

Changes in the position of skeletal elements of the ankle and foot during late embryonic and fetal periods

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Abstract

The morphology of the ankle joint and foot during early development exhibits distinct differences from that observed in adults, with physiological clubfoot being a well-documented phenomenon. To better understand this posture and its transformation, the skeletal elements in this region were three-dimensionally reconstructed using high-resolution phase-contrast X-ray computed tomography and magnetic resonance imaging of specimens (n=23) during the late embryonic and early fetal periods, before joint cavity formation. Sequential changes were analyzed both morphologically and morphometrically from anterior, lateral, posterior, and plantar views. The reduction in plantar flexion of the ankle joint rendered the positional change of the hindfoot substantially more complex, and three-dimensional reconstruction facilitated its comprehension. Continuous supination of the hindfoot, pronation of the forefoot along the foot axis, and reduced plantar flexion of the ankle joint were identified as key postural changes that contributed to the development of temporal physiological clubfoot, initiated as early as the late embryonic period. Twisting between the forefoot and hindfoot and the abduction of the ankle joint, resulting from the obliquity of the tibia–talus joint, were substantial. The offset effect of the two angle changes conceals such changes in most previous studies. Changes in the shape of the tarsal bones, especially the calcaneus and talus, affected the relative bone positions, indicating that the concept of "differential growth" may apply to ankle-joint and foot morphogenesis. Findings of the present study are expected to enhance understanding of the pathogenesis and mechanisms underlying clubfoot and facilitate fetal diagnosis via morphological assessments.

67 **Introduction**

68 The morphology of the lower limbs during the late embryonic and early fetal
69 periods exhibits distinct differences from that observed in adults. The "praying
70 feet", characteristic of the human lower leg at the end of the embryonic period
71 (Carnegie stage [CS] 23), is defined by the hallux being positioned cranially,
72 with the sole of the foot oriented medially (Figure 1a). This posture includes the
73 internal rotation of the lower leg during the late embryonic period and the
74 presence of physiological clubfoot at the ankle joint (O'Rahilly & Gardner, 1975;
75 Takakuwa et al., 2023; Sarrafian & Kelikian, 2011; Bardeen, 1905; Bardeen &
76 Lewis, 1910). Physiological clubfoot is observed during the late embryonic and
77 early fetal periods, persisting until approximately the end of 11 gestational
78 weeks (Kawashima & Uhthoff, 1990; Victoria-Diaz & Victoria-Diaz, 1984). This
79 condition is characterized by a reduction in the foot angle relative to
80 the frontal side of the leg (plantar flexion), as well as an initial increase, followed
81 by a subsequent decrease in the foot angle, relative to the lateral side of the leg
82 (inversion and adduction).

83 The human ankle joint is divided into proximal and distal components,
84 comprising the tibia, fibula, talus, and calcaneus, with the tarsal bones also
85 playing a crucial role in foot formation. These bones exhibit individual variations
86 in shape and size, as well as in their positional relationships during this period.
87 Previous studies employing gross anatomy and histology have endeavored to
88 elucidate the form and relative positioning of these bones (Bardeen, 1905;
89 Bardeen & Lewis, 1910; O'Rahilly and Gardner, 1975; Gardner et al., 1959;

90 Oliver 1962, 1962; Victoria-Diaz & Victoria-Diaz, 1984; McKee & Bagnall, 1987).

91 Several investigations have proposed interactions between the bones (O'Rahilly
92 and Gardner 1975, Gardner et al., 1959, Oliver 1962). The force exerted by the
93 fibula on the calcaneus has been proposed as the mechanism underlying
94 physiological clubfoot. (Victoria-Diaz & Victoria-Diaz, 1984)

95 During the emergence of physiological clubfoot, joint cavities with synovial
96 linings are not formed in any of the skeletal elements, including the ankle and
97 foot; therefore, extensive movements of the ankle and foot are not likely during
98 this period (de Palma et al., 2003; Kawashima & Uhthoff, 1990). Consequently,
99 changes in the ankle and foot may depend primarily on preprogrammed skeletal
100 and neuromuscular development (Kawashima & Uhthoff, 1990a; Kawashima &
101 Uhthoff, 1990b).

102 To examine the intricate and dynamic morphogenesis of the human body during
103 the embryonic period, it is imperative to stage each specimen and employ 3D
104 reconstruction (O'Rahilly, 1987). This necessarily extends to the ankles and
105 feet. However, few previous studies have adequately applied these two
106 methodologies (O'Rahilly and Gardner, 1975; Gardner et al., 1959; Oliver 1962,
107 1962; Victoria-Diaz & Victoria-Diaz, 1984; Kim et al., 2024). Descriptions from
108 earlier research lacking 3D reconstruction and staging are insufficient for
109 adequate reference and disputation, potentially leading to confusion.

110 Interpreting histological findings without 3D reconstruction risks
111 misunderstanding of the spatial relationships, particularly in the ankle and foot
112 regions (O'Rahilly & Gardner, 1975). Typically, the orthogonal plane is
113 determined by the trunk of the specimen, resulting in oblique sections of the

lower leg. The orientation of the lower leg is variable and differs considerably for each specimen, leading to variations in the sectional planes. McKee & Bagnall (1987) highlighted this risk, although their study is rarely cited. Kawashima & Uhthoff (1990) described the 3D formation of the ankle and foot through serial sections with three orthogonal planes that were accurately determined for this region. Although their descriptions appeared to be precise, verification using reconstruction is still necessary.

Growth and differentiation during the embryonic and early fetal periods proceed from cranial to caudal, indicating that differentiation of the ankle and foot continues beyond the embryonic period (O'Rahilly & Gardner, 1975). The use of Carnegie stages is limited to the embryonic period, up to approximately 56–60 days of gestation (O'Rahilly & Muller, 1987). The staged embryos and older fetal specimens were also measured in greatest length (GL, which is the maximum distance from the crown to the rump) and/or gestational week.

Notably, the interpretation of physiological clubfoot based on the relationship between skeletal elements during the late embryonic and early fetal periods has remained unresolved for decades. Kawashima & Uhthoff (1990b) proposed that obliquity in the two planes of the tibiotarsal joint leads to equinus and inversion. Kawashima & Uhthoff cite articles by Victoria-Diaz & Victoria-Diaz (1984), who make quite different arguments (although Kawashima & Uhthoff do not address those divergent ideas). A recent study introduced two ideas but did not discuss them (Bogers et al., 2019).

Understanding and adequately interpreting physiological clubfoot via skeletal elements are crucial for two primary reasons. First, it enhances understanding

of the pathogenesis and mechanisms underlying clubfoot, a well-known congenital anomaly (Miedzybrodzka, 2003). Second, it facilitates fetal diagnosis via morphological assessments such as sonography and magnetic resonance imaging (MRI); Bogers et al., 2019. Consequently, the present study aimed to analyze ankle and foot development using 3D reconstruction of bones from high-resolution MRI data. Specimens from the embryonic period were staged and also GL was measured, while those in the early fetal period were categorized using the GL alone.

METHODS

Ethical considerations

The ethics committees of Kyoto University Faculty, the Graduate School of Medicine, and Shimane University approved this study (approval numbers: E986, R0316, and R2224 for Kyoto University and 02150226-2 for Shimane University).

Human embryonic and fetal specimens

Seventeen human embryos (GL: 13.9–33.5 mm, approximately 51–60 days post-fertilization, Carnegie stage: 20–23, n = 3–6/stage) and six human fetal specimens (GL: 37.2–50 mm, 9–10 weeks from the early-fetal period) were selected for phase-contrast X-ray computed tomography (PCX-CT) and MRI (Table 1, Figure 1). The specimens were stored at the Congenital Anomaly Research Center at Kyoto University and Shimane University (Yamada, 2025; Yamaguchi & Yamada, 2018). The specimens were collected between 1963 and

1995 under the Maternity Protection Law of Japan, in accordance with relevant regulations. Written informed consent was not required, the parents provided verbal informed consent to deposit the specimens, and consent for each participant was documented in their medical records. All specimens were anonymized, de-identified, brought to the laboratory, measured, examined, and staged using the criteria proposed by O’Rahilly & Müller (1987).

Acquisition of digitized data from specimens

MRI scans were performed using a 7T preclinical MR system (BioSpec 70/20 USR, Bruker BioSpin MRI GmbH, Ettlingen, Germany) installed with ParaVision 5.1 software (Bruker BioSpin). A transmit–receive solenoid coil (inner diameter, 19 mm; Takashima Seisakusyo Co., Ltd., Tokyo, Japan) and quadrature transmit–receive volume coils (inner diameters of 35 and 72 mm; Bruker BioSpin and Takashima Seisakusyo) were used. Three-dimensional (3D) T1-weighted MR images were acquired using a spoiled gradient-echo sequence with appropriate acquisition parameters to obtain morphological information. Based on the specimen size, suitable data acquisition settings were selected from three conditions employing different MRI coils and acquisition parameters (Kanahashi et al., 2023).

PCX-CT images of the human embryos were acquired using a radiographic imaging system (BL14-C, 17.8 keV) at the Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization (Tsukuba, Japan). The methodology and conditions used to obtain the PCX-CT images of embryos have been previously documented (Yoneyama et al., 2013). The

spatial resolution ranged from 13 to 18 μm (isotropic). The image acquisition method was selected based on the specimen's resolution and volume. PCX-CT was employed to acquire images at a higher resolution than those obtained using MRI. However, PCX-CT is not appropriate for acquiring images of large-volume specimens. Therefore, we obtained images of specimens between CS20 and CS23 using PCX-CT.

Reconstruction

Phase CT and MRI data were analyzed using serial two-dimensional (2D) and 3D stereo-reconstructed images. Skeletal elements comprising the lower leg and foot were three-dimensionally reconstructed using Amira (version 5.5.0; Visage Imaging, Germany; Figure 1, Supplementary Videos S1 and S2) and underwent morphological observations and morphometry (length ratio and angle measurements).

The plantar view and coronal sections of the talus and calcaneus were used to observe the relationship between the two bones, which was visualized using MATLAB (ver. R2023b, MathWorks, USA).

Length ratio and angle measurements

The length ratios and angles were measured using the anterior, lateral, posterior, and plantar views (Figure 2).

The anterior view is defined with the tibia perpendicular to it, with maximum distance between the tibia and fibula (Figure 2a). The tibial axis by the anterior view was defined as the median line of the lateral and medial tangent lines

(indicated using red broken red lines in Figure 2a) of the tibia by the anterior view. The calcaneus–talus axis by the anterior view was defined as the median line of the lateral and medial tangent lines (indicated using broken blue lines in Figure 2a) of the calcaneus–talus complex by the anterior view. The tibial axis is set perpendicular to the horizontal line. The length ratio of the fibula to the tibia (F/Ti) and the angle between the tibial axis and the angle between the tibial axis and the calcaneus–talus axis by the anterior view ($\angle TiaCTa_A$) were measured. The posterior view is defined as follows. The lower leg was set on the ground contact plane, and the proximal medial part of the calcaneus and proximal part of the 3rd metatarsals overlapped (Figure 2b). The calcaneus–talus axis is defined as the median line of the lateral and medial tangent lines (blue broken lines in Figure 2b) of the calcaneus–talus complex in the posterior view. The tibial axis is defined as the median of the lateral and medial tangent lines (broken red lines in Figure 2b) of the tibia in the posterior view. The incline angle of the calcaneus–talus axis to the ground contact plane ($\angle Inc_CTa$), angle between the tibial axis and the perpendicular line of the ground contact plane (incline angle of the tibial axis: $\angle Inc_Tia$), and the angle between the tibial and calcaneus–talus axes ($\angle TiaCTa$: ankle joint abduction) were measured. The lateral and medial views were defined as follows: the calcaneus and the 1st and 5th metatarsals were set on the ground contact plane, and the Chopart joint plane was observed as a gap (Figure 2c). The plantar flexion of the ankle joint ($\angle PF$) and the angle between the Chopart joint plane and the tibia using the lateral view ($\angle TiCh_l$) were measured.

The plantar view was defined as the tarsal bone on the plane of the display (Figure 2d). The Chopart joint plane was used as a reference for each morphometry measurement. The following dimensions were measured, and the length ratio was calculated. Foot length (FL), foot width (FW), lateral segment length (LL), medial segment length (ML), hindfoot length (HL), 1st metatarsal length (1st), 3rd metatarsal length (3rd), 5th metatarsal length (5th). FW was measured in parallel, whereas FL, LL, ML, and HL were measured perpendicular to the Chopart joint plane.

The angle between the Chopart and Lisfranc joint plane in the plantar view ($\angle\text{ChLf_P}$), angle between the Chopart joint plane and 1st metatarsal ($\angle\text{ChM1}$:1st metatarsal abduction), angle between the Chopart joint plane and 5th metatarsal ($\angle\text{ChM5}$:5th metatarsal adduction), and angle between the 1st and 5th metatarsal ($\angle\text{M1M5}$) were measured.

RESULTS

Anterior view with the tibial long axis perpendicular

The tibia at the proximal region of the knee joint differentiates as early as CS20, and the medial and lateral condyles and the intercondylar eminence can be distinguished by the end of the embryonic period (Figure 3a). Consequently, the proximal part of the tibia is considerably larger than the shaft during the embryonic period. The fibula is located distal to the knee joint and does not form part of the knee joint.

The tibial and fibular shafts do not run in parallel, and the distal ends of the tibia and fibula are separated. The tibia and fibula extend toward the medial and

lateral sides of the talus, respectively, with the talus located between them during the embryonic period. During this period, the tibia is located more distally than the fibula. The distal end of the tibia, which forms the distal tibial articular surface, is tilted laterally to the tibial axis at CS20–22.

By the end of the embryonic period, the distal ends of the tibia and fibula begin forming the medial and lateral malleoli. These are located at approximately the same position at CS23. The fibula appeared to be in contact with the calcaneus in the frontal view (indicated by * in the CS23 reconstruction in Figure 3a).

However, the two bones were located apart and had gaps that were clearly visible in the posterior and lateral views.

The distal ends of the fibula were slightly more distal than the tibia. The distal ends of the tibia and fibula surrounded the talus and were located close to each other; the articular surface of the ankle joint was formed during the early fetal period.

During the embryonic period, the fibula was shorter than the tibia, with a length ratio (F/Ti length ratio) ranging from 0.6 to 0.8 at CS20 and gradually increasing to over 0.8 at CS23 and EF, but never exceeding 1.0 (Figure 3b_i). This indicated that the tibia was consistently longer than the fibula.

According to the growth of each skeletal element with joint formation, the angle between the tibial axis and the calcaneus–talus axis in the anterior view decreases. Morphometry revealed that the calcaneus–talus axis tilted approximately 80–86° to the tibial shaft axis at CS20–21, which decreased gradually, reaching 15–35° at EF (Figure 3b_ii). This decreased angle may be related to inversion of the whole foot and adduction of the forefoot.

Posterior view with the ground contact plane horizontal

During the embryonic period, the talus and calcaneus begin to align closely to form an articular surface (Figure 4a). The calcaneus–talus axis was almost parallel to the horizontal plane (ground plane) at CS20. As growth progressed, the calcaneus–talus axis gradually elevated from the ground. Morphometry ($\angle \text{Inc_CTa}$) revealed that the angle was 15–20° at CS20, increasing progressively with growth, exceeded 30° at CS23, and reached 45–55° at EF (Figure 4b_i). This angle reflects a twist in the forefoot, or alternatively, the reduced inversion of the forefoot.

The angle between the tibial and calcaneus–talus axes ($\angle \text{TiaCTa}$) exceeded 60° at CS20 and 21 and gradually decreased until the end of the embryonic period, reaching less than 20° at EF. This angle may reflect the formation of the tibia–talus joint (Figure 4b_ii).

The tibial inclination in the posterior view ($\angle \text{Inc_Tia}$) was measured; the values varied for each specimen at CS20–21, ranging from approximately -20 to 24°.

The inclination angle increased at CS22-23 and EF, reaching approximately 30° (Figure 4b, iii).

Lateral and medial views with the ground contact plane set horizontal

The ankle joint at CS20 displayed a significant degree of plantar flexion (equinus) (80–95°)(Figure 5a, b). Posterior curvature of the tibia was observed, which accentuating the equinus posture.

The talus was round at Cs20, which changed the shape (Figure 5b). The talus was close to the tibia by the medial posterior part. It elongated anterior-posteriorly, and its height increased, which differentiated the articular surface for the navicular and tibia and fibula, respectively. The degree of plantar flexion decreased gradually, reaching approximately 40–60° at EF. The tip of the metatarsal bone extends downward, and the arch at the sole becomes conspicuous with growth (Figure 5a). At the end of the embryonic period and EFs, the joint surface changes its position in a cranial direction. The long axis of the fibula was oriented towards the talus, with the tip of the fibula extending to the talus to form the ankle joint, which is distinct from the calcaneus (indicated by * in Figure 5a). The medial and lateral condyles elongate to cover both medial and lateral sides.

The angle of the Chopart joint plane to the ground was generally vertical in the lateral view and did not change significantly during the observation period.

The angle between the Chopart joint plane and the tibia in the lateral plane ($\angle Tch_I$) increased as plantar flexion decreased, which was negatively correlated with plantar flexion (Figure 5c_ii).

Plantar view of the tarsus

At CS20, the hindfoot was relatively short and narrow, while the forefoot exhibited a fan-like shape with each metatarsal spreading radially (Figure 6a). As growth progressed, each tarsal bone changed in size and shape. The proportion apparently differed from that of the hand. The fan-like forefoot disappeared as each metatarsal moved closer and adducted. The talus did not

contact the ground plane, and the medial posterior side of the calcaneus was obscured by the talus in the plantar view.

The detailed relation of the two bones is presented in Figure 6 b_i, ii. The articulating surfaces between the talus and calcaneus can be recognized as early as the CS21-22. The talus and calcaneus were closely positioned and overlapped. The degree of overlap increased with growth. The talus and calcaneus differentiated, and the tuberosity gradually became distinct during the embryonic period. Changes in the shape of the bones, such as the formation of the sustentaculum tali and the tuber calcanei, contributed to the positional change of the bone (Figure 6 b_ii).

The FW/FL ratio ranged from approximately 0.90 to 1.0 at CS20 (Figure 7a_i). With growth, the FW/FL ratio gradually decreased, reaching 0.5–0.65 at EF. The HL/FL value was approximately 0.3–0.4, and a slight increasing trend was observed, which suggests a higher rate of growth of the hindfoot. The 3M/FL value was distributed between approximately 0.35–0.5, and a local maximum was observed at the end of the embryonic period (CS22–23). ML/FL and LL/FL were considerably less than one, whereas ML was longer than LL at CS20 (Figure 7a_ii). The ML/FL and LL/FL ratios increased, reaching 0.85–1.00 of EF. This implies that the foot had a boxy shape (Figure 6a). The lengths of the first, third, and fifth metatarsals at CS20 were in the following order: third, fifth, and first (Figure 7a_iii). During the embryonic period, the length ratios of the 5th to 3rd and 1st to 3rd approached 1, and the fifth metatarsal becomes equal to or even longer than the third metatarsal at EF. The first metatarsal was still shorter

than the 3rd and 5th metatarsals, but gradually became less conspicuous. The plantar view confirms this trend (Figure 6a).

The angle between the Chopart and Lisfranc joints in the plantar view ranged from 10° to 30° in the majority of specimens (Figure 7b_i). A slightly decreasing trend was observed, which may enhance the adduction of the forefoot.

The adduction angle of the first metatarsal to the Chopart joint plane varied in each specimen (Figure 7b_ii). During the embryonic period, most specimens exhibited an adduction angle of more than 30° (range 25–55°), which decreased to approximately 30° or less (22–32°) at the EF.

The second to fifth metatarsals change direction to become gradually adducted during the embryonic and early fetal periods. The adduction angle of the fifth metatarsal to the Chopart joint plane decreased from approximately 60 °to less than 0° with growth (Figure 7_iii). The angle between the first and fifth metatarsals decreased from approximately 90° to less than 30° from CS20 to the EFs (Figure 7_iv).

DISCUSSION

Positional change of the talus and calcaneus

The talus and calcaneus are located close to each other during the embryonic period, as if in an intimate partnership, which the anterior, lateral, and posterior views in the present study confirmed. This implies that the future articular surface had already been determined during this period. The growth and differentiation of the hindfoot (talus and calcaneus) and the formation of joints with adjacent bones undoubtedly play essential roles in ankle joint and foot

formation and posture; therefore, many previous studies have focused on the positional change of the hindfoot (Bardeen, 1905; Bardeen & Lewis, 1910; O'Rahilly and Gardner, 1975; Gardner et al., 1959; Oliver 1962, 1962; Victoria-Diaz & Victoria-Diaz, 1984).

Our observations using 3D reconstruction indicated that the hindfoot exhibited supination along the lower limb axis according to growth, which was one long axis at CS20. When the plantar flexion of the ankle joint decreased, the lower limb was separated into the tibial and foot axes, and the hindfoot was supinated along the foot axis. The reduced plantar flexion of the ankle joint makes the positional change of the hindfoot much more complex. The positional change of the hindfoot changed in three dimensions when the lower leg was used as the reference. Therefore, it will be difficult to understand these changes without 3D reconstruction. Several previous studies using histological methods interpreted the position only in the local area or over-interpreted the 3D position.

Several studies have assumed the local interference of the fibula with the calcaneus. Gardner et al. (1959) and Oliver (1962) described the joint formation between the fibula and calcaneus. A recent study described the proximity of these two bones (Kim et al., 2024). In the lateral view of our reconstruction, in the lateral view, the fibula and calcaneus are located apart and have a significant gap compared to other bones, which later form joints. Moreover, the fibula is oriented toward the lateral side of the talus in the anterior view and is not oriented to the calcaneus, which is consistent with Oliver's (1962) description at CS18 and 19 in which the distal ends of the fibula and tibia are separated, and the talus is wedged between the tibia and the fibula. The long

fibular axis was oriented to the talus, which was never oriented to the calcaneus, even in the early fetal period (Lateral view in Figure 5a). Furthermore, the fibula exhibited a rapid elongation rate but stopped slightly beyond the medial malleolus, similar to that in adults (Figure 3). Although Victoria-Diaz & Victoria-Diaz (1984) proposed the mechanism that the force of the extra-elongated fibula pushes the calcaneus, which results in the formation of the club foot, i.e., the 'fibular phase' in the fetus with GL21–30 mm in their category, our anterior view (Figure 3) refutes their theory. McKee and Bagnall (1987), who analyzed 3D reconstructions from serial histological sections, reached the same conclusion, attributing the discrepancy to the oblique orientation of histological sections of the ankle joint, in which the calcaneus and fibula appeared closer when the plantar plane was used as the reference. Our present reconstruction suggests that changes in the shape of the calcaneus and talus, such as the formation of the lateral and medial tubercle in the talus and the sustentaculum tali and the tuber calcanei in the calcaneus, contribute to the positional change of the two bones. Specifically, the concept of "differential growth," which has been applied to various aspects of morphogenesis during the human embryonic period, such as the brain and external ear (Gasser, 2006; Kagurasho et al., 2012), may also be applicable to ankle-joint morphogenesis. In other words, positional changes in the developing structures may be attributable to alterations in the size and shape of the embryo and its components, rather than to the movement of structures from one region of the embryo to another. A recent histological study described the shape of the calcaneus and talus (Kim et al., 2024). However, because this study did not

apply Carnegie staging and 3D reconstruction, we could not compare our current results with those of the previous study, either in timing, spatial relationship, or their contribution to the positional change.

Plantar flexion of the ankle joint

A mechanism to reduce plantar flexion of the ankle joint is essential for understanding the ankle joint posture. The growth and differentiation of the talus and the formation of joints with adjacent bones, tibia, and talus play an essential role in reducing plantar flexion and the angle between the tibial and calcaneus–talus axes (Kawashima and Ulthoff, 1984). Even minor changes in the talus and lower part of the tibia will be noticed owing to amplified changes in ankle posture. During embryonic development, the talus is relatively flat, and the distal end of the tibia is oblique and concave (Kawashima and Ulthoff, 1984; Oliver, 1962; Straus Jr, 1927; de Palma et al., 2003). Our 3D reconstruction confirmed the previously described feature in the embryonic period and revealed that the talus formed a pulley-like structure during the early fetal period (Figure 5b). In the posterior view, with the ground contact plane set horizontally, the calcaneus–talus axis was tilted toward the ground (Figure 4). This observation indicates that the forefoot pronated along the foot axis and that the opposite change in position between the hindfoot and forefoot caused the foot to twist. The twisting could be quantified as $\angle \text{Inc_CTa}$ in Figure 4, which shows a continuous change of approximately 30 degrees from CS20 to EFs. In addition to the tarsal bones that compose the forefoot, the calcaneus and talus may also contribute to torsion by changing their shape (Straus Jr, 1927; Oliver, 1962).

This change is crucial for understanding foot arch formation. A detailed analysis will be required in future studies.

McKee and Bagnall (1987) also observed this view in their reconstructions and highlighted that previous studies had focused their attention on the change in the position of the hindfoot, while not evaluating the posture of the forefoot. The foot twisting appeared to be offset by a change in the angle between the calcaneus–talus and tibial axis by the posterior view ($\angle$ TiaCTa). The change in $\angle$ TiaCTa has been highlighted by Kawashima and Ulthoff (1984), who claimed that the obliquity in two planes of the tibia–talus joint is responsible for plantarflexion and inversion at the ankle joint. A change in angle of posterior view, observed as the $\angle$ TiaCTa in the present study, was as large as the plantar flexion of the ankle (Figure 4b_i). We believe that these two angle changes were not pointed out in previous studies, mainly because the offset effect of the two angles obscures such considerable changes in the angle.

McKee and Bagnall (1987) claimed that when this torsion of the hindfoot and forefoot was considered properly, the calcaneus appeared to be situated below the talus as early as stage 20, implying that the relationships in the embryonic hindfoot in the cartilaginous phase are similar to those in adults. Our observations of the posterior (Figure 4) and anterior views (Figure 3) and coronal section (Figure 6b_ii) support their claim at CS20 and later (cartilaginous period). Specimens before CS20 could not be reconstructed for analysis in the present study because the contrast of the MR was not sufficient to differentiate between precartilage and other mesenchymal tissues. It is crucial to determine whether the precartilages consisting of the ankle joint (tibia,

fibula, talus, and calcaneus) were on an identical plane. Knowing this would help to establish whether precartilag elements change from the embryonic to adult configuration, or whether the position is similar to the adult position at the initial precartilaginous stages (McKee and Bagnall 1984). This will be the subject of our future studies.

Foot proportion in the plantar view

Adduction of the forefoot and closing of the intermetatarsal have been reported in previous studies (Kawashima and Uhthoff 1990), which could be confirmed by 3D reconstruction and quantified in the present study by the foot proportion in the plantar view. The decrease in the adduction of the 1st metatarsal and the angle between the Chopart and Lisfranc joint surfaces was slight, suggesting that it contributes to the adduction of the forefoot. The elongation rate of the lateral part was slightly higher than that of the medial part, contrary to the results of a previous study (Victoria-Diaz & Victoria-Diaz 1984), which contradicted their theory.

Based on the present findings and those of previous studies, the assumed changes in the positions of the lower leg and foot are summarized in Figure 8. The continuous supination of the hindfoot, pronation of the forefoot along the foot axis, and reduced plantar flexion of the ankle joint may be key changes in understanding the posture that gives rise to a temporal physiological clubfoot. These three changes were initiated as early as the late embryonic period. However, the time to termination for these three factors was not determined in the present study.

494

495 **Limitations**

496 The present study has some limitations. First, the length and angle were
497 calculated on the 3D reconstruction image on the display, which may have
498 resulted in measurement errors. Second, deformities and shrinkage due to
499 fixation and preservation should be considered. Third, the artificial deformation
500 of the foot may have been due to an external force that was occasionally
501 observed. The phalanges were not included in the present study because they
502 were most affected by artificial deformations and defects. Finally, the present
503 study only analyzed the shape of the skeletal system around the ankle joint and
504 foot. The degree of differentiation in each cartilaginous bone and joint formation,
505 as well as other associated mesenchymal structures such as the muscle and
506 tendon, may affect the shape and position of each bone. A complementary
507 approach using 3D reconstructions and serial histological studies is necessary
508 for a detailed analysis and a deeper understanding.

509

510 **Conclusions**

511 The lower leg and tarsal bones were three-dimensionally reconstructed, and
512 sequential changes from CS20 to the early fetal period were analyzed
513 morphologically and morphometrically in the anterior, lateral, posterior, and
514 plantar views. The continuous positional change of the talus and calcaneus
515 (supination along the foot axis), pronation of the forefoot along the foot axis, and
516 reduced plantar flexion of the ankle joint may be pivotal changes in
517 understanding the posture that gives rise to a temporal physiological clubfoot.

The findings of this present study are expected to enhance understanding of the pathogenesis and mechanisms underlying clubfoot and facilitate fetal diagnosis via morphological assessments.

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Visualization: S.F., T.K.

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FIGURE LEGENDS

Figure 1: Representative gross images of the specimens, phase-contrast X-ray computed tomography (PCX-CT) and MR images, and 3D reconstructions used in the present study.

a) Specimens at CS 23 (ID. 29137)

- i) Lateral view of the gross image
- ii) Anterior views of the gross image
- iii) Plane PCX-CT image
- iv) 3D reconstruction

Blue: tibia, cyan: fibula, light green: talus, purple: calcaneus

Note the "praying feet", characteristic of the human lower leg at the end of the embryonic period

b) Specimens at the early fetal period (ID. F2510; GL 50 mm)

- i) Lateral view of the gross image
- ii) Plane MR image
- iii) 3D reconstruction

Blue: tibia, cyan: fibula, light green: talus, purple: calcaneus

Physiological clubfoot is still noted.

Figure 2 Morphometry

a) Anterior view with the tibial axis perpendicular to the ground plane

- b) Posterior view with the ground plane horizontal.
- Blue broken lines: lateral and medial tangent lines of the calcaneus–talus complex. Red broken lines: Lateral and medial tangent lines of the tibia.
- c) Lateral view with the ground plane horizontal.
- d) Plantar view.
- Double line: the Chopart joint plane used as a reference
- Blue: tibia, cyan: fibula, light green: talus, purple: calcaneus
- e) Abbreviations and definitions of the measurements are summarized in the panel.

Figure 3 Anterior view with the tibial long axis perpendicular

- a) 3D reconstructions: Six representative reconstructed images are illustrated.
- Blue: tibia, cyan: fibula, light green: talus, purple: calcaneus
- b) Morphometry using the anterior view
 - i) Fibula to tibial (F/Ti) length ratio,
 - ii) The angle between the tibial axis and the calcaneus–talus axis from the anterior view ($\angle\text{TiaCTa_A}$)

Figure 4 Posterior view with the ground contact plane horizontal

- a) 3D reconstructions: Six representative reconstructed images are illustrated.
- *: gap between the fibula and the calcaneus
- Blue: tibia, cyan: fibula, light green: talus, purple: calcaneus

b) Angle measurements

i) $\angle \text{Inc_CTa}$: incline angle of the calcaneus–talus axis to the ground contact plane

ii) $\angle \text{TiaCTa}$: angle between the tibial and the calcaneus–talus axis (ankle abduction)

iii) $\angle \text{Inc_Tia}$: incline angle of the tibial axis: angle between the tibial axis and the perpendicular line of the ground contact plane

Figure 5 Lateral and medial views with the ground contact plane horizontal

a) 3D reconstructions: four representative reconstructed images are illustrated. *: gap between the fibula and the calcaneus

b) Medial closer view closer to the talus. Skeletal elements, except the talus, were translucent to recognize the change in the shape of the talus and the site of its joint with the tibia.

Blue: tibia, cyan: fibula, light green: talus, purple: calcaneus

c) Angle measurements i) $\angle \text{PF}$: plantar flexion of the ankle joint, ii) $\angle \text{TiCh_I}$: angle between the Chopart joint plane and the tibia using the lateral view

Figure 6 Plantar view of the tarsus

a) 3D reconstructions: Six representative reconstructed images are illustrated.

Light green: talus, purple: calcaneus

b) Relative position of the talus and calcaneus

i) Plantar view of the talus and calcaneus. Red points indicated the

predicted articular surface between the bones. A black horizontal line depicted the position of the cross-section indicated in Figure 5b_ii.

Light green: talus, purple: calcaneus

- ii) Coronal section at the sustentaculum tali. mt: medial tuberosity, lt: lateral tuberosity, st: sustentaculum tali, tc: tuber calcanei

Figure 7 Morphometry of the tarsus using the plantar view

a) Proportion (length ratio)

- i) Proportion_1: FW/FL, HL/FL, 3rd/FL
- ii) Proportion_2: ML/FL, LL/FL
- iii) Metatarsal: 5th/3rd, 1st/3rd (Length ratio of the 1st and 5th to 3rd metatarsal)

b) Angle measurements

- i) $\angle \text{ChLf_P}$: angle between Chopart and Lisfranc joint planes
- ii) $\angle \text{ChM1}$: angle between Chopart joint plane and 1st metatarsal (1st metatarsal adduction)
- iii) $\angle \text{ChM5}$: angle between Chopart joint plane and 5th metatarsal (5th metatarsal adduction)
- iv) $\angle \text{M1M5}$: angle between the 1st and 5th metatarsals

Figure 8 Summary showing the assumed change in position of the lower leg and foot

Late embryonic period (CS20–CS23)

The limb axis is almost straight, running between the tibia, fibula and talus. The calcaneus was located on the fibular side of the talus. A 90° plantarflexion is observed.

The hindfoot (talus and calcaneus) initiates supination, whereas the forefoot pronates and adducts.

The ankle joint gradually reduces the plantar flexion, implying that the limb axis is separated into the lower leg and foot.

The hindfoot (talus and calcaneus) is located along the foot axis. The calcaneus gradually changes position posterior to the talus, which emerges as the heel formation because this supination axis runs through the talus.

Early fetal period (12 GA<)

The hindfoot (talus and calcaneus) was continuously supinated, whereas the forefoot pronated and adducted.

This positional change appears externally as an inversion of the ankle joint. The positional changes of the hindfoot and forefoot along the foot axis appear to be opposite, resulting in twisting. This positional change appears externally because of reduced inversion of the foot. Finally, the tibia, talus, and calcaneus are almost perpendicular to the vertical axis.

This change in the angle between the lower leg and the foot is related to a change in the technical terms describing the position of the ankle joint. It should be noted that the supination of the hindfoot to the foot axis becomes synonymous with inversion of the ankle joint, and the pronation of the forefoot to the foot axis becomes synonymous with the reduced inversion of the ankle joint,

when the plantarflexion is reduced (Kawashima and Ulthoff, 1984).
 When evaluating the ankle joint position, we estimated the tibia–talus joint, as
 well as other joint positions in the skeletal element. We believe that this makes
 the interpretation more complex.

Supplementary Videos

S1: Representative 3D reconstructions of the lower leg and foot skeletal
 elements rotating along the tibial long axis

S2: Representative 3D reconstructions of the lower leg and foot skeletal
 elements rotating with the ground contact horizontal plane.

Information about specimens used for each video are provided in the below
 panel.

stage	ID	GL (mm)
CS20	21594	16.6
CS21	24325	19.0
CS22	27050	19.6
CS23	52578	24.3
EF <40	52002	37.2
EF <50	F2307	43.0

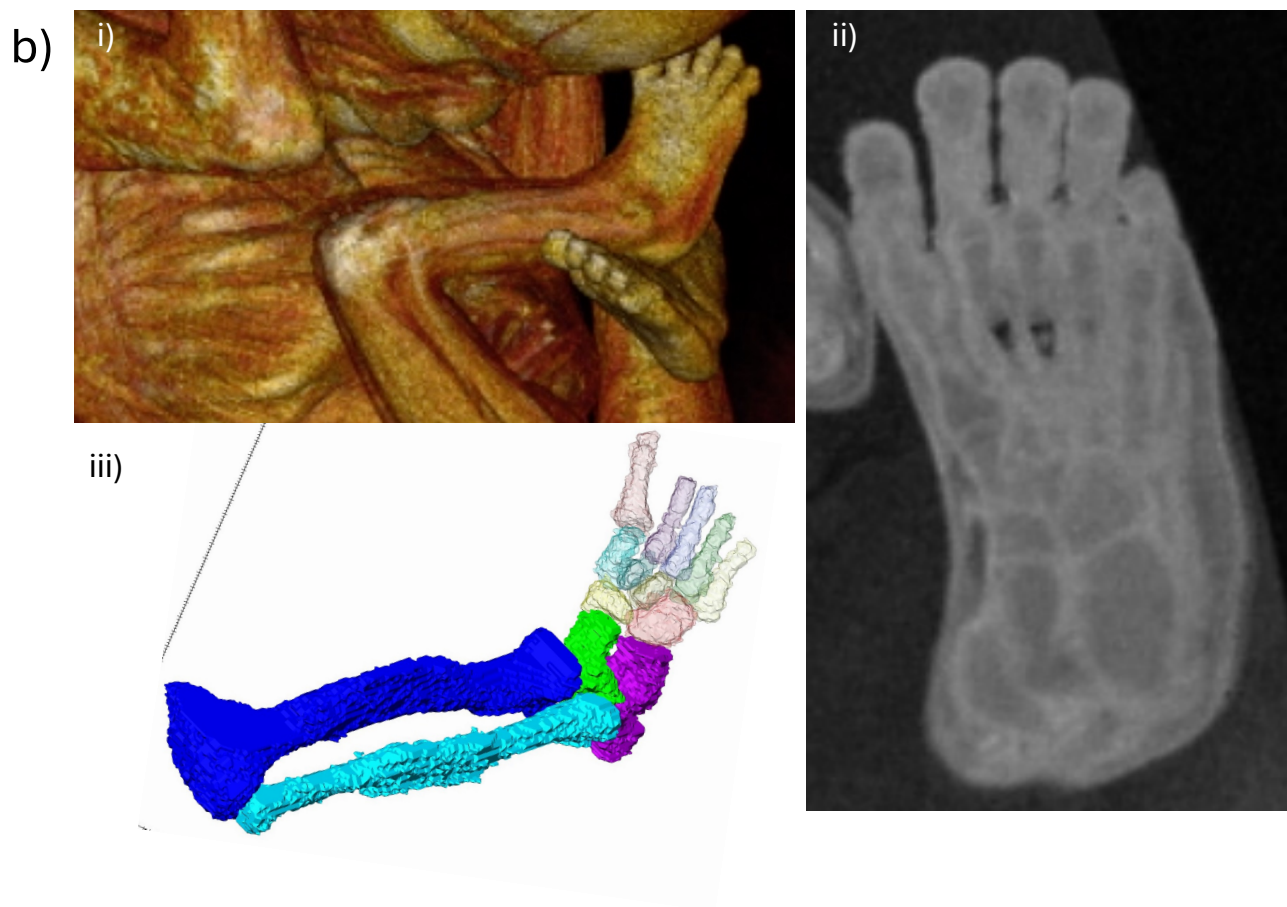
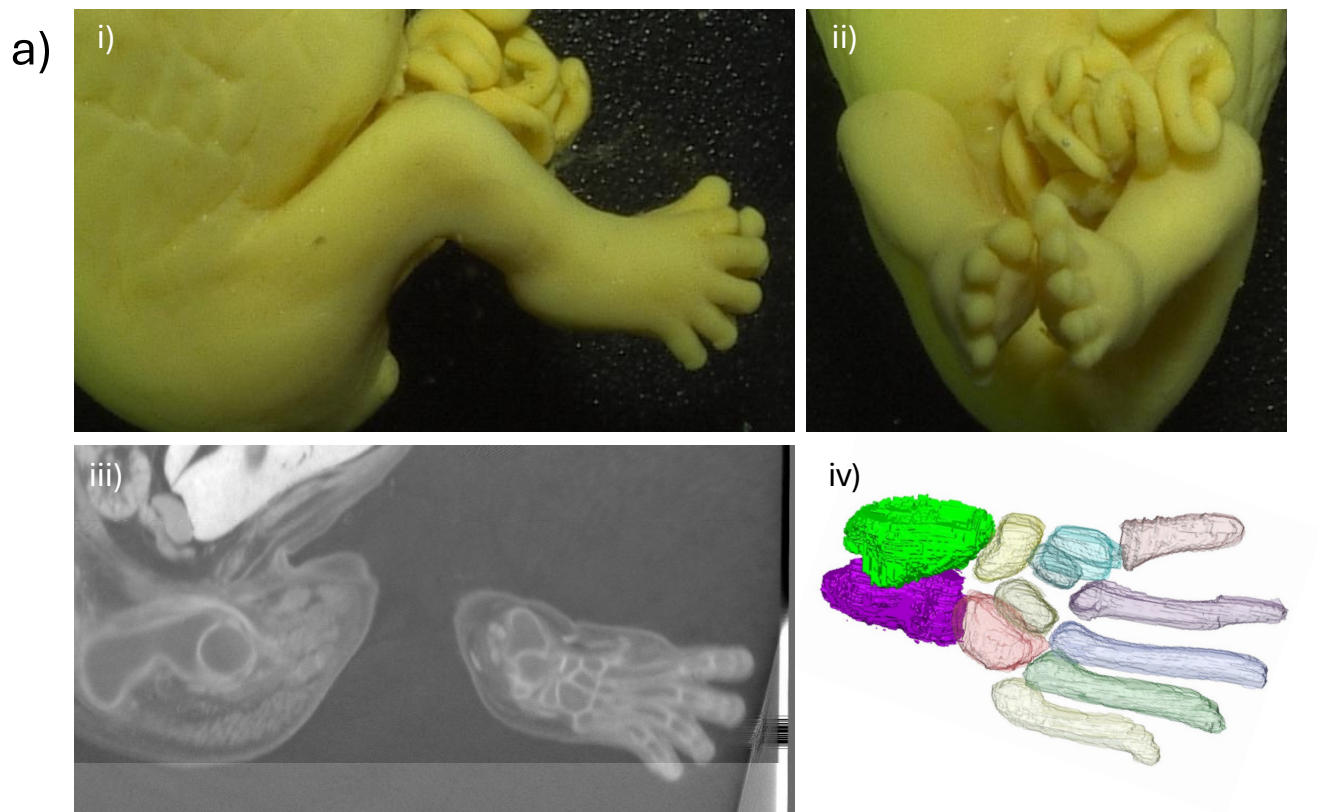
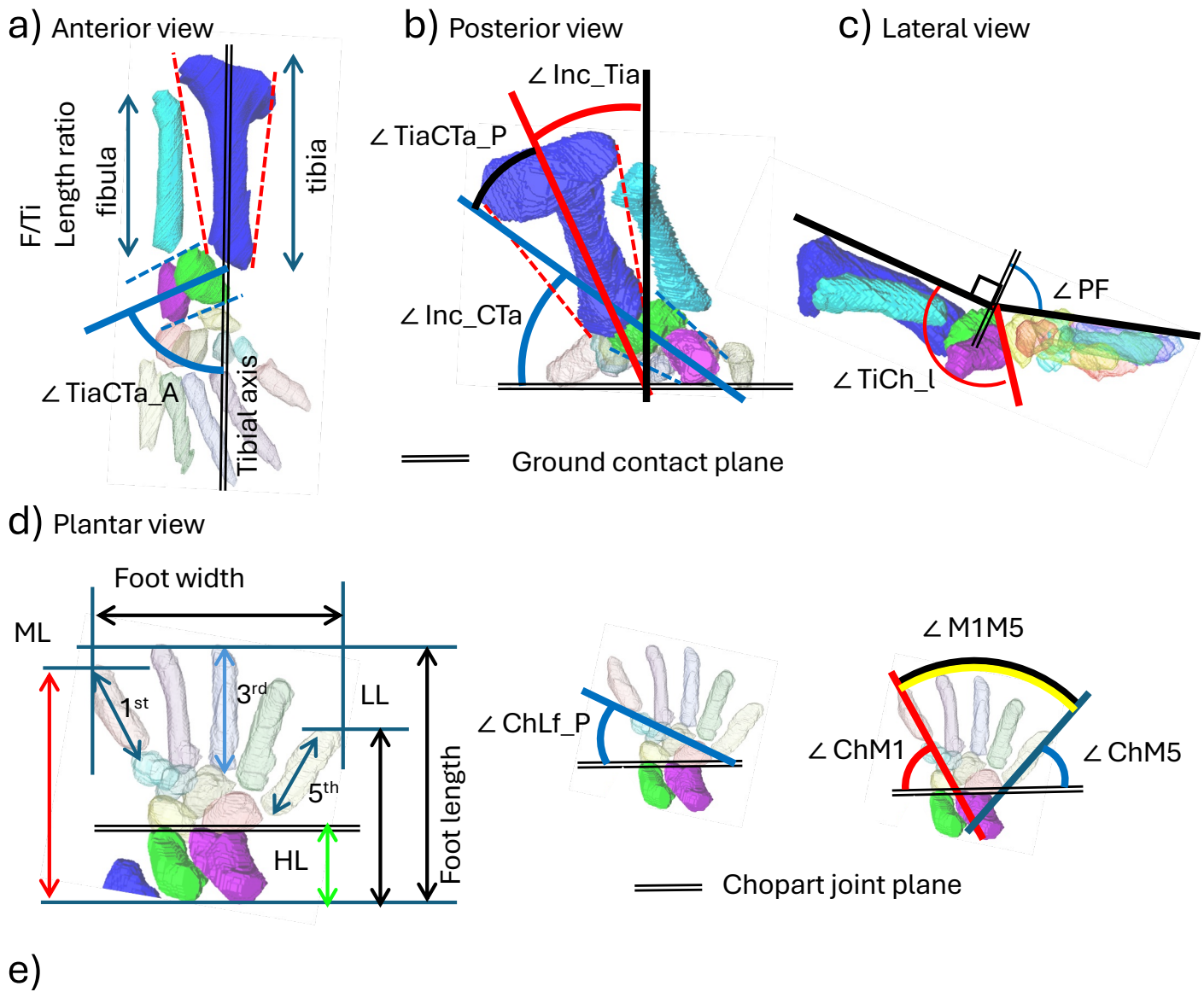


Fig1



view	abbreviations	note
Anterior	F/Ti	length ratio of the Fibula to the tibia
	$\angle \text{TiaCTa_A}$	the angle between the tibial axis and the calcaneus-talus axis by the anterior view
Posterior	$\angle \text{Inc_CTa}$	incline angle of the calcaneus-talus axis to the grand contact plane
	$\angle \text{Inc_Tia}$	incline angle of the tibial axis: angle between the tibial axis and the perpendicular line of the grand contact plane
	$\angle \text{TiaCTa}$	the angle between the tibial and calcaneus-talus axes (Ankle abduction)
Lateral	$\angle \text{TiCh_l}$	the angle between the Chopart joint plane and the tibia by lateral view
	$\angle \text{PF}$	plantar flexion of the ankle joint.
Plantar	FL	foot length
	FW	foot width
	LL	lateral segment length
	ML	medial segment length
	HL	hindfoot length
	3rd	3rd metatarsal length
	$\angle \text{ChLf_P}$	angle between Chopart and Lisfranc joint planes by plantar view
	1st/3rd	length ratio of the 1st to 3rd metatarsal
	5th/3rd	length ratio of the 5th to 3rd metatarsal
	$\angle \text{ChM1}$	angle between Chopart joint plane and 1st metatarsal (1st metatarsal adduction)
	$\angle \text{ChM5}$	angle between Chopart joint plane and 5th metatarsal (5th metatarsal adduction)
	$\angle \text{M1M5}$	angle between the 1st and 5th metatarsal

Figure 2

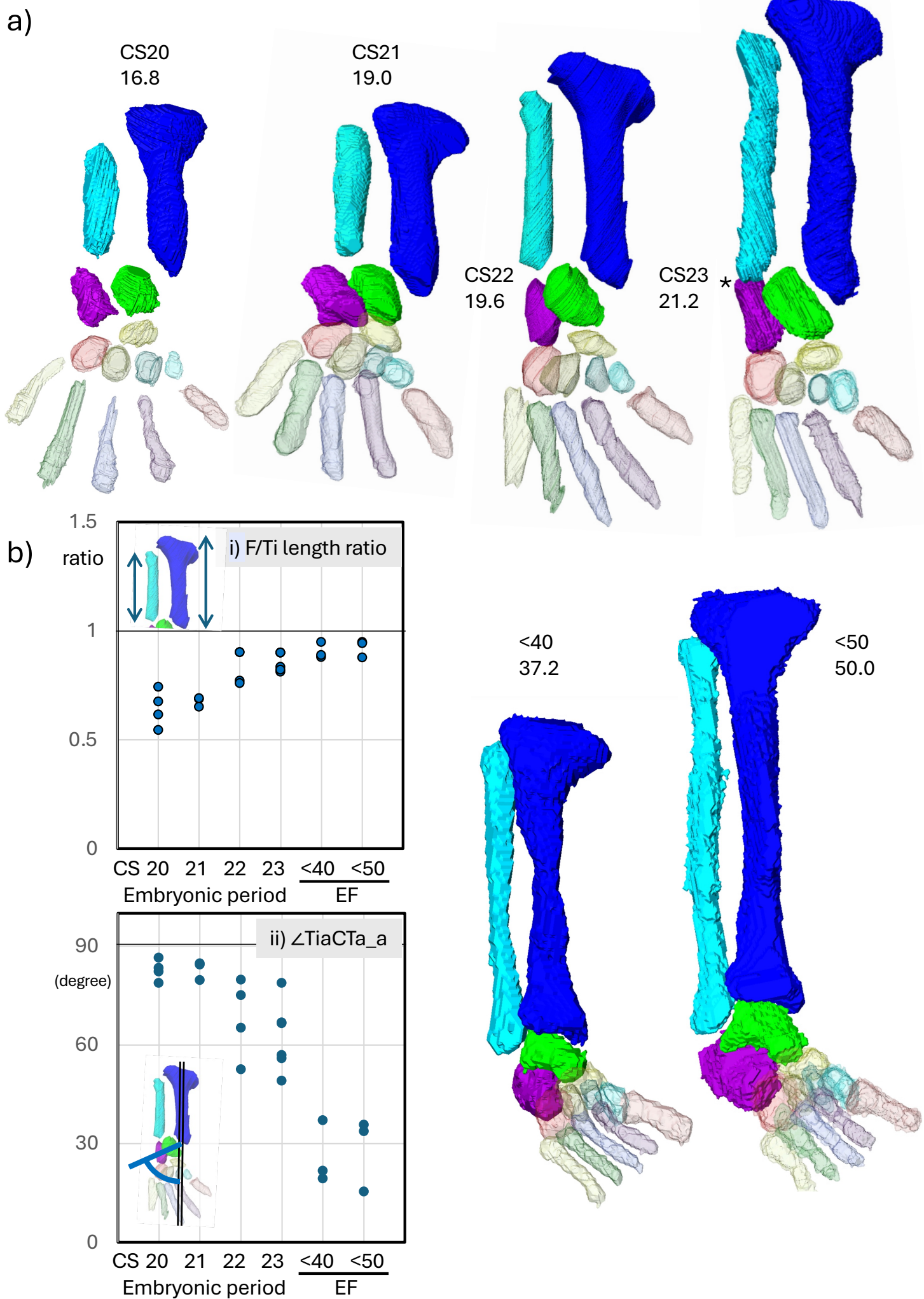
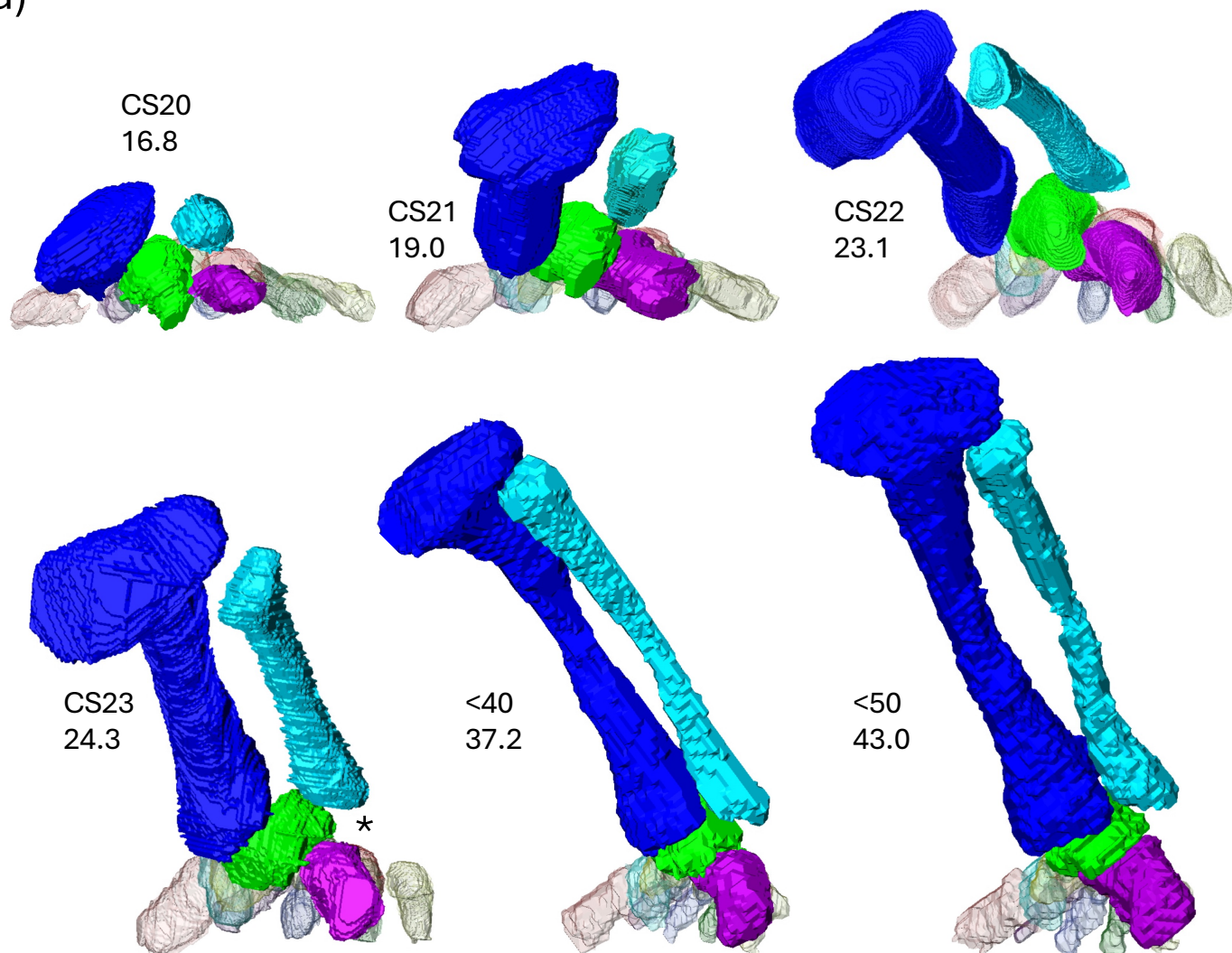


Figure 3

a)



b) Angle measurements (posterior view)

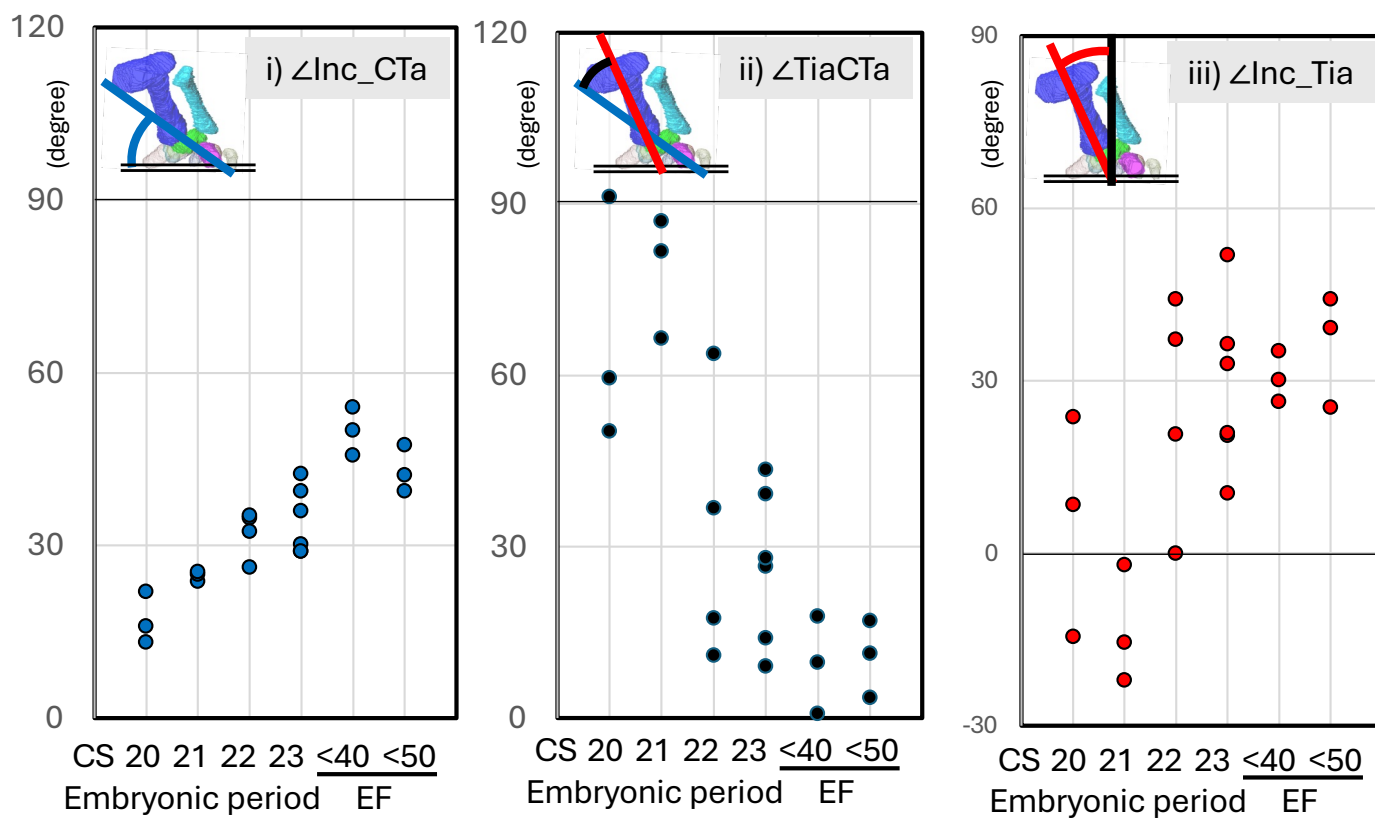


Figure 4

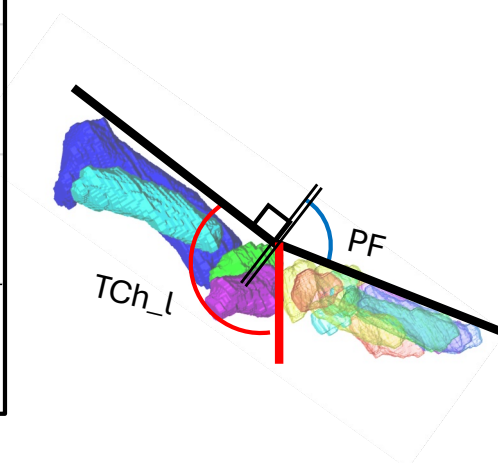
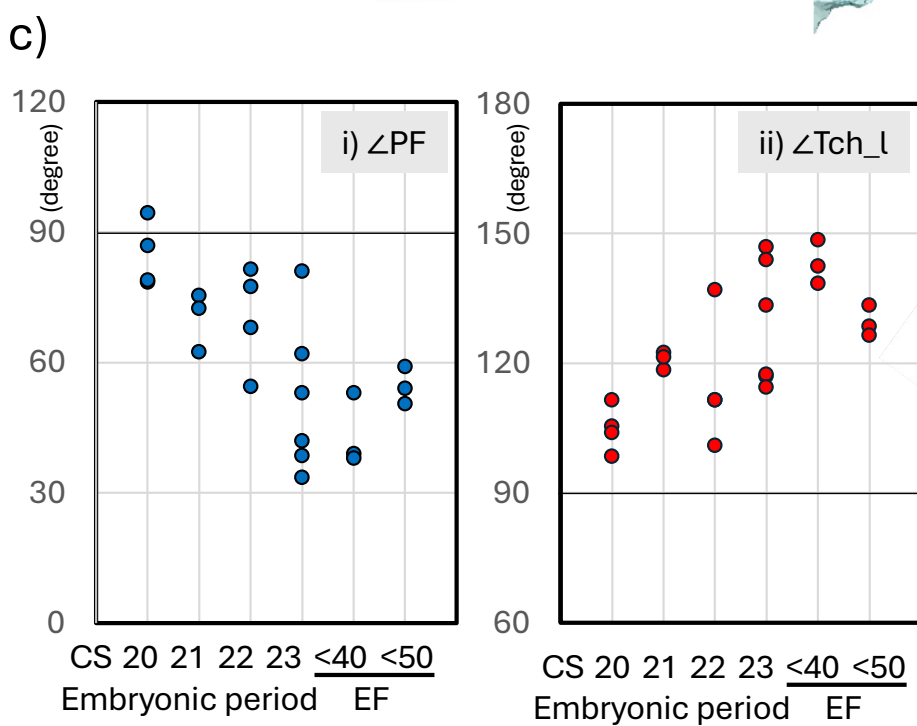
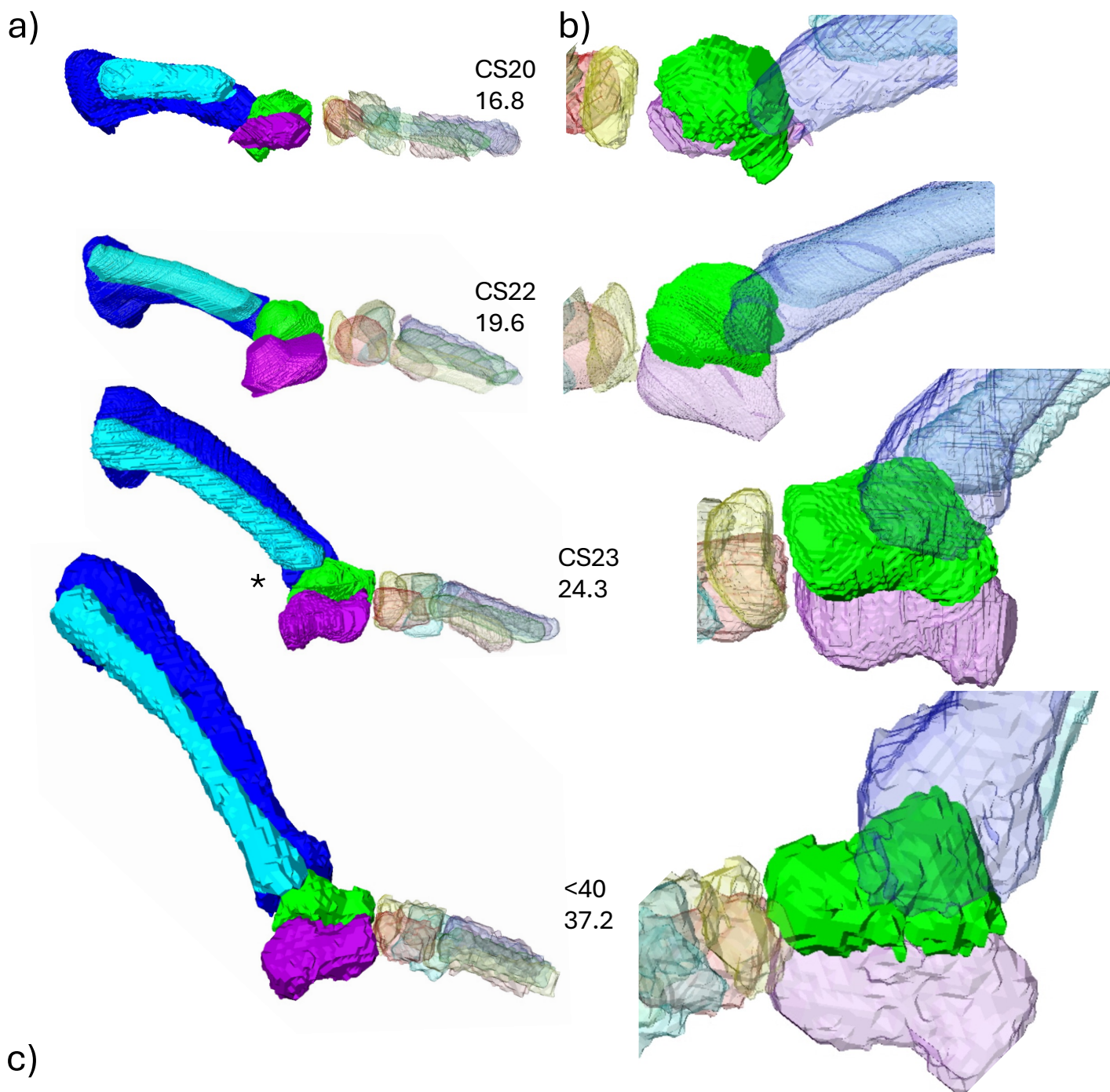


Figure 5

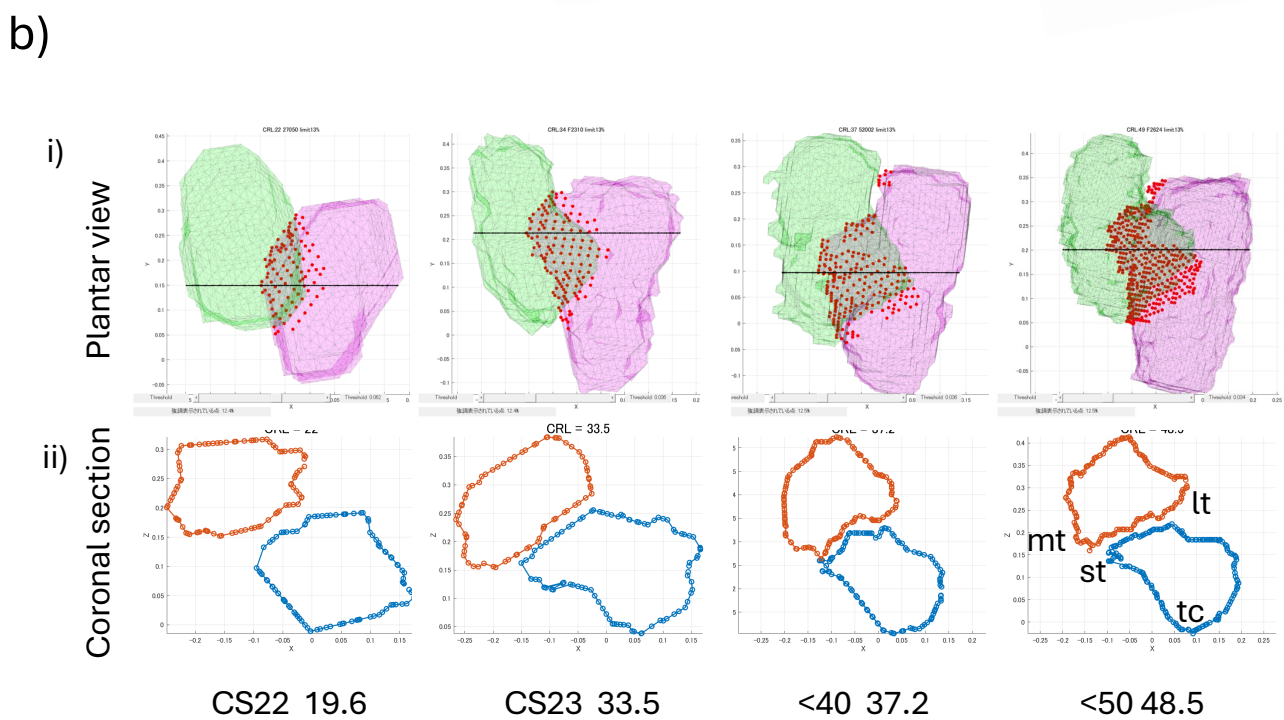
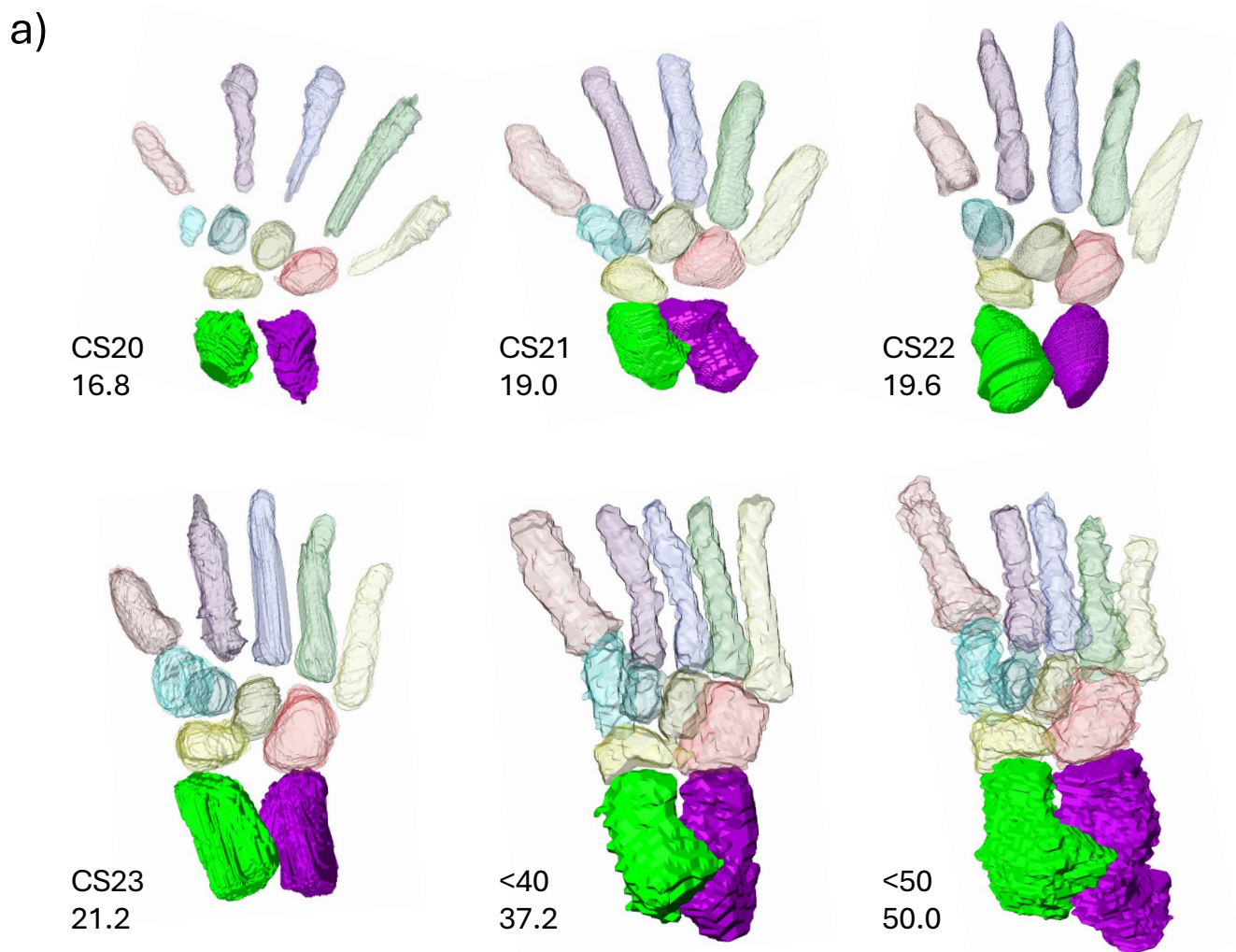
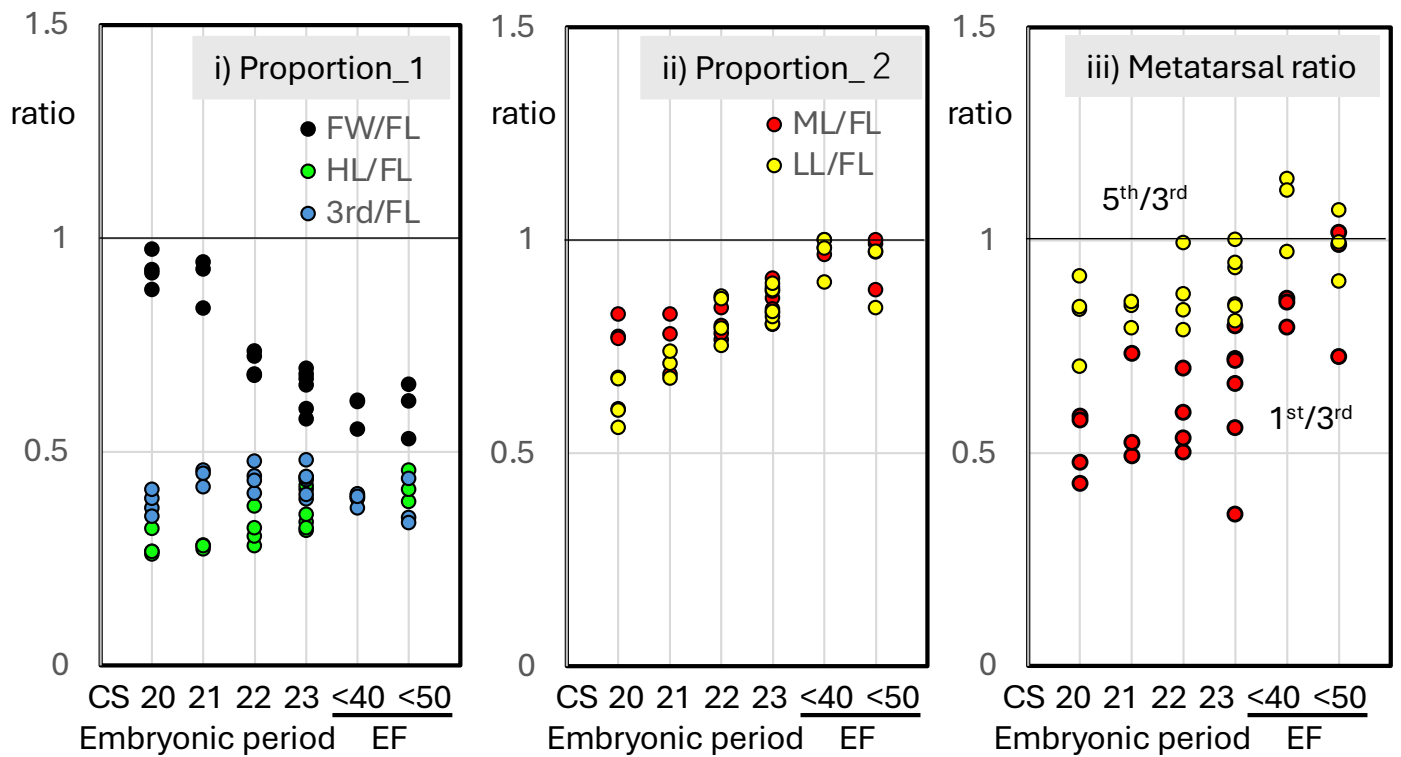


Figure6

a) Proportion (length ratio)



b) Angle measurements

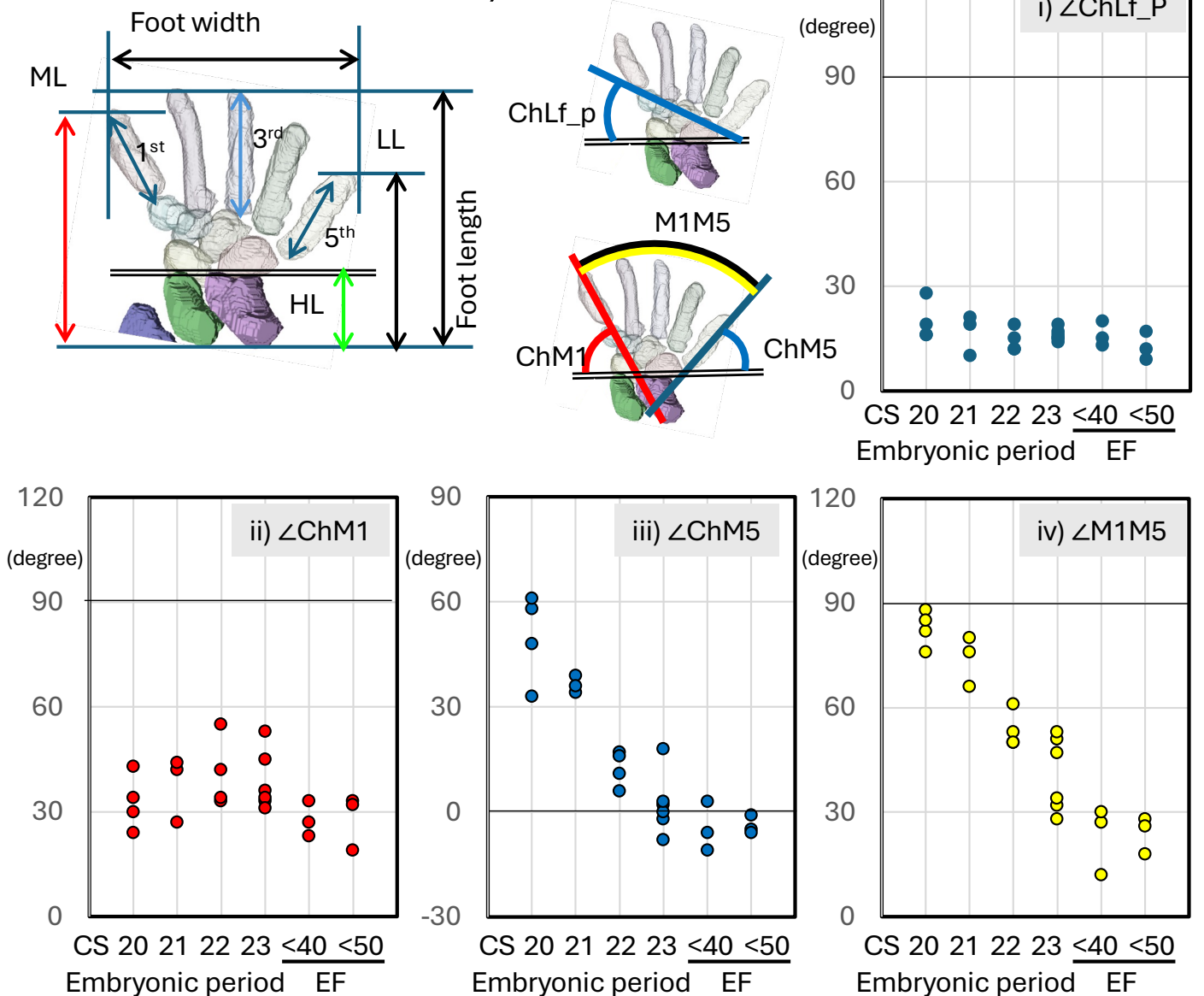


Figure7

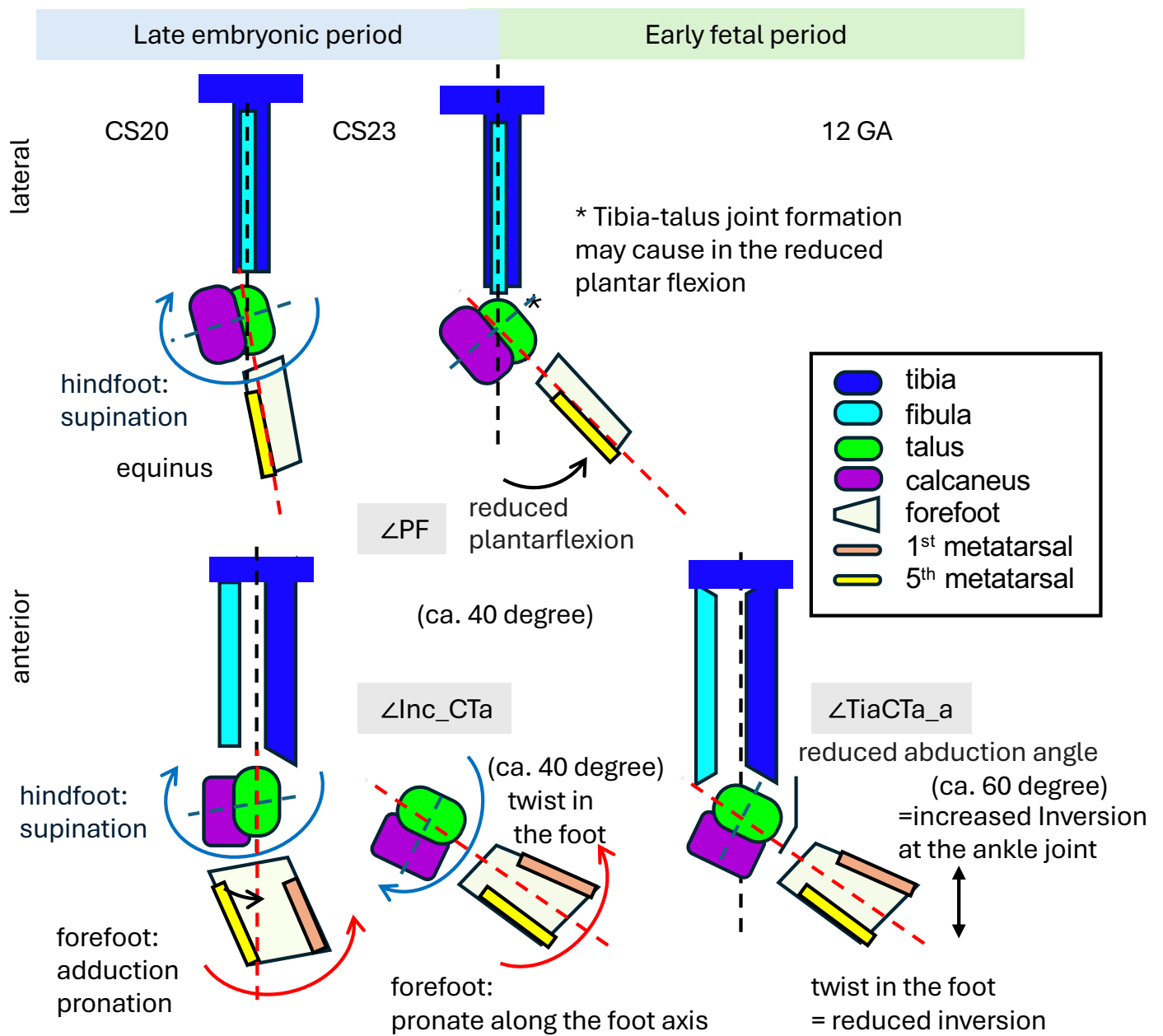


Figure 8

Table 1. Specimens used in the study

	Stage	GL	ID	Modality
Embryonic period	CS20	ND	23144	PCX-CT
		13.9	20268	PCX-CT
		16.6	21594	PCX-CT
		16.3	22403	PCX-CT
	CS21	19.0	24325	PCX-CT
		18.2	24787	PCX-CT
		17.0	30652	PCX-CT
	CS22	19.6	27050	PCX-CT
		19.7	27545	PCX-CT
		23.1	32163	PCX-CT
		21.4	35233	MRI
	CS23	21.2	20018	PCX-CT
		24.0	20249	MRI
		22.5	29137	PCX-CT
		24.3	52578	PCX-CT
		23.0	52817	MRI
		33.5	F2310	MRI
Early fetal period	<40 (CRL<40 mm)	37.2	52002*	MRI
		39.0	33467	MRI
		40.0	52730	MRI
	<50 (CRL<50 mm)	43.0	F2307	MRI
		48.5	F2624	MRI
		50.0	F2510*	MRI

CS: Carnegie stage, GL: greatest length, PCX-CT; phase-contrast X-ray computed tomography

*Shimane university