, A. Yamashita, T. Hirano, Y. Kirihata, Y. Sakai, S. Nishizawa, M. Fujiwara, H. Mineshima, M. Horimoto, M. Ema, M. Kuwagata,

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The 57<sup>th</sup> annual meeting of Japanese Teratology Society. Aug.26-28. 2017 (Tokyo, Waseda)

5) Preliminary study of mechanisms of thoracolumbar supernumerary ribs induced by 5-flucytocine in rats.

T. Kumamoto<sup>1)</sup>, G. Imai<sup>2)</sup>, R. Suzuki<sup>2)</sup>, T. Ogawa<sup>3)</sup>, K. Kobayashi<sup>4)</sup>, M. Horimoto<sup>5)</sup>, M. Senuma<sup>6)</sup>, M. Kuwagata<sup>6)</sup>

1) Ohu university, School of Pharmaceutical Sciences, 2) Ohu university, School of Dentist, 3) Faculty of Medicine, Saitama Medical University, 4) National Institute of Occupational Safety and Health, Japan, 5) Chiba University of Science, 6) Hatano Research Institute, FDSC.

The 57<sup>th</sup> annual meeting of Japanese Teratology Society. Aug.26-28. 2017 (Tokyo, Waseda)

6) Mechanisms of thoracolumbar supernumerary ribs induced by 5-flucytocine focusing on Hox genes.

T. Kumamoto<sup>1)</sup>, A. Suzuki<sup>1)</sup>, G. Imai<sup>2)</sup>, R. Suzuki<sup>2)</sup>, T. Ogawa<sup>3)</sup>, F. Kumagai<sup>4)</sup>, M. Todoroki<sup>4)</sup>, M.Senuma<sup>4)</sup>, M. Kuwagata<sup>4)</sup>

1) Ohu university, School of Pharmaceutical Sciences 2) Ohu university, School of Dentist、 3) Faculty of Medicine, Saitama Medical University、 4) Hatano Research Institute, FDSC.

The 45<sup>th</sup> Japanese Society of Toxicology, (Osaka) 2018. July. 18-20, 2018

7) Research of thoracolumbar supernumerary ribs induced by 5-flucytocine in rats.

T. Kumamoto<sup>1)</sup>, A. Suzuki<sup>1)</sup>, G. Imai<sup>2)</sup>, R. Suzuki<sup>2)</sup>, T. Ogawa<sup>3)</sup>, F. Kumagai<sup>4)</sup>, M. Todoroki<sup>4)</sup>, M. Senuma<sup>4)</sup>, M. Kuwagata<sup>4)</sup>

1) Ohu university, School of Pharmaceutical Sciences 2) Ohu university, School of Dentist、 3) Faculty of Medicine, Saitama Medical University、 4) Hatano Research Institute, FDSC.

The 58<sup>th</sup> Japanese Teratology Society (Shinjuku, Tokyo) July. 27-29, 2018

8) Effects of skeletal development on fetuses exposed to 5-FC at gestational days 9 and 13 in rats.  
M Senuma<sup>1)</sup>, M. Todoroki<sup>1)</sup>, F. Kumagai<sup>1)</sup>, T. Kumamoto<sup>2)</sup>, T. Ogawa<sup>3)</sup>, M. Kuwagata<sup>1)</sup>

1) Hatano Research Institute, FDSC. 2)Ohu university, School of Pharmaceutical Sciences, 3)  
Faculty of Medicine, Saitama Medical Universit

The 58<sup>th</sup> Japanese Teratology Society (Shinjuku, Tokyo) July. 27-29, 2018

9) Postnatal observations of thoracolumbar supernumerary ribs in rats

M. Kuwagata, F. Kumagai, M. Senuma, M. Todoroki

Hatano Research Institute, FDSC.

The 58<sup>th</sup> Japanese Teratology Society (Shinjuku, Tokyo) July. 27-29, 2018

10) Research on the mechanism of thoracolumbar supernumerary rib development after birth using  
CT scanning

Makiko Kuwagata

Hatano Research Institute, Food and Drug Safety Center,

9th Berlin-Workshop on developmental toxicology as satellite event to the 46th Annual meeting  
of the European teratology society

13. Sep. 2018- 14. Sep. 2018 (Berlin, Germany) *Invited lecture.*

3. The number and summary of patents and patent applications

Not applicable

4. Others (awards, press releases, software and database construction)

Not applicable