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

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# Our Mission

To investigate the toxicological significance of thoracolumbar supernumerary ribs (TSR) after birth.

# Thoracolumbar supernumerary ribs (TSR)

- Classified as a variation.
- Observe with relatively high incidence in a rodent study.

- Researchers' opinions split on the significance of TSR after birth.
- Little reliable data on TSR after birth.
- Difficult to distinguish chemically induced effects from spontaneous development based only on statistically significant results.
- Toxicological meaning is still debatable.

# Historical control data on rat developmental toxicity test in Japan (2011-2015)

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ORIGINAL ARTICLE

## Historical control data on developmental toxicity studies in rats

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According to this survey, TSR(%) is observed at 0.07% to 12.98% in SD rats, and 4.89% to 58.10% in Wistar Hannover rats.

- Data collected from 24 Japanese laboratories, 15 pharmaceutical and chemical companies, and 9 contract research organizations.
- Sprague-Dawley (Crl:CD(SD)) and Wistar Hannover (RccHan:WIST and BrlHan:WIST@Jcl(GALAS)) were used.

# Today's talk

- Using CT scanning, monitor TSR morphological changes after birth in the same animal.

# 5-FC induced TSR rat model
## (postnatal observation)

Chemical : 5-flucytocine (5-FC)
Dose : 0, 35 or 75 mg/kg
Treatment : GD9 (orally)
No. dams : 20 dams per group

After delivery, offspring were culled to 8 offspring per litter (4 males and 4 females) on PND4.

CT scanning : PNDs 4, 14, 26, 35 (male), 42 (female), 53 (male), 61 (male) and 62 (female).

# 5-FC induced TSR rat model (Cont.)

Developmental landmarks : BW, FC, onset of sexual maturation

Organ weights and histopathology at autopsy at terminal point : liver, spleen, kidneys, adrenal glands, testes, epididymides, ovaries, uterus

Autopsy at Terminal point : PNDs 61-63

Skeletal morphology of all offspring will be observed by double skeletal staining.

# Results

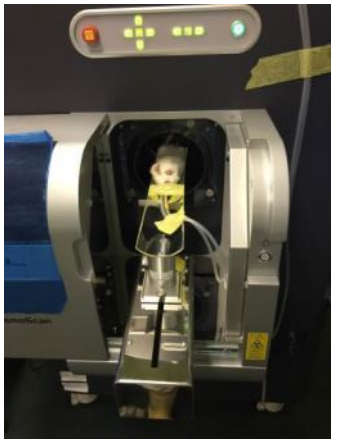
- Dams: No adverse effects on BW, delivery index, number of pups alive, and nursing.
- Offspring: No adverse effects on viability, BW, FC, onset of sexual maturity, OWs.

# Postnatal TSR observation by CT scanning

Monitor TSR during postnatal development in the same animal.

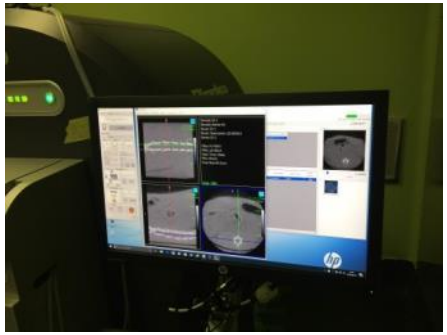
3D micro X-ray computed tomography (CT) for laboratory animals
CosmoScan GXII (RIGAKU, Japan)

1. Anesthesia



2. Set animal

3. Scanning

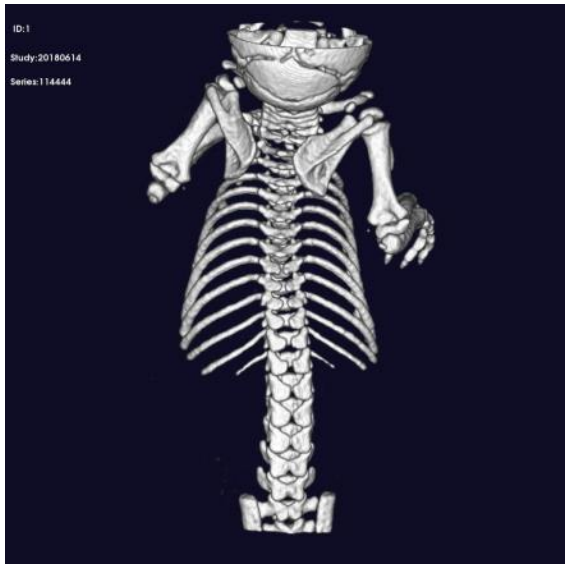


4. Analysis

# Postnatal TSR observation by CT scanning

## Analysis

1. 3D picture




Monitor rib morphology
(types of TSR; rudimentary, short, full)

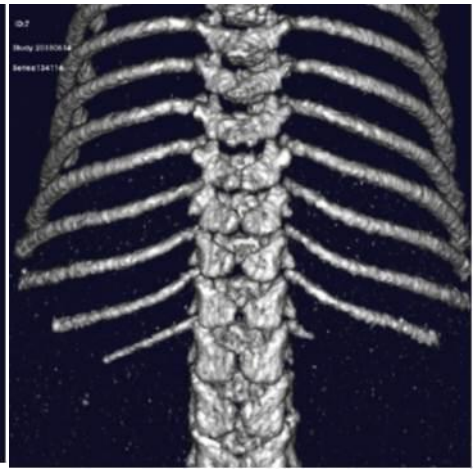
2. MIP (maximum intensity projection) picture

Measurement the length of ribs
(ratio of $14^{th}$ rib to $13^{th}$ rib)

# Postnatal TSR observation by CT scanning

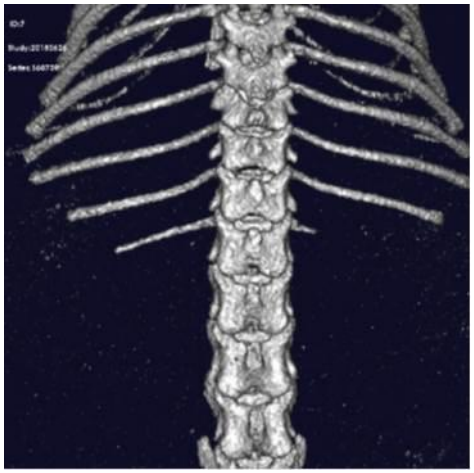
1. 3D picture


PND4

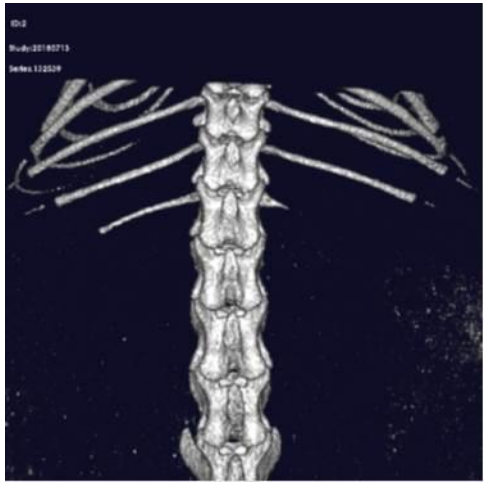


PND14

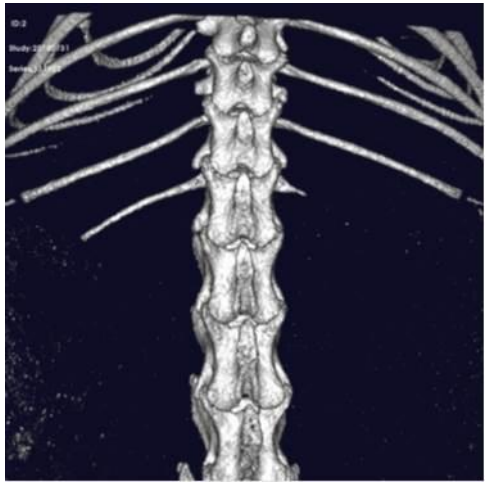
PND26



PND35 (pre-puberty)



PND43 (after-puberty)



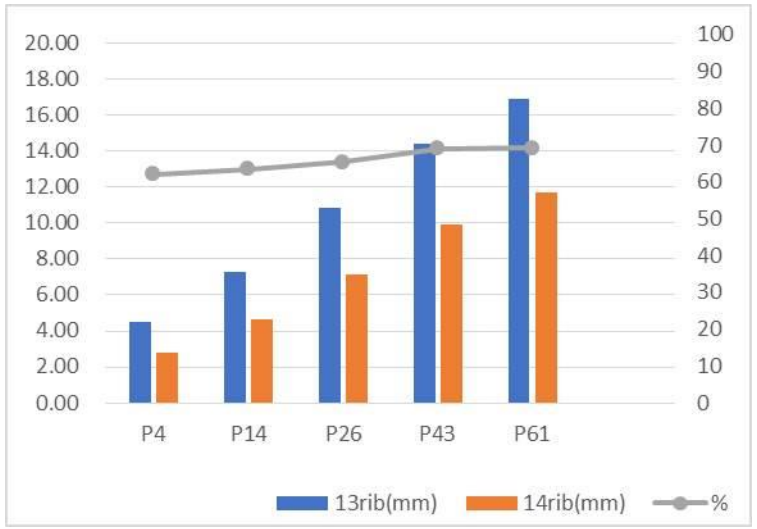
PND61

Animal no.5FC-H2, F7
Left side: full type
Right side: short type

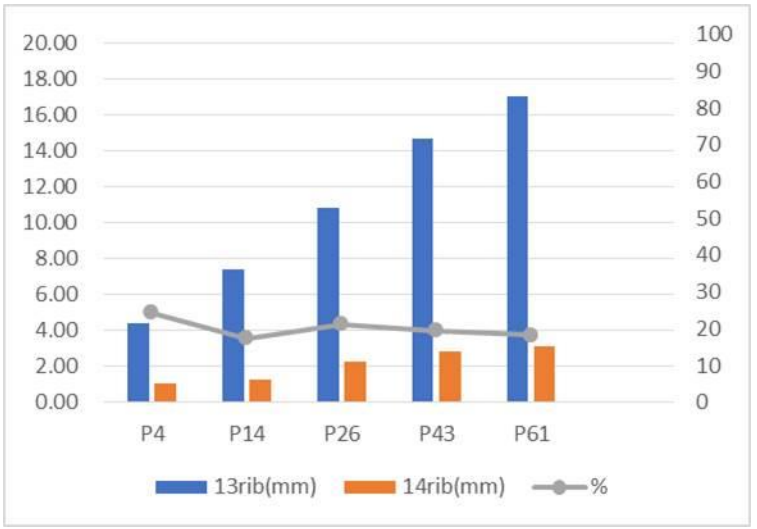
# Postnatal TSR observation by CT scanning

## 2. MIP picture

Left (full type)




Right (short type)




- Measure length of the 13$^{th}$ and 14$^{th}$ ribs (mm)
- Calculation of the ratio of 14$^{th}$ rib to 13$^{th}$ rib (%)

TSR develop within the normal range, but do not exceed the normal range after birth.

- Sexual maturation did not affect the features of TSR.

Animal no.5FC-H2, F7
Left side: full type
Right side: short type

# Ongoing project

- Using CT scanning, monitor TSR morphological changes after birth in the same animal. (Today's talk)
- Characteristics of 5-FC-induced TSR (The critical window is narrow and earlier than the ordinary administration time : *under submission*).
- Researching the mechanism of TSR induced by 5-FC (Contribution on HOX10 gene : *in preparation*).

**Final goal**

Determine the toxicological significance of TSR in ReproTox studies.

# A Goal to reach for…

The results obtained hint at the significance of TSR in reproductive and developmental toxicity studies.

Now, proceeding with the analysis.

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