Research on the mechanism of thoracolumbar supernumerary rib by use of computed tomography (CT)

Makiko Kuwagata DVM PhD

National Institute of Health Sciences, CBSR,
Division of Cellular and Molecular Toxicology,
Kawasaki, Japan

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Our Mission

To investigate the toxicological significance of thoracolumbar supernumerary ribs (TSRs) in a reproductive and developmental toxicity study.

Normal

TSR

CT image of PND14 rat offspring

Epidemiolocal survey in Japan（Nakajima et al., 2014）

Insights Imaging (2014) 5:77–83
DOI 10.1007/s13244-013-0286-0

ORIGINAL ARTICLE

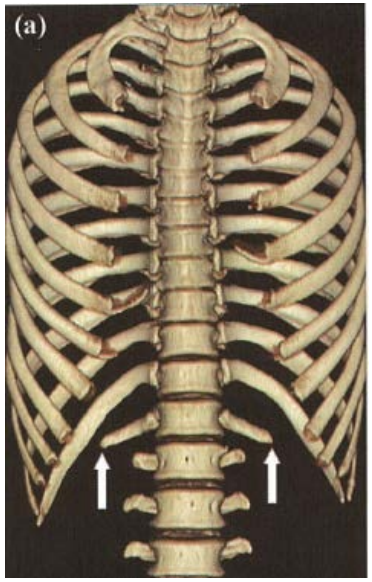
The prevalence of morphological changes in the thoracolumbar spine on whole-spine computed tomographic images

Aya Nakajima · Akihito Usui · Yoshiyuki Hosokai · Yusuke Kawasumi · Kenta Abiko · Masato Funayama · Haruo Saito

13/211 (5.8%)

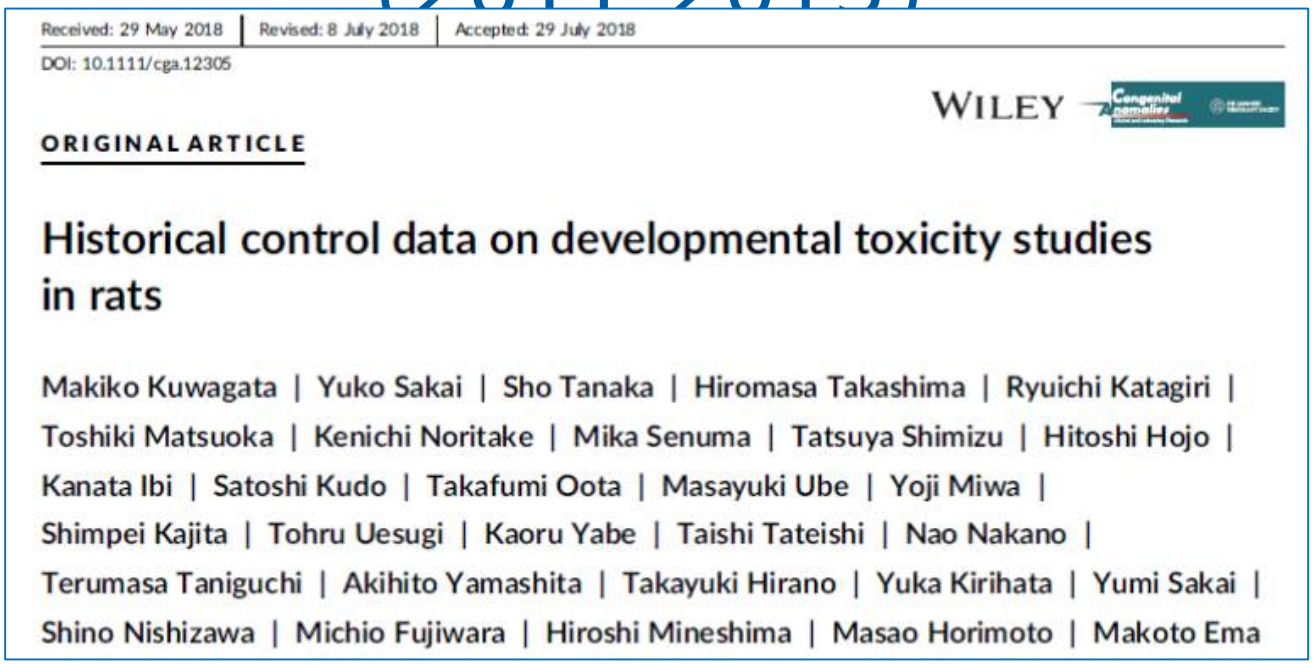
Table 2 The prevalence of different rib configurations by vertebral configuration type, subdivided into lumbarisation, normal and sacralisation

Rib configuration	Lumbarisation	Vertebral configuration Normal configuration	Sacralisation	Total
Agenesis of 12th ribs	0 -	1 (0.5 %)	1 (16.7 %)	2 (0.9 %)
Normal configuration	3 (21.4 %)	203 (98 5 %)	5 (83.3 %)	211 (93.4 %)
Lumbar ribs	11 (78.6 %)	2 (1.0 %)	0 -	13 (5.8 %)
Unilareral	2	1		3
Bilateral	9	1		10
No. of cases	14	206	6	226



		Male	Female	Total
Rib anomalies	Lumber ribs (interpreted as 13 pairs of ribs)	11 (7.4 %)	2 (2.6 %)	13 (5.8 %)

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



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WILEY

ORIGINAL ARTICLE

Historical control data on developmental toxicity studies in rats

Makiko Kuwagata | Yuko Sakai | Sho Tanaka | Hiromasa Takashima | Ryuichi Katagiri | Toshiki Matsuoka | Kenichi Noritake | Mika Senuma | Tatsuya Shimizu | Hitoshi Hojo | Kanata Ibi | Satoshi Kudo | Takafumi Oota | Masayuki Ube | Yoji Miwa | Shimpei Kajita | Tohru Uesugi | Kaoru Yabe | Taishi Tateishi | Nao Nakano | Terumasa Taniguchi | Akihito Yamashita | Takayuki Hirano | Yuka Kirihata | Yumi Sakai | Shino Nishizawa | Michio Fujiwara | Hiroshi Mineshima | Masao Horimoto | Makoto Ema

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

- Data were 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.

Thoracolumbar supernumerary ribs (TSR)

- Classified as a variation.
- Relatively high incidence observed in a rodent study.

- Researchers' opinions split on the significance of TSR.
- 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.

Today's talk

- Using CT, we monitored morphological changes in TSR after birth in the same animal.

Discuss toxicological significance of TSR.

Chemically induced TSR animal model

Chemical: 5-flucytocine (5-FC)

- Antifungal compound, inhibits cell division
- TSR was induced in rat fetuses after treating dams orally with 5-FC on GD9 (Horimoto et al., JSOT meeting, 2014).

Incidence of TSR in GD20 fetuses treated with 5-FC

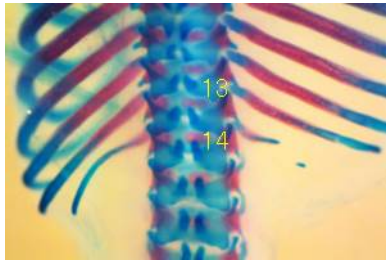
Table S3 Skeletal examination of rat fetuses following exposure of dams to 5-FC on GD9.

Group	Control	5-FC-a	
Number of dams	9	8	
Number of fetuses examined	111	99	
Each type of anomaly			
TSR (total)	2	73	
	1.6 ± 4.8	75.8 ± 25.7	**
Types of TSR			
Rudimentary	0	48	
	0.0 ± 0.0	46.8 ± 19.6	**
Short	2	14	
	1.6 ± 4.8	14.6 ± 13.9	
Full	0	30	
	0.0 ± 0.0	34.3 ± 32.0	**

Dose:75 mg/kg (5 mL/kg)
Vehicle: 0.5 % CMC-Na

(Kuwagata et al., Accepted; Nov.2018)

Reproducibility of the previous study (Horimoto et al.) was confirmed.



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: 9 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: liver, spleen, kidneys, adrenal glands, testes, epididymides, ovaries, uterus

Autopsy: PNDs 61-63

Results

- Dams: No adverse effects on BW, delivery index, number of pups alive, or nursing.
- Offspring: No adverse effects on viability, BW, FC, onset of sexual maturity, organ weights, or histopathology.

Postnatal TSR observation by CT

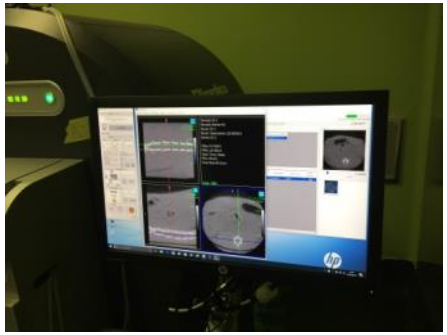
Monitor TSR during postnatal development in the same animal.

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

1. Anesthesia

2. Set animal

3. Scanning

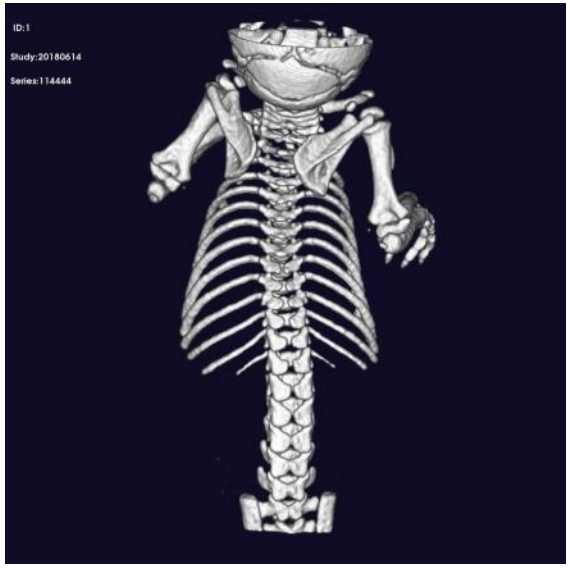


4. Analysis

Postnatal TSR observation by CT

Analysis

1. 3D picture



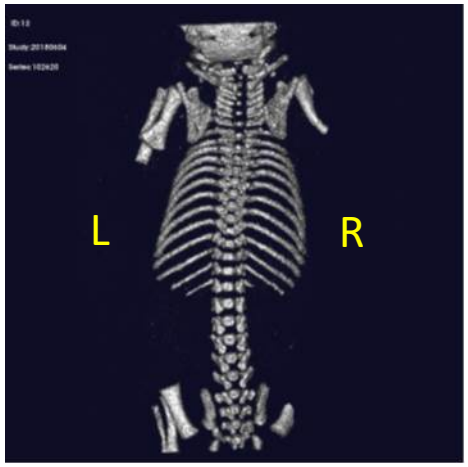
Monitor rib morphology
(types of TSR: rudimentary, short, full)

2. Maximum intensity projection (MIP) picture

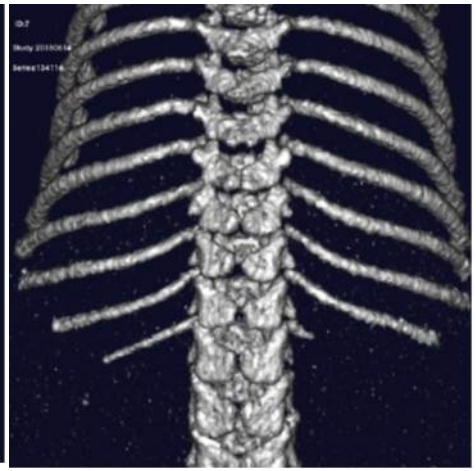
Measurement the rib length
(ratio of 14th rib to 13th rib)

Postnatal TSR observation by CT

1. 3D picture

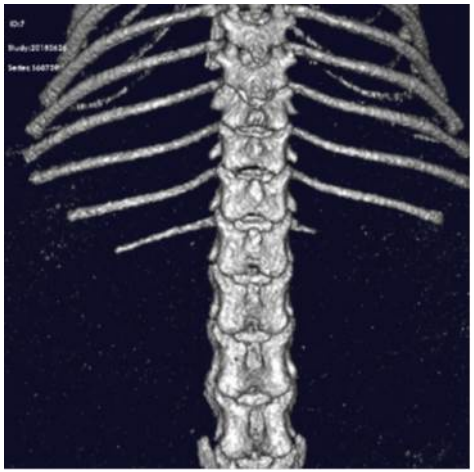


PND4

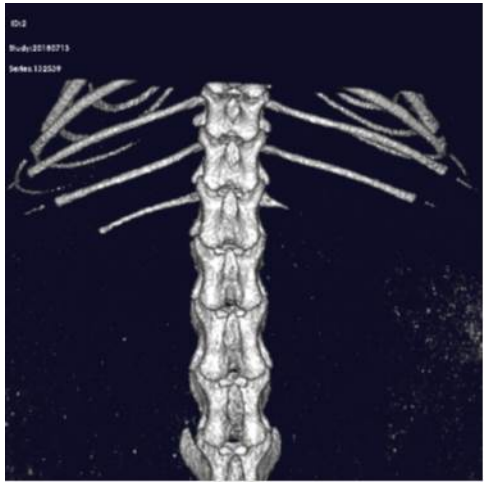


PND14

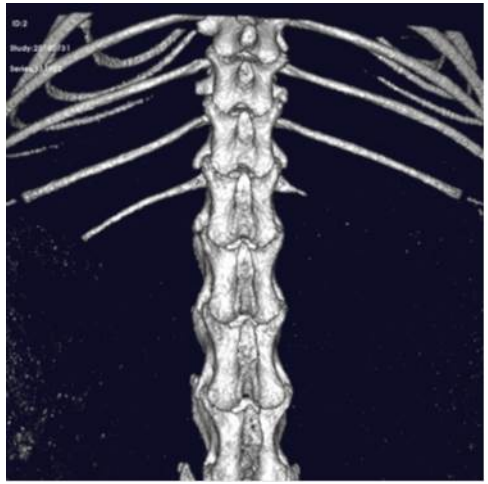
PND26



PND35 (pre-puberty)



PND43 (post-puberty)



PND61

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

Incidence of TSR (Male offspring)

Group	Control				5FC-L				5FC-H				
Number of dams	9				9				8				
No. of male offspring examined (pre- culling)	(70)				(62)				(61)				
Male offspring showing TSR (pre-culling)	8.5	±	20.1	(7)	30.6	±	32.0	(19)	61.0	±	33.0	*	(24) *
P4 (pre-culling)													
Rudimentary	11.6	±	21.3	(9)	26.2	±	20.5	(18)	44.4	±	27.9	*	(28) *
Short	0.0	±	0.0	(0)	4.2	±	9.6	(3)	10.2	±	13.0		(6)
Full	0.0	±	0.0	(0)	3.2	±	6.3	(2)	12.5	±	23.1		(7)
Offspring for CT examination (after culling)													
No. of male offspring examined	36				36				34				
Male offspring showing TSR	13.9	±	22.0	(5)	44.4	±	42.9	(16)	67.3	±	41.3	*	(24)
P4													
Rudimentary	13.9	±	22.0	(5)	33.3	±	30.6	(12)	60.0	±	36.7	*	(21) *
Short	0.0	±	0.0	(0)	5.6	±	11.0	(2)	12.5	±	26.7		(4)
Full	0.0	±	0.0	(0)	5.6	±	11.0	(2)	11.5	±	24.0		(5)
P14													
Rudimentary	13.9	±	22.0	(5)	33.3	±	30.6	(12)	60.0	±	36.7	*	(21) *
Short	0.0	±	0.0	(0)	5.6	±	11.0	(2)	12.5	±	26.7		(4)
Full	0.0	±	0.0	(0)	5.6	±	11.0	(2)	11.5	±	24.0		(5)
P26													
Rudimentary	13.9	±	22.0	(5)	33.3	±	30.6	(12)	60.0	±	36.7	*	(21) *
Short	0.0	±	0.0	(0)	5.6	±	11.0	(2)	12.5	±	26.7		(4)
Full	0.0	±	0.0	(0)	5.6	±	11.0	(2)	11.5	±	24.0		(5)
P35													
Rudimentary	13.9	±	22.0	(5)	33.3	±	30.6	(12)	60.0	±	36.7	*	(21) *
Short	0.0	±	0.0	(0)	5.6	±	11.0	(2)	12.5	±	26.7		(4)
Full	0.0	±	0.0	(0)	5.6	±	11.0	(2)	11.5	±	24.0		(5)
P53													
Rudimentary	13.9	±	22.0	(5)	33.3	±	30.6	(12)	60.0	±	36.7	*	(21) *
Short	0.0	±	0.0	(0)	5.6	±	11.0	(2)	12.5	±	26.7		(4)
Full	0.0	±	0.0	(0)	5.6	±	11.0	(2)	11.5	±	24.0		(5)
P60													
Rudimentary	13.9	±	22.0	(5)	33.3	±	30.6	(12)	60.0	±	36.7	*	(21) *
Short	0.0	±	0.0	(0)	5.6	±	11.0	(2)	12.5	±	26.7		(4)
Full	0.0	±	0.0	(0)	5.6	±	11.0	(2)	11.5	±	24.0		(5)

Rudimentary > Full $\geq$ Short

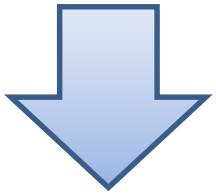
Short, less than half the length of the 13th thoracic rib; Full, half or greater than half the length of the 13th thoracic rib.

Value, Mean ± SD (n)

*, Significantly different from the control group at $p<0.05$

Incidence of TSR (Male offspring)

Group	Control	5FC-L	5FC-H
Number of dams	9	9	8
Offspring for CT examination (after culling)			
No. of male offspring examined	36	36	34
Male offspring showing TSR	13.9 ± 22.0 (5)	44.4 ± 42.9 (16)	67.3 ± 41.3 * (24)
PND 4			
Rudimentary	13.9 ± 22.0 (5)	33.3 ± 30.6 (12)	60.0 ± 36.7 * (21) *
Short	0.0 ± 0.0 (0)	5.6 ± 11.0 (2)	12.5 ± 26.7 (4)
Full	0.0 ± 0.0 (0)	5.6 ± 11.0 (2)	11.5 ± 24.0 (5)
PND 60			
Rudimentary	13.9 ± 22.0 (5)	33.3 ± 30.6 (12)	60.0 ± 36.7 * (21) *
Short	0.0 ± 0.0 (0)	5.6 ± 11.0 (2)	12.5 ± 26.7 (4)
Full	0.0 ± 0.0 (0)	5.6 ± 11.0 (2)	11.5 ± 24.0 (5)



No change in TSR type

Incidence of TSR (Female offspring)

Group	Control				5FC-L				5FC-H				
Number of dams	9				9				8				
No. of female offspring examined (pre-culling)	53				55				46				
Female offspring showing TSR (pre-culling)	7.4	±	16.9	(3)	33.0	±	34.4	(16)	63.5	±	36.9	*	(29) *
P4 (pre-culling)													
Rudimentary	7.4	±	16.9	(3)	27.9	±	18.6	(15) #	53.1	±	25.5	*	(27) *
Short	0.0	±	0.0	(0)	16.1	±	27.8	(6)	13.8	±	18.7		(4)
Full	0.0	±	0.0	(0)	2.8	±	8.3	(1)	16.9	±	18.2		(5)
Offspring for CT examination (after culling)													
No. of female offspring examined	36				36				30				
Female offspring showing TSR	8.3	±	17.7	(3)	33.3	±	37.5	(12)	65.2	±	42.2	*	(19) *
P4													
Rudimentary	8.3	±	17.7	(3)	25.0	±	25.0	(9)	48.5	±	36.4	*	(15) *
Short	0.0	±	0.0	(0)	16.7	±	28.0	(6)	16.7	±	19.4		(4)
Full	0.0	±	0.0	(0)	2.8	±	8.3	(1)	16.7	±	19.4		(4)
P14													
Rudimentary	8.3	±	17.7	(3)	25.0	±	25.0	(9)	48.5	±	36.4	*	(15) *
Short	0.0	±	0.0	(0)	16.7	±	28.0	(6)	16.7	±	19.4		(4)
Full	0.0	±	0.0	(0)	2.8	±	8.3	(1)	16.7	±	19.4		(4)
P26													
Rudimentary	8.3	±	17.7	(3)	25.0	±	25.0	(9)	48.5	±	36.4	*	(15) *
Short	0.0	±	0.0	(0)	16.7	±	28.0	(6)	16.7	±	19.4		(4)
Full	0.0	±	0.0	(0)	2.8	±	8.3	(1)	16.7	±	19.4		(4)
P43													
Rudimentary	8.3	±	17.7	(4)	25.0	±	25.0	(9)	48.5	±	36.4	*	(15) *
Short	0.0	±	0.0	(0)	16.7	±	28.0	(6)	16.7	±	19.4		(4)
Full	0.0	±	0.0	(0)	2.8	±	8.3	(1)	16.7	±	19.4		(4)
P61													
Rudimentary	8.3	±	17.7	(4)	25.0	±	25.0	(9)	48.5	±	36.4	*	(15) *
Short	0.0	±	0.0	(0)	16.7	±	28.0	(6)	16.7	±	19.4		(4)
Full	0.0	±	0.0	(0)	2.8	±	8.3	(1)	16.7	±	19.4		(4)

Short, less than half the length of the 13th thoracic rib; Full, half or greater than half the length of the 13th thoracic rib.
Value, Mean ± SD (n)
*, Significantly different from the control group at $p<0.05$
#, Significantly different from the 5FC-H group at $p<0.05$

Incidence of TSR (Female offspring)

Group	Control				5FC-L				5FC-H				
Number of dams	9				9				8				
No. of female offspring	36				36				30				
Female offspring showing TSR	8.3	±	17.7	(3)	33.3	±	37.5	(12)	65.2	±	42.2	*	(19) *
PND 4													
Rudimentary	8.3	±	17.7	(3)	25.0	±	25.0	(9)	48.5	±	36.4	*	(15) *
Short	0.0	±	0.0	(0)	16.7	±	28.0	(6)	16.7	±	19.4		(4)
Full	0.0	±	0.0	(0)	2.8	±	8.3	(1)	16.7	±	19.4		(4)
PND 61													
Rudimentary	8.3	±	17.7	(4)	25.0	±	25.0	(9)	48.5	±	36.4	*	(15) *
Short	0.0	±	0.0	(0)	16.7	±	28.0	(6)	16.7	±	19.4		(4)
Full	0.0	±	0.0	(0)	2.8	±	8.3	(1)	16.7	±	19.4		(4)

No change in TSR type

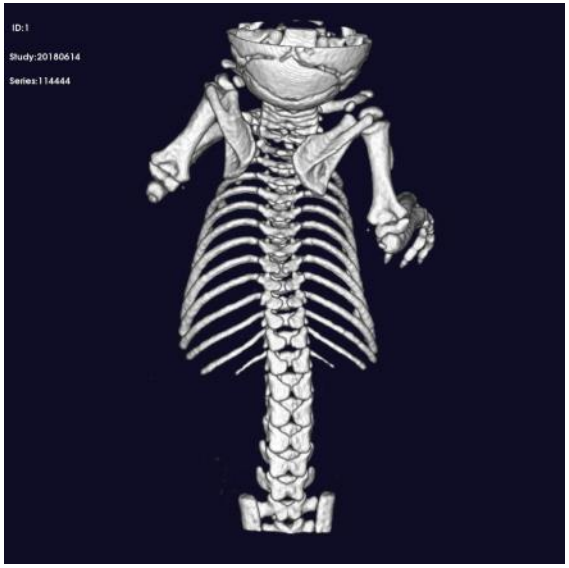
No gender difference !

No left and right difference !!

Postnatal TSR observation by CT

Analysis

1. 3D picture



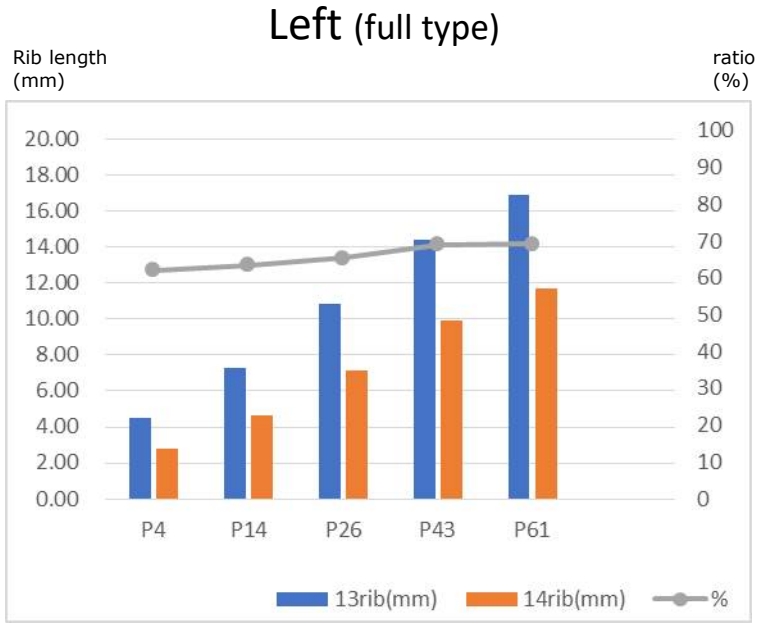
Monitor rib morphology
(types of TSR: rudimentary, short, full)

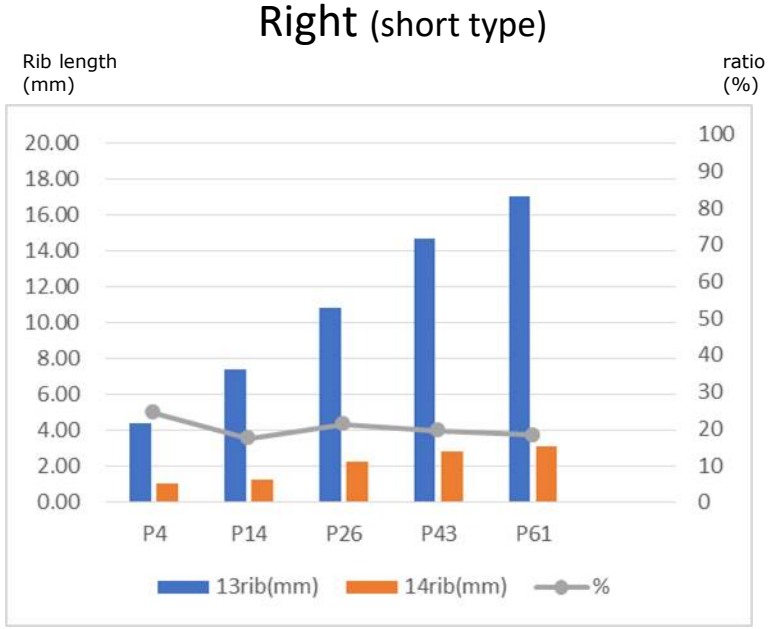
2. Maximum intensity projection (MIP) picture

Measurement the rib length
(ratio of 14th rib to 13th rib)

Postnatal TSR observation by CT

2. MIP picture





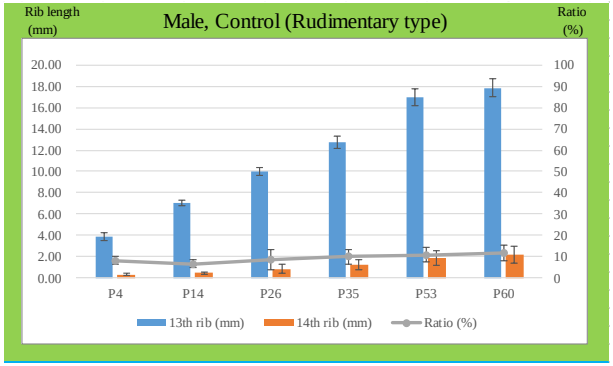
Animal no.5FC-H2, F7
Left side: full type
Right side: short type

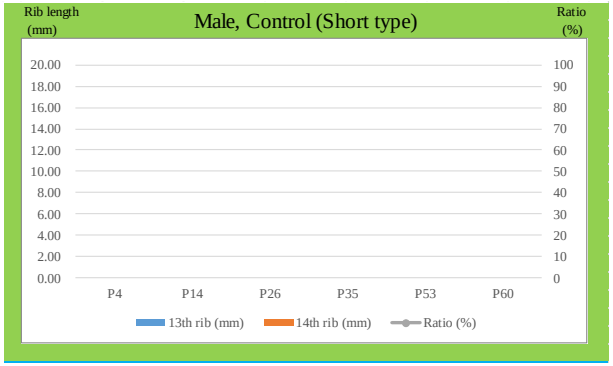
- Measure length of the 13th and 14th ribs (mm)
- Calculate of the ratio of 14th rib to 13th rib (%)

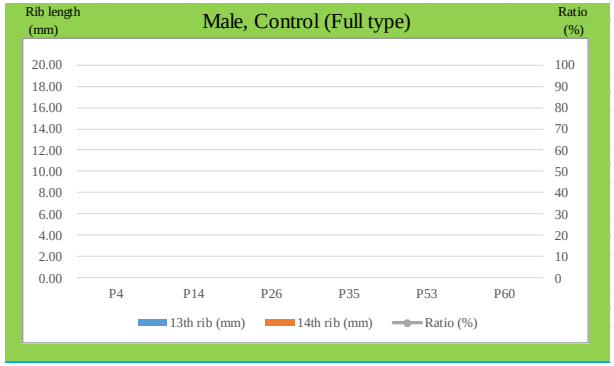
TSR develops within the normal range, but does not exceed the normal range after birth.

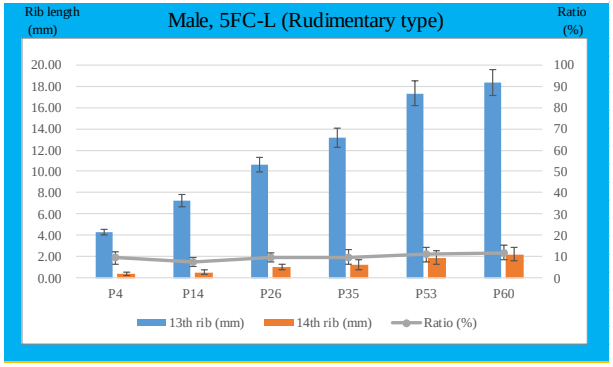
- Sexual maturation did not affect the TSR features.

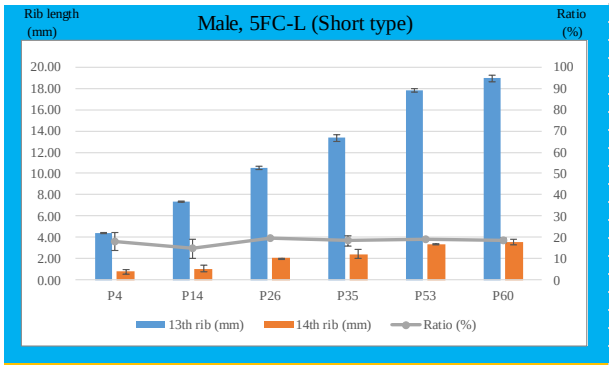
Postnatal TSR observation by CT （Male offspring）

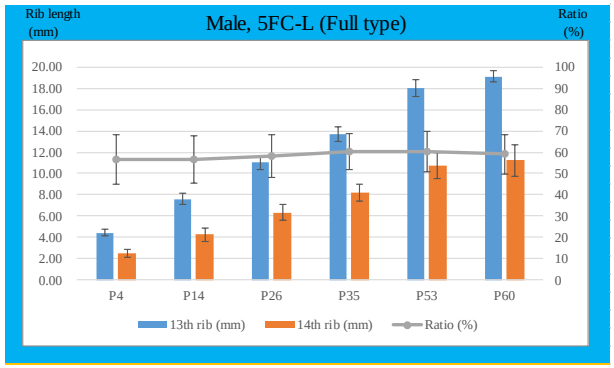


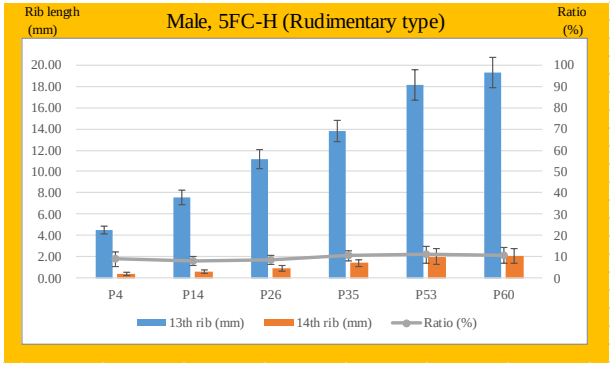


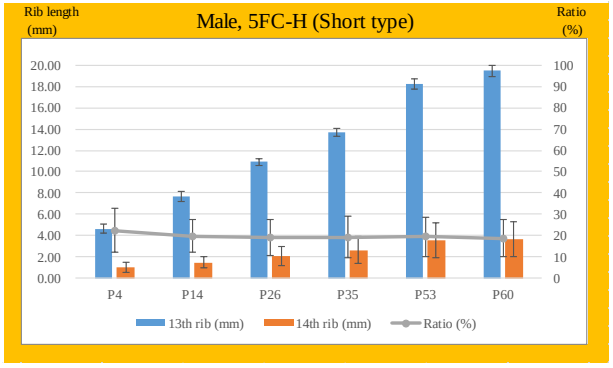


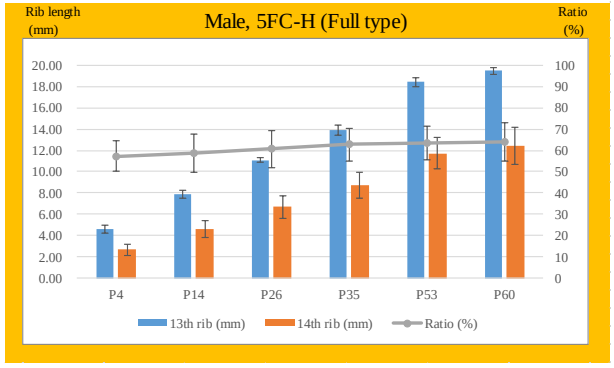










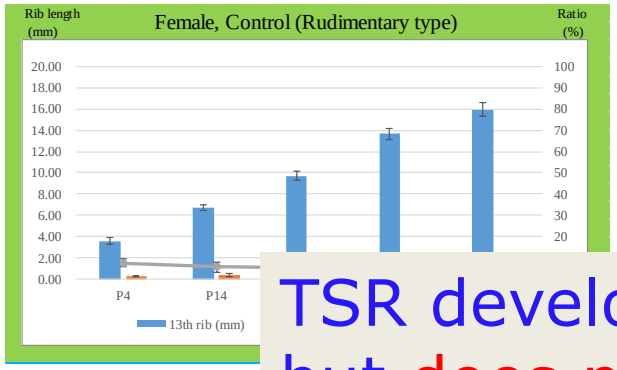


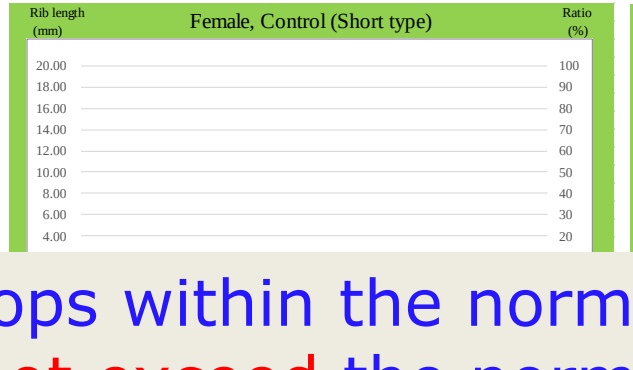
Rudimentary

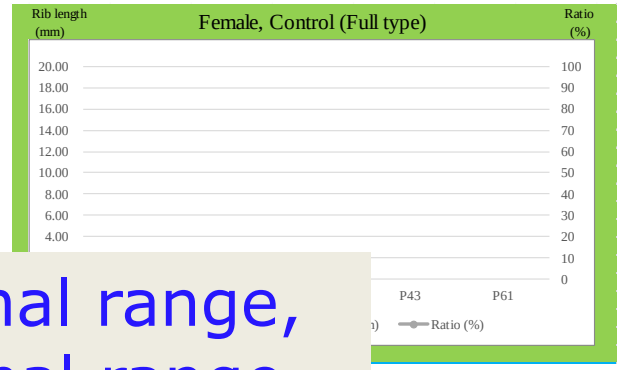
Short

Full

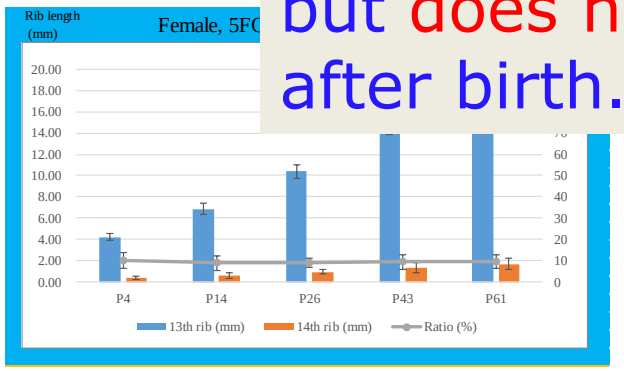
Postnatal TSR observation by CT (Female offspring)

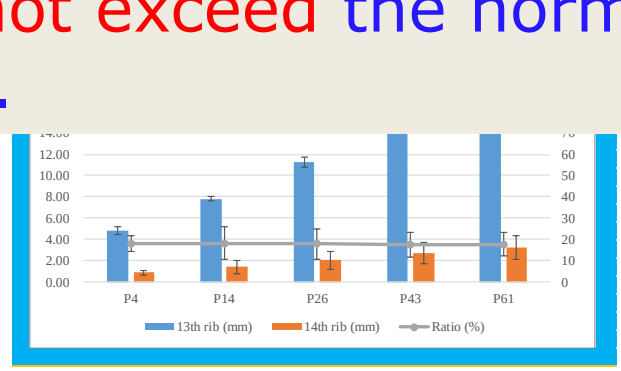


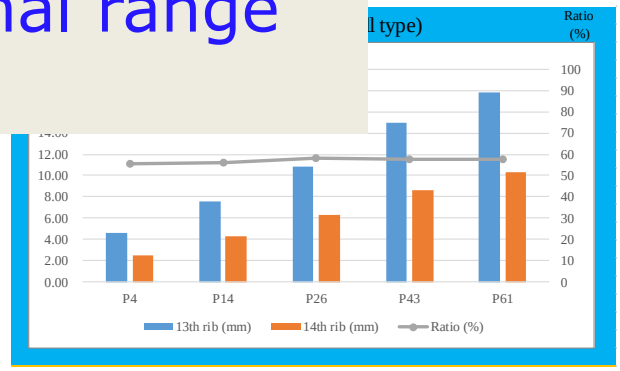


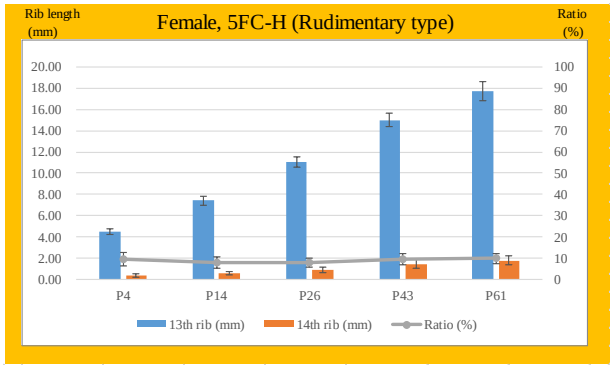


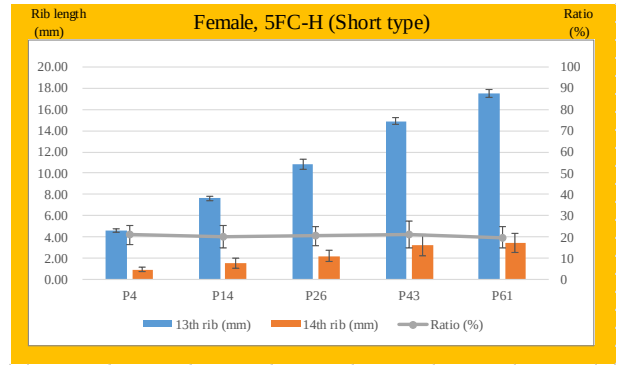
TSR develops within the normal range, but does not exceed the normal range after birth.

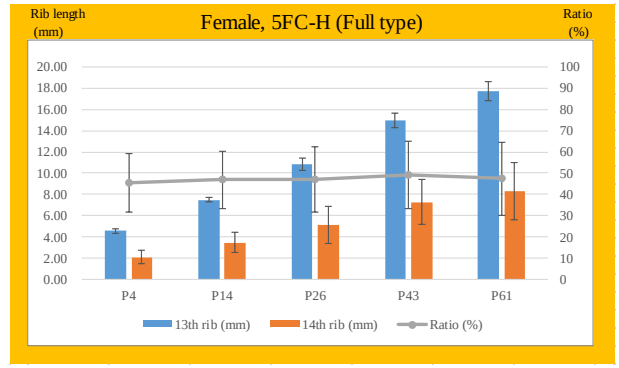












Rudimentary

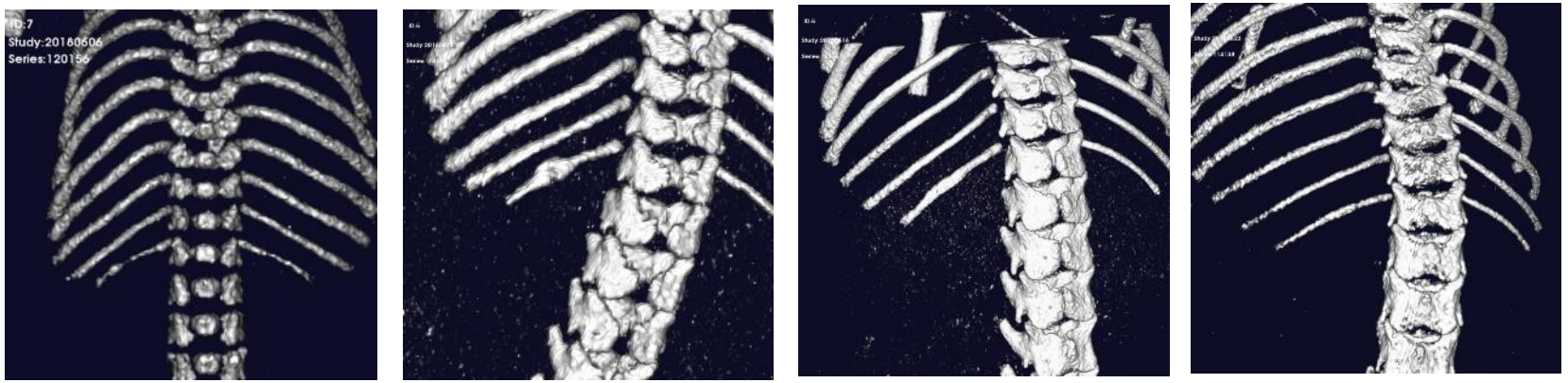
Short

Full

Other findings

From my desk

1. Rib nodule



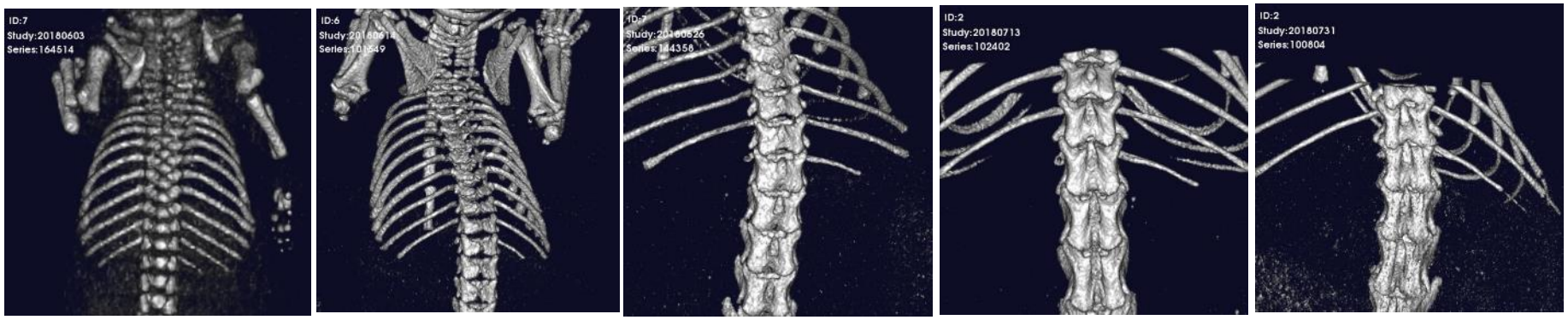
PND4 PND11 PND16 PND21

Animal no.5FC-H11, F5
Left side: nodule of the 13th rib

The nodule was gradually less clear on the CT image as it grew, and after weaning the nodule was not observed.

Rib nodule: Recovery after birth

2. Short rib (the 13th rib)



PND4 PND14 PND26 PND43 PND61

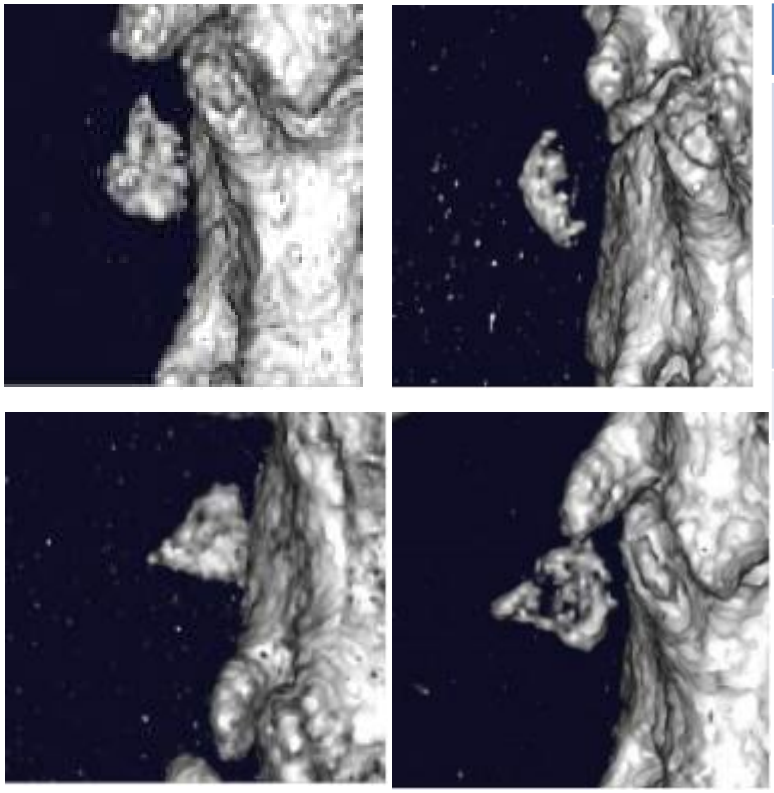
Animal no. C6, F6
The 13th short rib in the left side

The short rib was observed until terminal autopsy.

Short rib: No recovery after birth

3. Various types of rudimentary TSR

Various shape



Fusion rate with the vertebra

	Male offspring		Female offspring	
Group	Fusion rate (%)	Fusion type / Total rudimentary (Litter)	Fusion rate (%)	Fusion type / Total rudimentary (Litter)
Control	37.5	3/8 (4) [a]	57.1	4/7 (2)
5FC-L	26.3	5/19 (6)	23.5	4/17 (6)
5FC-H	21.7	10/46 (8)	30.0	6/20 (6)

a, the ratio of the number of rudimentary TSRs fused with the vertebra to the total number of rudimentary TSRs.

Some were incorporated into the thoracic vertebra, but not all.

And…

Tokyo station

The characteristics of the critical window for chemically induced TSR (5-FC and SAL)

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SHORT COMMUNICATION

Induction of a thoracolumbar supernumerary rib in rat developmental toxicity studies: A short discussion on the critical window

Makiko Kuwagata[1] | Mika Senuma[1] | Mai Todoroki[1] | Fumiaki Kumagai[1] | Takayuki Kumamoto[2] | Tetsuo Ogawa[3]

Comparison with characteristics of the critical window for TSR induced by 5-FC and sodium salicylate (SAL).

To investigate the characteristics of the critical window for chemically induced TSR, SD rats were administered 5-FC or SAL at one of three time points on GD9: early morning, midday, or late afternoon.

TABLE 1 TSR incidences in rat fetuses following exposure of dams to 5-FC or SAL at three treatment times on GD9

a) 5-FC[a] (75 mg/kg)				(on GD20 fetus)
Group	**Control**	**5-FC-a**	**5-FC-b**	**5-FC-c**
Number of dams	9	8	8	7
No. of fetuses examined	111	99	100	71
Incidence of TSR (%)				
Mean ± S.D. per dam (no. of fetuses)	1.6 ± 4.8 (2)	75.8 ± 25.7 (73)[b]	25.7 ± 32.5 (26)	13.5 ± 29.0 (9)[c]
		+++		

b) SAL[d,e] (180 mg/kg)				(on GD20 fetus)
Group	**Control**	**SAL-a**	**SAL-b**	**SAL-c**
Number of dams	7	7	7	7
No. of fetuses examined	82	79	78	66
Incidence of TSR (%)				
Mean ± S.D. per dam (no. of fetuses)	11.4 ± 15.2 (8)	41.1 ± 26.0 (32)	32.3 ± 30.3 (24)	19.8 ± 31.7 (9)
		++	++	

[a] 5-FC-a, treated with 5-FC at 7:00 AM; 5-FC-b, treated with 5-FC at 1:00 PM; 5-FC-c, treated with 5-FC at 7:00 PM.
[b] $P < 0.01$ compared with the control group.
[c] $P < 0.01$ compared with the 5-FC-a group.
[d] Not significantly different among any group.
[e] SAL-a, treated with SAL at 7:00 AM; SAL-b, treated with SAL at 12:00 PM; SAL-c, treated with SAL at 4:00 PM.

Different characteristics of the critical period for TSR between chemicals

Variability in the incidence of TSR observed in DevTox studies

Not a good toxicological landmark ?

The critical window for TSR is different between two drugs. 5-FC has a shorter critical window of TSR than SAL.

Conclusion and Discussion

1. Historical control data

・Relatively high incidence in rats

・Strain difference between SD rats and Wistar Hannover rats

Incidence: Wistar Hannover >>SD

2. Postnatal TSR change

・TSR develops within the normal range, but does not exceed the normal range after birth.

* Based on fetal observations in a developmental study, it is possible to predict postanal changes in TSR.

3. Different characteristics of the critical period for TSR between chemicals

・May cause variability in the incidence of TSR observed in developmental toxicity studies

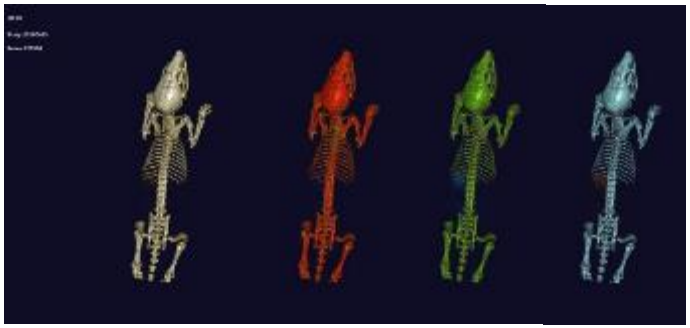
* Not a good toxicological landmark ?

Conclusion and Discussion (cont.)

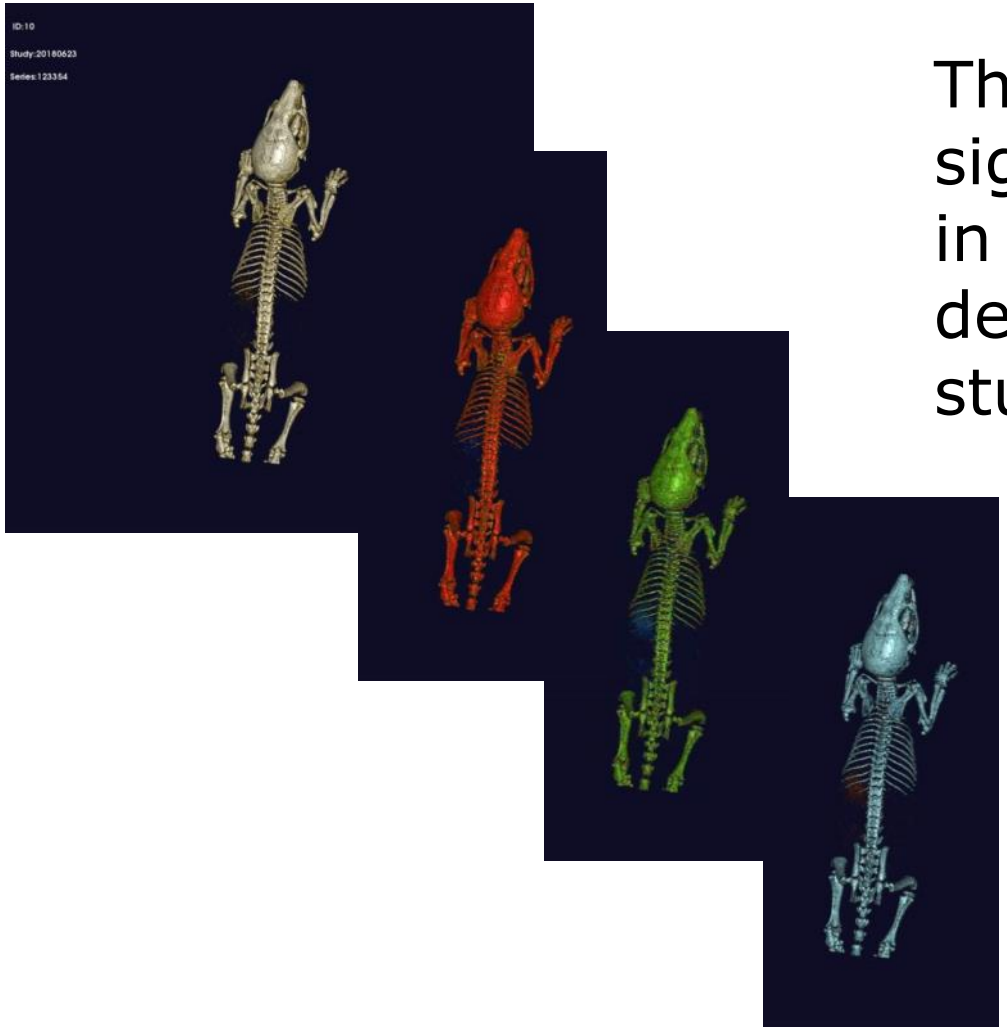
4. TSR is a target for ARfD？

Not suitable.
TSR was observed to have a relatively high incidence spontaneous incidence and exhibited strain differences .
Different critical windows for TSR induced by chemicals are considered.

* Not all chemically induced TSRs are detected.
* Wide variation in the incidence of TSR between studies



A goal to reach for…



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

Thank you for your attention

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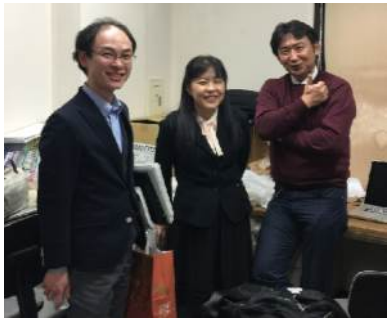
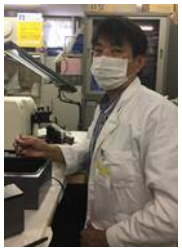
CT experiment
Hatano Research Institute, Food and Drug Safety Center, Hadano, Kanagawa, Japan

Moved

My team
(In front of the front door at NIHS)



Hox gene analysis
Dr. Kumamoto
Dr. Imai, Dr. Suzuki (Ohu Univ.)



Fetal histopathology
Dr. Ogawa
(Saitama Med. Univ.)

Division of Cellular and Molecular Toxicology
Biology Safety Research Center,
National Institute of Health Sciences
Kawasaki, Kanagawa, Japan