THE MISSING SEGMENT OF THE AUTOPOD 1st RAY: NEW INSIGHTS FROM A MORPHOMETRIC STUDY OF THE HUMAN HAND.

Length and shape homology of hand long bones and the relationship with cartilage anlagen ossification.

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ABSTRACT

Whether the 1st segment of the human autopod 1st ray is a “true” metapodial with loss of the proximal or mid phalanx or the original basal phalanx with loss of the metacarpal has been a long-lasting discussion.

The developmental pattern of upper autopod segments at fetal age 20th-22nd.weeks, combined with X-ray morphometry of normal hand long bones in the growing age was used for analysis of the parameters % length, epiphyseal ossification centers position and prox/distal growth rate.

The symmetrical growth pattern in the fetal anlagen changed to unidirectional in the postnatal development in relation to epiphyseal ossification formation. The % length assessment, the epiphyseal ossification centers distribution and differential prox/distal growth rate among the growing hand segments supported homology of thumb most proximal segment with the 2nd 5th proximal phalanges and that of the thumb proximal phalanx with the 2nd-5th mid phalanges in the same hand.

Either metanalysis of “triphalangeal thumb” and “prox/distal epiphyseal ossification centers” published case reports was used to support the applied morphometric methodology: particularly the latter did not give evidence of growth pattern inversion of the thumb proximal segment.

The presented datasupported the hypothesis that the lost segment of the autopod 1st ray during evolution is the metacarpal.

Key Words

Autopod fetal anlage growth. Fetal ossification pattern.

Postnatal ossification pattern. Morphometric and patterning homology

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INTRODUCTION

During the fetal period, the hand long bone anlagen of modern humans undergoes symmetric longitudinal growth of both the proximal and distal ends (Pazzaglia et al, 2017). However, this symmetric growth pattern changes with the onset of the epiphyseal ossification; this change is plainly evident in the postnatal age when the ossification centers can be routinely documented by X-rays. In contrast, the symmetric growth pattern of the proximal and distal anlagen ends is maintained in the stylopod and zeugopodof the upper limb (arm and forearm) until the closure of the growth plate cartilages (Caffey, 1948; Christie1949). In the lower limb, the cartilage anlagenossification pattern is similar to that of the upper limb.

X-rays of the normally developing hand and foot tubular bonesshowonly one epiphyseal ossification center and the related growth plate cartilage, whereas the opposite end is described as undergoing direct ossification, indicated by the term “pseudo-epiphysis” (Heines, 1938 1974; Ogden et al. 1994). The distribution of the epiphyseal ossification centers is distal in metacarpals and metatarsals from the 2^{nd} to 5^{th}ray, while the 1^{st} is proximal similar to those of all the phalanges.

The first ray of the hand and foot has only two phalanges (ph. formula = 2-3-3-3-3); this similar patterning and epiphyseal ossification center distribution in theautopodshas raised a long-lasting debate about homology and phylogenetic evolution of this ray in mammalian and non-therian-tetrapods (Reno et al. 2013). In this discussion, there are two hypotheses. 1) The 1^{st} metacarpal/metatarsal is the original basal phalanx and the corresponding metapodial has been lost during evolution. If this hypothesis is accepted it may solve the discrepancy of the epiphyseal center’s position between the 1^{st} and 2^{nd}-5^{th}metapodials. 2) The metacarpal/metatarsal is a “true” metapodial with loss of one element of the 1^{st} ray (the proximal or the mid phalanx). In this case, the 1^{st}metapodial ossification pattern must have been reversed in respect to those of the 2^{nd}–5^{th} rays.Apart from these morphological considerations, other advanced hypotheses consider the fusion between the thumb metacarpal with the same ray proximal phalanx (symbrachydactyly) or that of the distal with the mid phalanx of the thumb (Guillem et al. 1999). The epiphyseal end’s growth asymmetry in autopod metapodials and phalanges has been recently addressed in a morphological study by (Reno et al. 2006) in an attempt to identify the cellular events underlying the induction of growth plate formation; this was followed by a comparative study in theriantetrapods(alligators), which form growth plates at both ends of their metapodials (Reno et al. 2007). These authors suggested in a recent review paper

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that an answer to the question needs to be considered in a larger phylogenetic context and supported the view that the 1^{st} ray proximal segment is a “true” metapodial (Reno et al, 2013).

Anthropoids and hominins exhibit differential adaptation in the autopod segment proportions and number. This differential adaption is needed to satisfy similar functional demands related to climbing, suspension, bipedal posture and hand tool-use (Almecija et al. 2015; Marzke, 1997; Marzke & Marzke,2000; Young & Hallgrimsson, 2005). Both the molecular and fossil evidence have had important consequences for interpreting theevolutionary history of the hand within the Hominidae family and the Hominin tribe (Tocheri et al. 2008).

Histomorphology of fetal autopod segments and the postnatal morphometric study based on hand metacarpal and phalangeX-rays through the developmental age can integrate the knowledge derived from human and animal model histomorphology, developmental patterning studies and phylogenetic history. In this context, the present study offers several hints that may be summarized as follows: - 1) a well-established knowledge of the appearance of the tubular and carpal bones ossification centers, which have been developed for clinical use (Caffey, 1948; Christie, 1949; Vogt & Vickers, 1938); - 2) the availability of normal hand X-rays from hospital archives; and - 3) the wide documentation of congenital hand defects reported in radiology, hand and plastic surgery journals and the increasing number of gene analyses in syndromes that include hand development defects.

The aim of this study is to analyze the following: - a) the histology of human autopod segments in the 20^{th}-22^{nd} week of fetal age; - b) X-ray morphometry of normal hand long bones from postnatal age to 16 years old; and - c) metanalyses of congenital human phenotypes consistent with the metacarpal and phalanges development, such as the “triphalangeal thumb” and “prox/distal epiphyseal ossification centers”. The latter two phenotypes are related to autopod segment patterning, growth, and genetic controlled morphogenesis. Specifically, the problem rising from the thumb biphalangeal pattern in the length measurement was determined using the triphalangeal thumb metanalysis to set in the normal hand series as the reference ray for calculating the % length of the thumb segments. Otherwise, the distribution of the epiphyseal ossification centers, the epiphyseal shape and the proximal/distal growth rate index were evaluated and compared between the ray elements independently from the two or three phalangeal ray patterns.

The morphometric data of the study were limited to the development and ossification of the skeletal segments. To the best of our knowledge, combined metanalysis of human phenotypes with X-ray morphometry of normal hand series in

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the developmental period represents an original methodology for the analysis of autopod segment variance and covariance in the more general context of the molecular control and the evolutionary phylogenetic line.

MATERIALS AND METHODS

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Fetal anlagen histomorphology

The histological slides of the autopod anlagen were selected from a larger retrospective analysis of the Morbid Anatomy Archives where all pregnancy terminations are routinely examined. Only cases of cardiovascular and brain malformation without skeletal dysmorphia were considered (Pazzaglia et al, 2016). The examined material was in the developmental interval between the $20^{th}$and 22^{nd} week; the inclusion criteria required that slides were comprehensiveof the whole bone cartilage anlagen in longitudinal section. The study protocol was approved by the DSMC Council of the University of Brescia.

The tissue specimens had been fixed in neutral formaldehyde solution (10%), dehydrated in a series of increasing ethanol solution concentrations and embedded (undecalcified) in paraffin blocks. Sections 10 µm thick were stained with hematoxylin-eosin and observed with an Olympus BX51 microscope.

X-ray postnatal, normal hands series

A total of 53 hand X-rays of 47 normal children were selected from the Pediatric Radiology archives (Spedal iCivili di Brescia). The patients' ages were between 8 months and 15 years old and equally distributed for sex; in three patients, both hands were available. For 30 hands, both an X-ray antero-posterior view of the whole hand and of the 1^{st} ray was performed. The radiographic survey was carried out for trauma of the wrist/fingers to exclude fracture or joint dislocation. Other X-rays were taken for assessment of the skeletal age. X-rays were taken in an a-p projection of the hand, at a standard distance of 50cm from the radiogenic tube.Those of the thumb were obtained while changing the position of the thumb on the X-ray plate holder (Fig. 1).The selected 47 hand X-rays (only one for the three subjects with right and left hand available) were divided for the morphometric analysis into six age groups: A) 6 months-2 years; B) 3-4 years; C) 5-6 years; D) 7-8 years; E) 9-10 years; and F) over 10 years.

Length analysis

The length of each segment (metacarpals and phalanges) was assessed from the proximal to the distal end on the median axis; the epiphyseal ossification center (if present) was included in the measurement. The ray total length was calculated as the sum of the metacarpal and that of the corresponding phalanges. The absolute lengths were ordered transversally from the 1^{st} to 5^{th} ray. The % length of each element in the same hand was calculated on the total length of the corresponding ray. The thumb metacarpal, proximal and distal phalanx % lengths were calculated either on the 1^{st}or 3^{rd} ray total length of the same hand.The purpose of performing two measurements of the 1^{st} ray elements % length was to consider the bias due to the biphalangism of this ray (see triphalangeal thumb case report metanalysis).

Two series of comparison were carried out as follows:

-1 the thumb distal phalanx % length (calculated on the 1^{st} ray total length and that of the 3^{rd} ray of the same hand) versus the 2^{nd} - 5^{th}distal phalanges % length (calculated on its own ray);

-2 the thumb metacarpal and proximal phalanx % length (calculated on the 3^{rd} ray of the same hand) versus the corresponding 2^{nd} – 5^{th} metacarpals and proximal phalanges % lengths (each calculated on its own ray) or the thumb proximal phalanx versus the proximal and mid phalanges of the 2^{nd}- 5^{th} fingers respectively.

In the first comparison, the difference between the thumb distal phalanx % length with regard to the 1^{st} and 3^{rd} ray quantified the bias due to the missing segment of the thumb (the 3^{rd} ray length of the same hand was assumed as that of a hypothetical, ancestral thumb with the regular number of phalanges). Indeed, the homology of all the distal phalanges cannot be questioned because of the apical tuft specific morphology.

In the second comparison, the degree of length homology was tested for the following: thumb metacarpal **vs** the 2^{nd}–5^{th} metacarpals or the 2^{nd}–5^{th}prox phalanges and thumb proximal phalanx **vs** the 2^{nd}- 5^{th}prox phalanges or the 2^{nd}-5^{th} mid phalanges.

Epiphyseal ossification centers distribution and shape analysis

The time of appearance and distribution of the epiphyseal ossification center' was analyzed in the normal hand series separated into the earlier reported age groups by counting the mean number of ossification centers in the carpus and tubular bones.

The shape of the ossification centers was classified as "rounded" when the ratio between the longitudinal and transverse diameter was 1.0 – 0.5, "flattened" when it was 0.4 – 0.2 and "not-assessable" in the earlier phase of ossification.

Regarding the profile of the non-epiphyseal ends and the geometry of the meta-epiphysis some typical patterns characterized proximal and distal extremity of each bone. They could be distinguished as follows: A) "rounded", B) "cone-shaped", and C. "flat". A further, characterizing element was "metaphyseal flaring" (D). This evaluation was not enforceable before the appearance and sufficient organization of the ossification center; therefore, this feature could be defined only in the older age groups D, E and F (Fig. 1.1 and 1.2).

Proximal/distal growth rate index assessment

In all the analyzed phalanges and metacarpal X-rays the narrower part of the diaphysis did not correspond to the mid longitudinal length. Otherwise, in the early fetal period, the primary ossification center developed in the middle of the long bone cartilage anlage, which then provided the scaffold for the structuring diaphyseal cortex (Pazzaglia et al, 2016). Postnatally, the distance of the narrower, transverse diameter from the proximal and distal ends of each phalanx and metacarpal resulted from the longitudinal growth rate of the proximal and distal transition zone of the fetal anlage and from the metaphyseal growth plate when it was formed at the end of the fetal period. The ratio between these two measurements provided an index of the anlage proximal and distal growth.

To evaluate the normal hand series, the narrower, transverse diameter was traced in the diaphysisof the digitalized X-ray images (Fig. 2.1) and the distance from the proximal and distal ends was measured with the program "Cell"(Soft Imaging System GmbH, Munster, Germany). When the definition of the latter was uncertain, the proximal and distal boundaries of the narrower, central segment of the diaphysis were traced; the mid point of the latter was assumed as the level of the narrower diameter (Fig. 2.2). The ratio between the proximal/distal longitudinal segments was determined and was expressed numerically (IGR). It represented the differential growth rate of the anlage during the fetal and the early postnatal periods: the value 1 corresponded to a proximal longitudinal growth rate equal to

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the distal; the values > 1 to a higher proximal growth rate and those < 1 to a slower growth rate.

The shape analysis and the IGR of the thumb segments were not feasible in the standard a-p projection of the hand because the position of the1st ray corresponded to an oblique projection.Appropriate a-p thumb projections were available for 30 hands of the normal series. A further limitation of this evaluation was represented by the not yet sufficiently developed epiphyseal ossification centers. Therefore, statistical comparison of IGR and shape analysis was restricted to a smaller population of hands than that used for % length assessment ,including only the older age groups D, E and F.

Triphalangeal thumbs and prox/distal epiphyseal ossification centers metanalysis

Triphalangeal thumbs with completely developed phalanges (a condition which excluded delta or severely underdeveloped phalanges) was an uncommon pattern, which to the best of our knowledge has been documented only in the human species (Tab. 1). The morphometric analysis was carried out on a selected number of the published X-ray images. The inclusion criterion were the quality and definition of the scanned image, which should allow reliable measurements of the ray total length, the segments % length and IGR. All the analyzed triphalangeal hands were in young adults.The thumb metacarpal % length (on its own ray) and that of the 2nd-5th fingers was compared with the proximal and mid phalanges of the corresponding rays. Further, the % length of each 2nd- 5th ray segment was compared transversally with the corresponding segments of the 1st ray.

Proximal and distal epiphyseal ossification centers (in the same bone) were also uncommonly reported phenotypes. In the former, one or more autopod segments presented a longitudinal growth pattern through a proximal and a distal epiphyseal ossification center (Zuidam et al, 2006). In one case, it was reported to be associated with a triphalangeal thumb, but most frequently in hands with normal digital patterning (Tab. 2). In this hand series, the cases associated with polydactyly and those defined on the basis of the radiographic signs “notch”, “fissure” or “incomplete pseudoepiphysis” were not considered. The quality and definition of the scanned X-ray images of this series did not allow reliable measurements of the morphometric parameters; therefore, the metanalysis was limited to the distribution in each hand of the double epiphyseal ossification centers. Only in the

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deJong et al. (2014) case report could IGR be calculated and compared among all the hand segments.

Statistical Analysis

Repeated measurements of 380 hand segments were obtained independently by two investigators (AGS and AM) from a sample equal to 40 % of the total number of examined hands. Each data set was measured twice at an interval of one month in two series of paired measurements. The difference of each paired measurements (intra-observer and inter-observer) was plotted against the difference in individual segments and total ray lengths. By analyzing the differences between the paired measurements, the only error was that which was likely to follow a normal distribution. The variation in the differences for the two series of measurements was wider in the inter-observer paired data set than in the corresponding intra-observer set with a degree of agreement above the 95% confidence interval for both (Bland & Altman, 1986).

The % of finger segment length, the IGRs and the number of ossification centers were expressed as the mean ± SEM. Statistical analysis was performed with a statistics package (Graph Pad prism 5, Graph Pad Software, San Diego, CA, USA). Non-parametric data were analyzed by a Kruskall-Wallis test followed by Dunn's test or the Mann-Whitney test when appropriate.

The trend followed by the % measurements of finger segment lengths (each measured on its own ray) polled/age group over all age groups was analyzed by the area under the curve (AUC) calculated by trapezoidal approximation. Differences with $p<0.05$ were considered significant.

RESULTS

Fetal anlagen histomorphology

The autopod anlagen in the fetal period from the 20th-22nd week showed a more advanced chondrocyte maturation stage (hypertrophy) and inter-territorial matrix calcification with a proximal-distal progression along each ray. All the metacarpal

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anlagen central sector was calcified, providing the mineral scaffold for the apposition of the first periosteal lamellae. The longitudinal growth proceeded symmetrically provided by the aligned proliferation of the two transition zone chondrocytes (Fig. 3.1). A similar aspect could be observed in the proximal phalanges, while in the mid phalanges, chondrocytes had undergone hypertrophy with initial matrix calcification, and in the distal phalangeshypertrophy had occurred but without evidence of mineral deposit (Fig.3.2).

X-ray postnatal, normal hand series

% length of metacarpals and phalanges

The mean total length of the finger rays in the normal hand population increased from R1 to R3 and then decreased from R3 to R5 in all age groups, which represented the most common pattern of the species phenotype (Fig 1.1 and 1.2). In the comparison among the ray segments, the % length assessment was further biased by the missing segment 1st ray. The % length calculation of the thumb segments on the 3rd ray total length, rather than on the 1st, produced the same % correction among all age groups. The adjusted % length of the thumb distal phalanx was significantly higher than that of the 2nd-3rd phalanges of the younger age groups (A and B) and of the 4thphalanges of the older age groups (C, D and F) (Fig. 4). However, the homology of the distal phalanges was not questionable because they share the unique apical tuft feature (Mittra et al, 2007).

The profile (from age groups A to F) of the mean thumb metacarpal % length was lower than those detected in the 2nd-5th metacarpals (Fig. 5a) and superimposable on the profile of the 2nd-5th proximal phalanges (Fig. 5b). In line with these observations are the AUC data reported in Tab 1, which show a significant difference in AUC % length of the thumb metacarpal throughout the age groups versus the 2nd-5th metacarpals. The profile of the mean proximal phalanx % length was lower than those detected in the 2nd-5th proximal phalanges (Fig. 5c), reaching a high statistical significance as reported in the AUC analysis (Tab. 1), whereas it did not differ when compared with the 2nd-5th mid phalanges (Fig. 5c and Tab. 1).

These figures and data supported the % length parameter homology thumb metacarpal ≈ 2nd – 5th proximal phalanges and thumb proximal phalanx ≈ 2nd – 5th mid phalanges.

Distribution and shape of the epiphyseal ossification centers.

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The analyzed hand X-ray series covered a range of ages from 8 months to 16 years. The appearance time of the carpals and long bone epiphyseal ossification centers had avariable agreement with the chronological age; in the hands of the early age groups (A-B), few had appeared, while their number increased with age. In the older groups some had undergone a partial fusion: these could also be counted only if the ossification center shape and morphology were still recognizable.

The first evidence of epiphyseal ossification in the group from 8 months to 2 years was observed in the central rays (2^{nd}–3^{rd}-4^{th}) at the base of proximal phalanx; however, in two hands of this group no evidence of ossification was present in any of the long bones. Two carpal ossification centers had developed in all hands, and three carpal ossification centers had developedin one hand. However, the sequence of the appearance of the long bone center' did not follow a regular transverse or longitudinal order, so that occasionally one center could be absent or less developed either in the transverse line of the metacarpals and phalanges or along the digital ray. The mean number of centers increased in groups A and B and decreased later with the advancement of age, due to the fusion of the epiphyseal ossification center with the diaphysis. Only the number of carpal ossification centers showed a regular increment during the whole developmental period, thus validating their use for the assessment of the skeletal age (Fig. 6).

All the distal, mid and proximal phalanges ossification centers were type "flattened"and proximally positioned; those of the 2^{nd}-5^{th}metacarpals were type "rounded" and distally positioned (Fig.1.1 and 1.2). The shape description of the thumb metacarpal and proximal phalanx was uncertain because in the standard hand X-ray the thumb projection was a ¾ oblique, but the metacarpal ossification center was always proximal. The available thumb a-p projectionsof age groups D-F documented the appearance sequence of the 1^{st} ray ossification centers from the distal phalanx to the metacarpal and the apical tuft of all the distal phalanges. Both the proximal ossification centers of the 1^{st} ray phalanx and metacarpal were classifiable as "flat"; however, the joint outline of the latter was unique because it was modeled on the shape of the saddle joint with the trapezius. All the segments of the thumb had larger transverse diameters than those of the other fingers (Fig. 1.3).

Regarding the shape of the 2^{nd} – 5^{th}ray segments, metaphyseal flaring characterized the proximal end of all phalanges in contrast to the inverted cone-shape of the distal end. In metacarpals before the appearance of the ossification centers, flaring was less evident than in the phalanges, but with the development of the distal centers and the cortical remodeling, the bone had an elongated, clepsydra-like shape (Fig. 1.2).

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Proximal/distal growth rate index assessment

The proximal/distal growth rate of each thumb and finger segment class could be determined only in the older age groups D, E and F, because the definition of the narrower, transverse diameter was uncertain until the diaphysis was modeled. An IGR = 1 indicated a symmetric proximal/distal longitudinal growth rate, while a comprehensive description of the distribution and growth rate difference among segments in the age groups is given in Fig. 7. All the phalanges showed an IGR>1, with an increase from the age group D to the older ones. In the 2nd -5th metacarpals, the index documented a higher distal growth rate, while in the thumb the higher rate was proximal. Significant differences were observed comparing homologous segments in the three age groups and between the segments of each ray (Fig. 8).

Triphalangeal thumb and prox/distal epiphyseal ossification centers metanalysis

Case reports of triphalangeal thumb and proximal and distal epiphyseal ossification centers were both uncommon phenotypes.

The first cases maypresent with different degrees of expression such as hypoplastic or dysplasic supernumerary segments (known as delta phalanx), while they are rare when the extra-phalanx is fully developed. In the metanalysis of “triphalangeal thumb” we found 12 hands in 9 reported cases, which were mostly young adults or adolescents. Based on the quality of the published X-rays, 8 hands were suitable for measurements (Tab. 2). These rare phenotypes were relevant for the aims of the study because the homology of the 1st metacarpal with the other four was not questionable.

The length of the hand segments was compared along each ray axis between the metacarpal and the proximal, mid and distal phalanx using the % length the segments in relation to each ray’s own total length (Fig. 9.1) and transversally between the series of the five segments in line (Fig. 9.2). The % length was significantly different between the ray segments in the longitudinal sequence metacarpal – proximal - mid - distal phalanx but not significant in the transverse line. Further, the mean 1st metacarpal IGR (calculated in 8 hands) was not significantly different from the mean of the 2nd-5th metacarpals in the same hand. This suggested the homology between the 1st and the 2nd-5th metacarpals in this phenotype. Moreover, one case of this group (Heiss, 1957) documented that an autopod ray

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pattern = 4-4-4-4-4 in humans could occur through a genetic mutation, since it was present bilaterally in the mother and in her newborn (Fig. 10).

The metanalysis of complete double ossification centers case reports included 9 hands with an irregular distribution among the involved segments with a prevalence of metacarpals on the proximal and mid phalanges but never in the distal ones (Tab. 3). No % length measurements were enforceable in this hand series. However, in both hands of the case reported by de Jong et al (2014), all the 1^{st}-5^{th} proximal and mid phalanges and the thumb metacarpal had double, well developed epiphyseal ossification centers, while in the 2^{nd} -5^{th} metacarpals, the ossification pattern was regular (Fig. 11.1), enabling the IGR evaluation of this hand. It is worth to pointing out that this case was also the result of a genetic mutation because the younger sibling presented with the same bilateral pattern. The IGR of regular patterned hand segments (2^{nd}- 5^{th} metacarpals and 1^{st}-5^{th} distal phalanges) and those of the double epiphyseal centers (all the other) ,documented (Fig. 11.2) a significantly higher index in the former (coherently with the position of the unique ossification center) compared with the double ossification center, where the mean IGR result was 0.74 for the 2^{nd} – 5^{th}metacarpals and 1.38 for proximal and mid phalanges, and still higher (1.82) for the distal phalanges.

DISCUSSION

Skeletal morphometryis a currently applied methodology in anthropology, paleontology, zoology and anatomy (Kivell, 2015). Since diversification is the key issue of biological development and evolution, homology, topology and typology represent basic concepts to deduce the phylogenetic history of the skeleton in the the Kindom Animalia (Rieppel, 1993). Several parameters may be used to define the origin and the transformation of the vertebrate skeletal elements; they include size, shape, structural morphology, growth patterns , biochemistry, genetic transmission and control.

The autopod anlagen histomorphology and the X-ray morphometry examined in this normal hand series during the postnatal developmental age addresses the question of homology of the thumb segments with the posterior metacarpals and phalanges. The answers to the above questions lead to the identification of the missing thumb

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segment and the different interpretations of the human autopod development given so far.

The comparative analysis of the homologous autopod segments and the measurement methodology required some statistical contrivances in relation to the developmental age of the studied population and to the somatic individual phenotypic variations. Regarding the first point, the length of the metacarpal and phalanges was divided into classes by age; for the second point, the metacarpal or phalanx length was expressed as % of the corresponding ray total length in the same hand. However, a comparative evaluation of the thumb segments homology with that of the posterior fingers in the same hand was hampered by the yet unsolved question of the missing 1st ray segment.

To the best of our knowledge, the hand ray pattern = 4-4-4-4-4 (triphalangeal thumb) was seldom reported in modern humans and never in therian tetrapods and anthropoids. However, this statement does not mean that this phenotype can be expressedonly in the *Family Hominidae*, rather than in *Homo Sapiens* (modern humans), the most monitored species in the Kindom Animalia because of medical care. The metanalysis of reported triphalangeal thumb cases was used to reduce the missing element bias of the 1st ray measurements because this allowed extension to a more reliable % length comparison to the thumb segments. Beyond the methodological considerations, the triphalangeal thumb series gave evidence of a gene mutation that produced a phenotype with an evident length homology among the 1st segments of the hand rays. The familiar transmission of this phenotype from the mother to the newborn was documented by the case report of Heiss (1953) and by the genealogical tree of five families (Girisha et al, 2014; Heutink et al, 1994; Warm et al, 1988; Wieczorek et al, 2010), where gene mutations were reported in the subtelomeric region of chromosome 7q or in the zone of polarizing activity regulatory sequence (ZRS) of Werner Mesomelia.

The opinion of the thumb metacarpal as a modified phalanx was bolstered by many authors (Guillem et al, 1999; Jay, 1978; Thompson, 1869; Valenzuela et al, 2009), who considered primarily the parameter length and epiphyseal ossification center position, proximal in the phalanges and distal in metacarpals, respectively. The comparative % length analysis between the thumb and the posterior fingers in this study was original and allowed a crossed, statistical comparison of the thumb metacarpal **vs** the 2nd-5th proximal phalanges and the thumb proximal phalanx **vs** the 2nd-5th mid phalanges. Regarding the epiphyseal ossification centers, we also considered the position in addition to the shape and the bone segments' growth rate index (IGR). The first could be properly evaluated only with the a-p projection X-

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ray of the thumb because the standard hand a-p projection gave an oblique and distorted image of the ossification centers. The second was directly correlated to the growth pattern allowing a quantitative evaluation of the growth process dynamics.

In the context of the debated question, the assignation of the thumb proximal segment to the metacarpal or phalanx class is a cornerstone for the autopod development and evolution understanding; in particular, the epiphyseal ossification pattern deserved a thorough analysis. Reno et al (2006), using an experimental model with mouse posterior metatarsals, observed at one end the formation of a typical growth plate interposed between the primary and the epiphyseal ossification centers, while at the opposite end a disorganized ossification replaced the cartilage epiphysis directly. The same pattern was also described in children's growing bones by Heines (1974) and Ogden et al. (1994). Further, Reno et al (2007) demonstrated the presence of growth plates at both cartilage anlage ends in alligator metapodials. More recently, the same authors (Reno et al, 2013) reviewed the literature reports of bidirectional growth in several therian tetrapod species and birds, concluding that the latter was the ancestral condition, which was lost in both placental and marsupial tetrapod mammals (therian synapomorphism). Their conclusions were that, despite the anatomical similarities shared by thumb metacarpal and phalanges, which continue to be the primary basis for the hypothesis of a modified phalanx, the question should be considered in a larger phylogenetic context because the comparative developmental biology suggested that MP1 was not a phalanx.

The bidirectional growth as an ancestral condition of the autopod growth pattern, which changed to unidirectional in tetrapod mammals in the phylogenetic lineage is not in contrast with the histomorphology of human hand development. Indeed, up to the 23rd week of fetal age, growth was characterized by a symmetrical proximal and distal ends length increment in metacarpals, proximal or mid phalanges (Pazzaglia et al, 2016, 2017). The data presented in this study confirmed that two different patterns of growth can be distinguished in human hand development related to age: the fetal phase with bidirectional and balanced growth in both metacarpals and phalanges and the postnatal, with growth in length restricted to the cartilage bone model extremity, where the epiphyseal center had initially formed; later, the metaphyseal growth plate cartilage provided the remaining longitudinal growth up to skeletal maturity.

The IGR assessment in the normal hand series (age groups D-F) measured the whole growth period comprehensive of the fetal phase (growth bidirectional) and of the postnatal period (growth unidirectional). This index documented the growth dynamics of metacarpals and phalanges, with full conformity to the epiphyseal

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ossification centers' position. However, the relevant point for the aim of the paper was the documented, significant difference between the IGR of the 1^{st} metacarpal and that of the 2^{nd}-5^{th} ray. Therefore, the hypothesis that the thumb 1^{st} segment is a "true" metacarpal implies the need to explain the inversion of this segment's unidirectional growth pattern.

In the detailed review of the evolutionary development and patterning digit identity, Reno et al. (2013) stated that the profound difference in selector gene expression territories during the 1^{st} ray evolution had so altered the morphologies, growth patterns and responses of the 1^{st} ray to the downstream gene expression, that it was impossible to resolve the question of identity and homology of the mammalian 1^{st} metacarpal". Further, they interpreted the triphalangeal thumb phenotype in humans as a complete homeotic transformation into an ancestral index finger associated with a proximal and distal ossification center and bidirectional growth.

The triphalangeal thumb (TPT) phenotype in humans is expression of a transmittable mutation producing an anlage epiphyseal ossification and growth pattern (abridged by the parametric length) similar to that of the other four rays segments. It is also worth emphasizing that the latter was associated in almost all cases with a trapezius-1^{st} metacarpal saddle joint dysmorphism and with failure of the related muscle and tendon system development, which produced a non-opposable 1^{st}ray (also indicated by the term "five-fingered hand"). The metanalysis for morphometry required selection of published X-rays that satisfied the basic conditions of having fully developed hand segments, absence of other congenital defects and good quality of the X-ray reproduced image. All the analyzed cases were young adults with ossified epiphyses; therefore, the ossification center position or the presence of a proximal and distal center was not assessable. However, the TPT 1^{st} metacarpal IGR showed the same growth pattern of the 2^{nd}-5^{th} hand metacarpals (IGR <1), in contrast to that of the five proximal phalanges of the corresponding rays (IGR >1). Therefore, these data did not give useful insights to explain the proximal location of the metacarpal ossification center in the normal hand.

Proximal and distal epiphyseal ossification centers were seldom reported in both metacarpals and phalanges of otherwise normal hands, without an exclusive localization in the 1^{st} ray metacarpal. The deJong et al. (2014) report of two siblings with the widest distribution so far documented of true, double ossification centers in both the hands and feet suggested a mutation that did not change the patterning of the autopod segments, but whose expression was limited to the anlage epiphyseal ossification and longitudinal growth pattern. From the metanalysis carried out in this study, the number of true, double ossification centers was difficult

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to ascertain because the earlier papers (Brailsford, 1943; Dreizen et al, 1965; Garn et al, 1972; Posener et al, 1939; Snodgrasse et al, 1955) also included features such as epiphyseal notches or partial clefts, which were interpreted as an incomplete or a late phase of the supernumerary ossification center fusion. The variability of the epiphyseal center's time of appearance and the age of the child when the X-rays were taken contributed to the uncertainty of frequency figures in the hand tubular bones. In general, proximal and distal epiphyseal centers in the same bone were rare observations that had a variable distribution in the autopod segments. Zuidam et al. (2006) calculated the length ratio between 6 metacarpals (with double ossification centers) and the corresponding 2nd metacarpal in the same hand. This ratio was compared with the values of the normal population given by Garn et al. (1972), resulting an increase in the 1st group compared to the normal population. More extensive research, based on X-ray IGR assessment in a normal hand series could give a more reliable incidence of this growth pattern, since an IGR ≈ 1 should correspond to a bidirectional, longitudinal growth pattern.

In the discussion of the 1st ray segments anatomical definition, the TPT and bidirectional growth pattern are of particular interest. To the best of our knowledge, there have been no reports of a ray patterning = 4-4-4-4-4 in the evolutive lineage of therian tetrapods and anthropoids, which suggests that possible gene mutations similar to those documented in modern humans had not given a reproductive advantage and did not survive natural selection. Exclusive reports among human subjects can be explained by the incomparable, wide diffusion of research and medical care in this species. Beside TPT and the hand segment bidirectional growth pattern, the congenital hand malformations extensively studied in modern humans express in general mutations involving the Hox genes and the signaling pattern through overexpression or repression of Shh regulatory region of the limb bud(Burke et al, 1995; Reno et al, 2008; Rosello-Diez et al, 2011; Tickle et al, 1975). The oldest classifications were exclusively based on the appearance of the clinical defect (Swanson, 1964). The increased knowledge of the molecular basis of limb development prompted new classification schemes that also considered genetic and molecular pathways involved in skeletal segment patterning (Oberg, 2014; Oberg et al, 2010). In relation to the present discussion and the point concerning the missing thumb segment, the thumb hypoplasia (radial longitudinal deficiency) of the Blauth (1981) classification, may be relevant, which was updated by Manske et al (1995) who provided examples of severe metacarpal underdevelopment or absence as a specific entity.

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In conclusion, the normal hand X-ray morphometric study suggested that the missing thumb segment was the metacarpal. The proportion of the hand segments along each ray was respected if a correction for the 1st ray missing segment was introduced. The ray formula 3-4-4-4-4, the directional growth pattern, and the shape of the epiphyseal ends (including apical tufts of distal phalanges) remained remarkably constant in the tatrapods evolution lineage with only two examples of a different formula 2-4-4-4-4 in extant primates (Patel & Maiolono, 2016).

Variations of segment length and width occurred among taxa as an evolutive adaptation to tetrapedal and bipedal walking, climbing and suspension up to upper limb and tool manipulation. Otherwise, the lack in the phylogenetic lineage of triphalangeal thumb and other human phenotypes did not seem sufficient to support the opposing theory of the proximal thumb segment as a modified metacarpal against the data provided by morphometry.

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None of the authors had conflicts of interest.

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Legends of Figures

Fig. 1

Fetal anlagen (22ndweek), hematoxlin-eosin, bar = 500 μm.

.1 The primary ossification center (diaphyseal) of the metacarpals and proximal phalanges documents the complete calcification of the interterritorial matrix between the hypertrophic chondrocytes and the initial deposition of periosteal cortical bone (arrowheads). The process is more advanced in metacarpals than in the phalanx. The symmetric length growth is documented by the equal distance between the mid anlage transverse plane (dotted line) and the epiphyseal ends (proximal and distal arrows).

.2 Initial mineral deposition in the hypertrophic, central zone of the mid phalanx (asterisk), which is delayed in respect to the proximal phalanx and metacarpals, however, the periosteal apposition is already evident (arrowheads). The images are taken from the same autopod of Fig. 1.1. The distal phalanx does not show evidence of calcification, but in the central zone the chondrocytes are undergoing the hypertrophy process (dotted circle). In the mid phalanx, symmetric longitudinal growth is evident; in the distal phalanx, cartilage growth of the basal end is higher than that of the apical tuft.

Fig. 2

.1 Right hand X-ray, a-p projection (age 8 months, group A).

Early stage of ossification with two centers of the carpal short bone anlagen and with basal , epiphyseal ossification centers of the 3rd and 4th ray proximal phalanges. The thumb bone segments are taken in an oblique projection, not comparable for shape analysis with those of the 2nd and 5th rays.

.2 Right hand X-ray, a-p projection (age 13 years, group F).

Advanced stage of ossification with all eight carpal bones ossification centers and the presence of all the long bones ossification centers: proximal position of the 1st – 5th phalanges and inverted position of the thumb metacarpal to the 2nd – 5th metacarpals. The shape of the thumb ossification center can be classified as flattened even if it is thicker than the phalangeal center, but it certainly is not similar to the rounded-shaped distal epiphyses of the 2nd – 5th metacarpals. The thumb bone segments are taken in oblique projection as in age group A.

.3 Hand X-ray, 1st ray a-p projection (age 9 years, group E).

Shape analysis of the thumb segments in this projection allows comparison with the other rays segments.

Fig. 3

Graphic illustration of the growth rate index (IGR) measurement method in postnatal long bones (see details in materials and methods). This assessment could be applicable only in segments with a well-developed epiphyseal ossification center (age groups D-F).

Fig. 4

The 1st ray distal phalanx mean % length (measured on the 3rd ray total length) was compared with the 2nd – 5th ray distal phalanges mean % length (measured on each ray’s own total length). Result was significantly higher than that of the 2nd – 3rd ray distal phalanges in all age groups A – F ; not significantly different than that of the 4th – 5th rays of age groups C – D. The typology of the 1st ray distal phalanx cannot be questioned because of the characterizing apical tuft morphology. Therefore, the observed differences documented a “true” major growth of the latter segment versus the 2nd – 3rd rays; this is independent from the % measurement method, when it was assumed that the reference to the 3rd ray total length corrected the bias due to the missing segment of the thumb.

(* $p<0.05$; ** $p<0.01$; *** $p<0.001$)

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Fig. 5

1 (**a-b**) Graphic profile of R1-R5 metacarpals % length total length (R1 measured on R3 total length, R2-R5 on each ray's own total length) in age groups from A to F. This documents the % length dishomology of Mtc R1 (red) in respect to Mtcs R2-R5 (red) and the homology of the same Mtc R1 (red) with respect to the % length of Ph-p R2-R5 (blue).

(**c-d**) Corresponding graphic profile of R1-R5 metacarpals % length (R1 measured on R3 total length, R2-R5 on each ray's own total length) documenting the % length dishomology of Ph-p R1 (blue) in respect to Ph-p R2-R5 (blue) and the homology of the same Ph-p R1 (green) with respect to % length of Ph-m R2-R5 (blue).

Tab. 1

(**Tab AUC**) Quantitative assessment of differences among individual profiles was carried out and compared through the trapezoidal rule of AUC (Area Under Curve).

(Mtc R1 vs Ph-p R2-R5   * $p<0.05$;   ** $p<0.01$;   *** $p<0.001$)

(Ph-p R1 vs Ph-m R2-R5   ° $p< 0.05$;   °° $p<0.01$;   °°° $p<0.001$)

Fig. 6

The regular progression of the number of carpal ossification centers with age confirmed the current use for clinical assessment of skeletal age (Vogt & Vickers, 1938; Greunlich & Pyle, 1959). The different slope of the tubular bone epiphyseal ossification center number among the age groups is representative of variability of epiphyseal centers ossification time of appearance in these segments. The reduction in number between age groups A and F corresponds to fusion with the ossified diaphyses.

Fig. 7

Proximal-distal growth rate index (IGR) compared among R1-R5 metacarpals (Mtc), proximal phalanges (Ph-p) and mid phalanges (Ph-m) in age groups D – E. This parameter was not assessable in age groups A – C.

With reference to IGR $\cong 1$ corresponding to symmetrical, bidirectional growth, the index was inverted at the passage from the 1^{st} and the 2^{nd} metacarpals with an evident relationship with the epiphyseal ossification center position (and later growth plate cartilage). Significant differences in proximal and mid phalanges (not reported in the histograms) but without inversion.

(* $p<0.05$;   ** $p<0.01$;   *** $p<0.001$ versus R1 Mtc)

Fig. 8

Triphalangeal thumb metanalysis.

Comparison of the mean % length (measured on its ray's own total length) 1^{st}-5^{th} ray metacarpals (Mtc), proximal (Ph-p), mid (Ph-m) and distal (Ph-d) phalanges of TPT series (mean ± SEM of 8 subjects). No significant difference when each segment type is considered in the transverse sequence R1-R5. The % length in all rays decreases from metacarpal to distal phalanges.

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Fig. 9

Image of triphalangeal thumb of the right and left hand of the mother (.1) and her newborn (.2) reported by Heiss (1953) and reproduced from Zeitschrift fur Anatomie und Entwicklungsgeschicte with permission of Springer Nature (license n. 4334811065195).

Fig. 10

.1 Image of the hand with the widest distribution of proximal and distal epiphyseal ossification centers, reported in two siblings by deJong et al (2014) and reproduced from The Journal of Hand Surgery with permission of Elsevier (license n. 4280070488758).

.2 Table reporting the IGR calculationof each hand segment.

Tab. 2

Case reports used for metanalysis of triphalangeal thumbs.

Tab. 3

Case reports used for metanalysis of prx/distal epiphyseal ossification centers and distribution in hand long bones segments.

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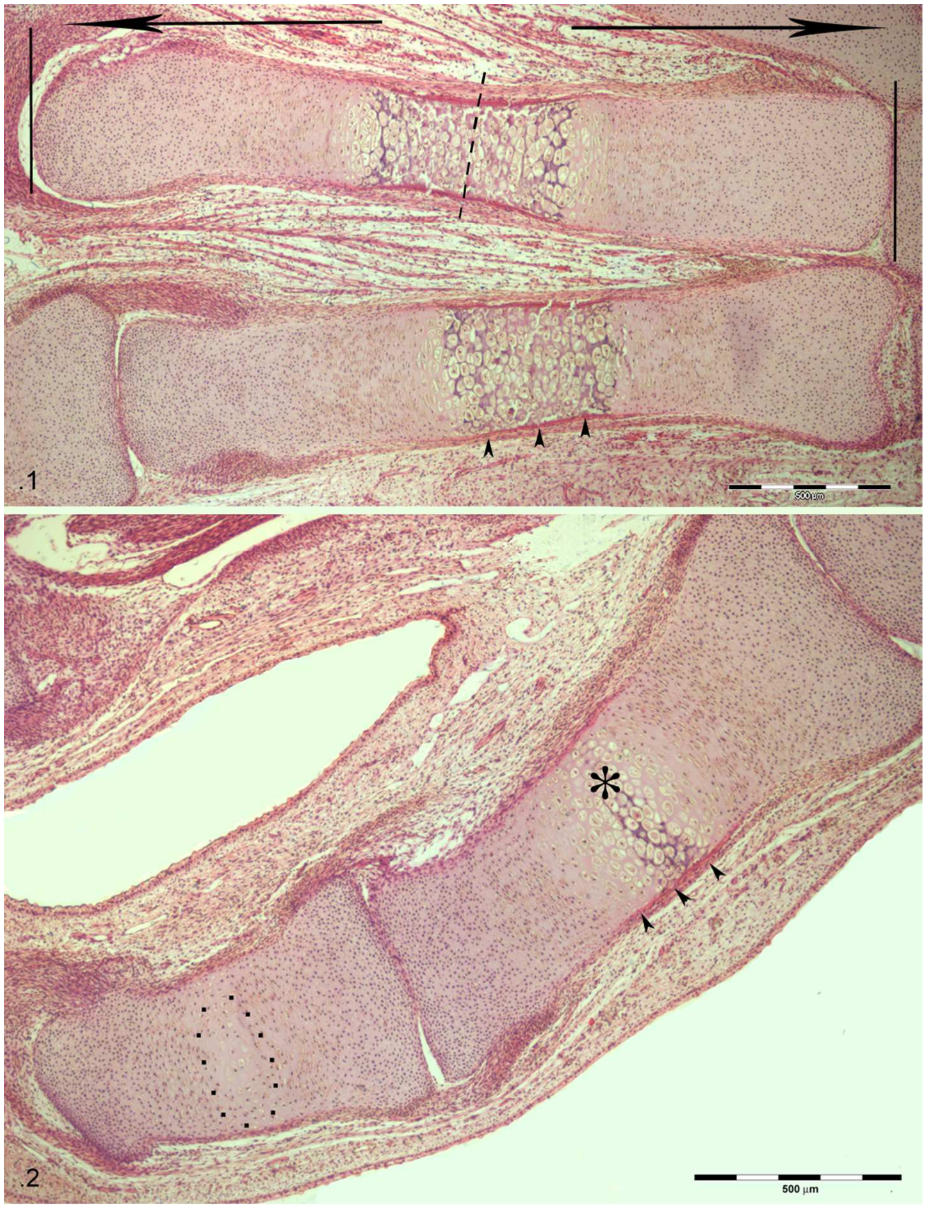

Fig. 1
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.1 The primary ossification center (diaphyseal) of the metacarpals and proximal phalanges documents the complete calcification of the interterritorial matrix between the hypertrophic chondrocytes and the initial deposition of periosteal cortical bone (arrowheads). The process is more advanced in metacarpals than in the phalanx. The symmetric length growth is documented by the equal distance between the mid anlage transverse plane (dotted line) and the epiphyseal ends (proximal and distal arrows).
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97x126mm (300 x 300 DPI)

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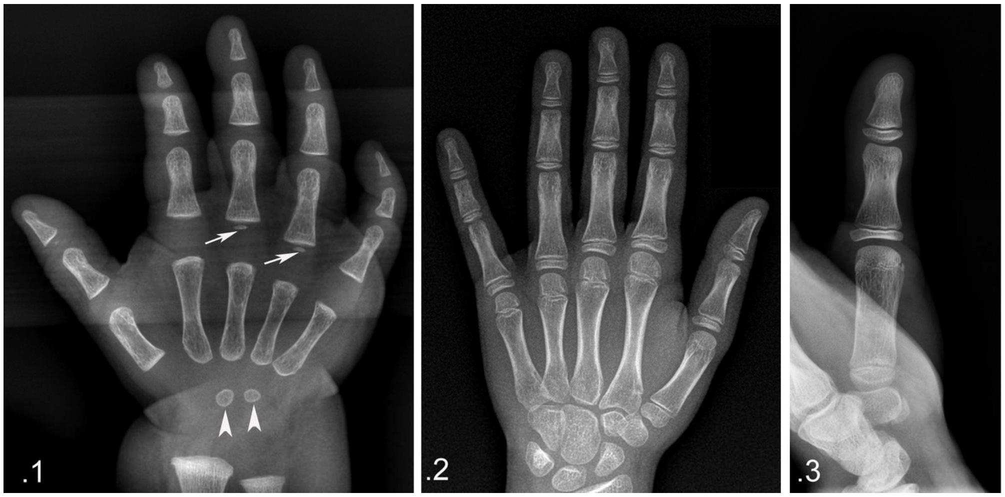

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.3 Hand X-ray, 1st ray a-p projection (age 9 years, group E).
Shape analysis of the thumb segments in this projection allows comparison with the other rays segments.

664x324mm (72 x 72 DPI)

Only

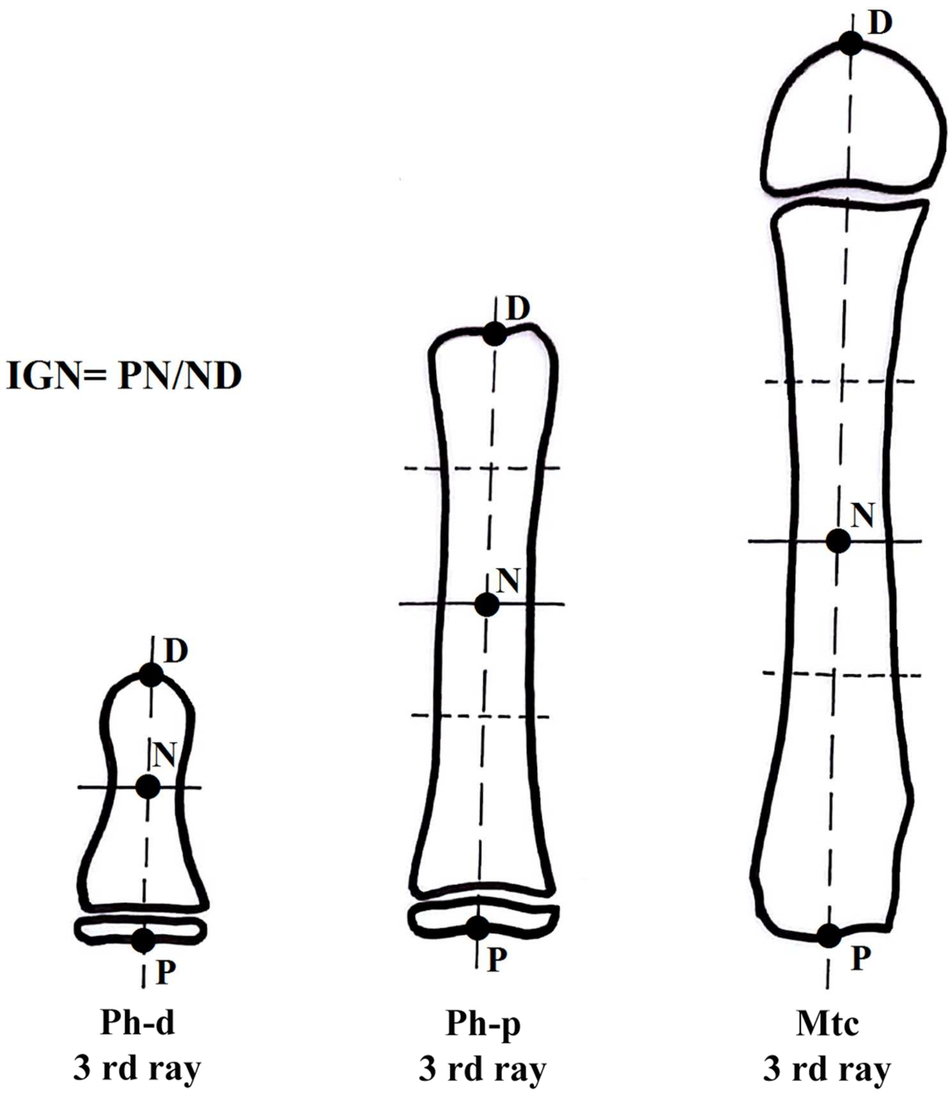

Fig. 3
Graphic illustration of the growth rate index (IGR) measurement method in postnatal long bones (see details in materials and methods). This assessment could be applicable only in segments with a well-developed epiphyseal ossification center (age groups D-F).

437x488mm (72 x 72 DPI)

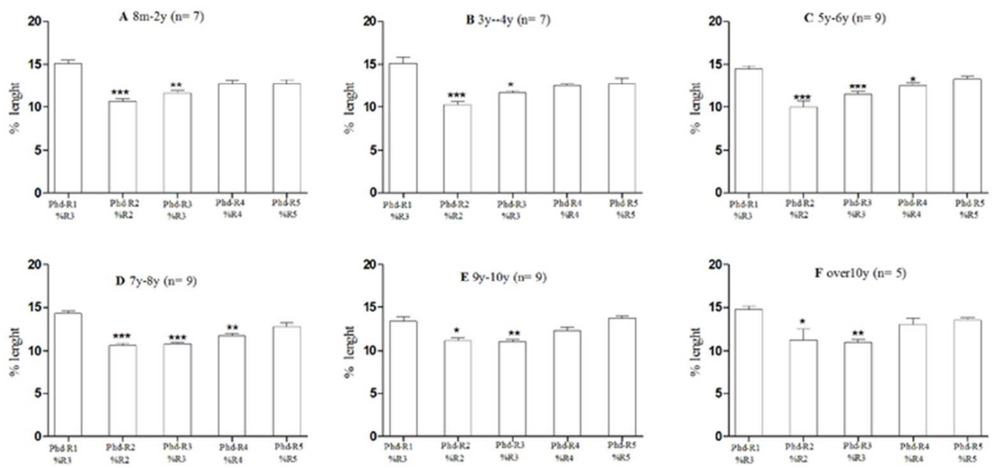

Fig. 4
The 1st ray distal phalanx mean % length (measured on the 3rd ray total length) was compared with the 2nd – 5th ray distal phalanges mean % length (measured on each ray's own total length). Result was significantly higher than that of the 2nd – 3rd ray distal phalanges in all age groups A – F ; not significantly different than that of the 4th – 5th rays of age groups C – D. The typology of the 1st ray distal phalanx cannot be questioned because of the characterizing apical tuft morphology. Therefore, the observed differences documented a "true" major growth of the latter segment versus the 2nd – 3rd rays; this is independent from the % measurement method, when it was assumed that the reference to the 3rd ray total length corrected the bias due to the missing segment of the thumb.
(* $p<0.05$; ** $p<0.01$; *** $p<0.001$)

66x32mm (300 x 300 DPI)

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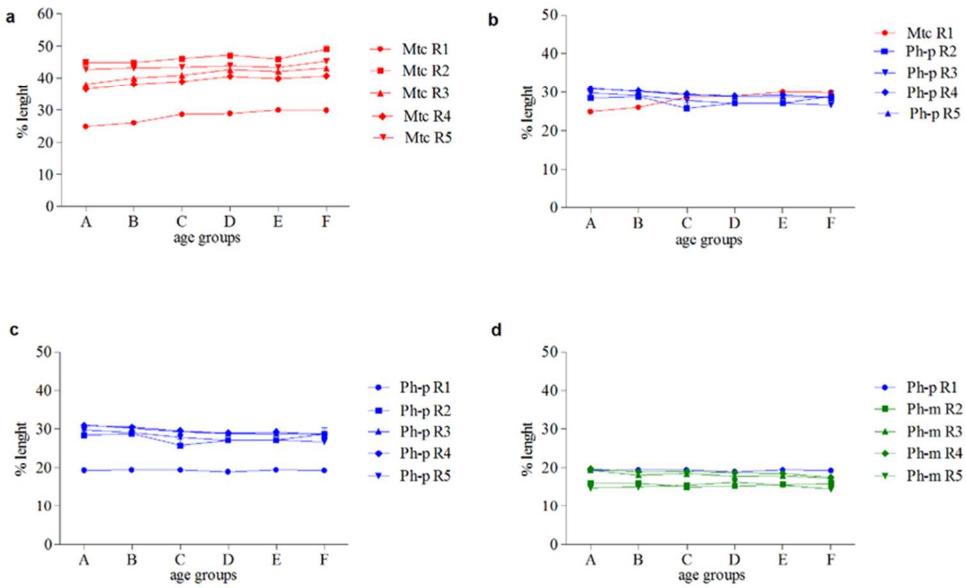

Fig. 5 ‼ ┼ 1 (a-b) Graphic profile of R1-R5 metacarpals % length total length (R1 measured on R3 total length, R2-R5 on each own ray total length) in age groups from A to F, documenting the % length dishomology of Mtc R1 (red) in respect to Mtcs R2-R5 (red) and the homology of the same Mtc R1 (red) respectively to the % length of Ph-p R2-R5 (blue). ‼ ┼ (c-d) Corresponding graphic profile of R1-R5 metacarpals % length (R1 measured on R3 total length, R2-R5 on each own ray total length) documenting the % length dishomology of Ph-p R1 (blue) in respect to Ph-p R2-R5 (blue) and the homology of the same Ph-p R1 (green) respectively to % length of Ph-m R2-R5 (blue).‼ ┼ 2 Quantitative assessment of differences among individual profiles was carried out and compared through the trapezoidal rule of AUC (Area Under Curve).‼ ┼ (Mtc R1 vs Ph-p R2-R5 * $p<0.05$; ** $p<0.01$; *** $p<0.001$)‼ ┼ (Ph-p R1 vs Ph-m R2-R5 ° $p< 0.05$; °° $p<0.01$; °°° $p<0.001$)‼ ┼

75x47mm (300 x 300 DPI)

Only

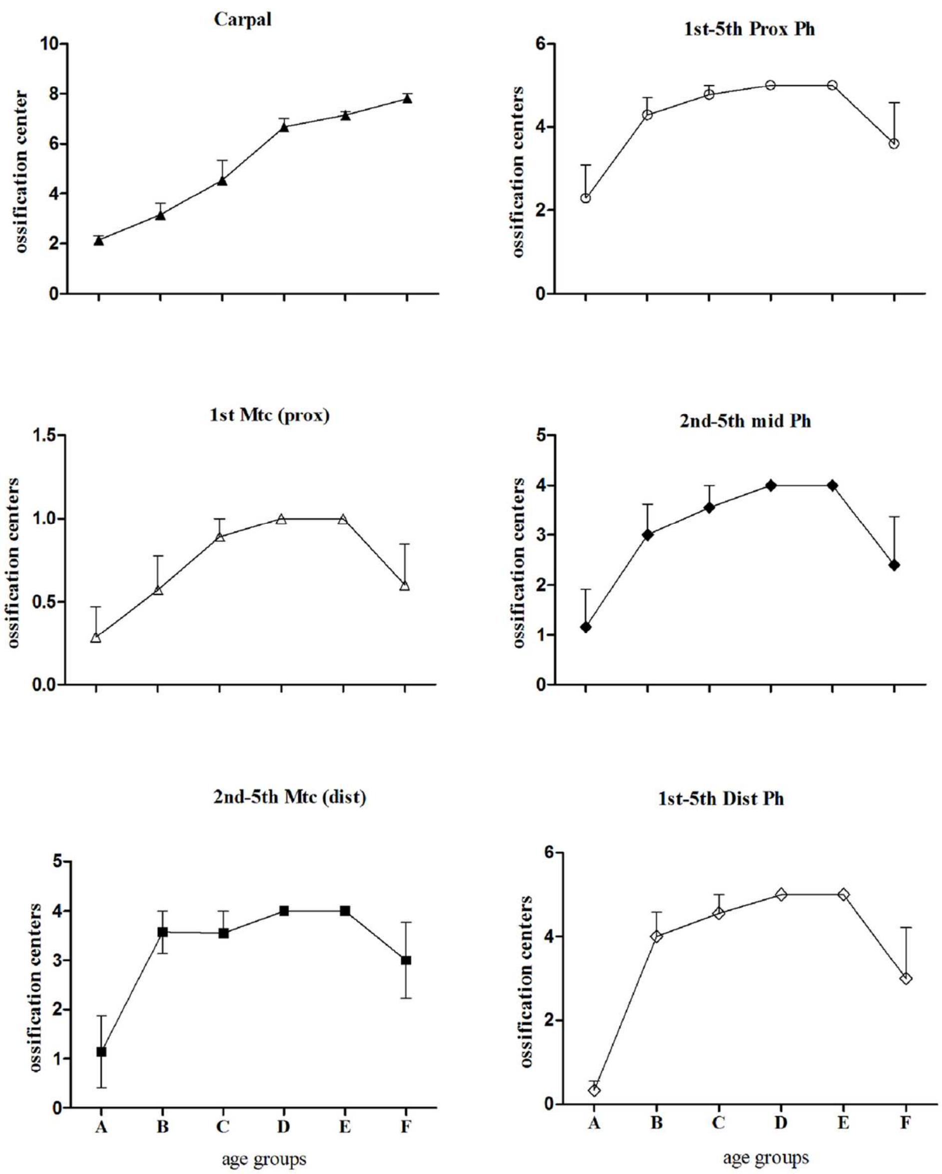

Fig. 6‼ ┼ The regular number progression of carpal ossificati on centers with the age confirmed the current use for clinical assessment of skeletal age (Vogt and Vickers, 1938; Greunlich and Pyle, 1959). The different slope of the tubular bone epiphyseal ossification center number among the age groups is representative of variability of epiphyseal centers ossification time in these segments. The number reduction between age groups A and F corresponds to fusion with the ossified diaphysis.‼ ┼

104x129mm (300 x 300 DPI)

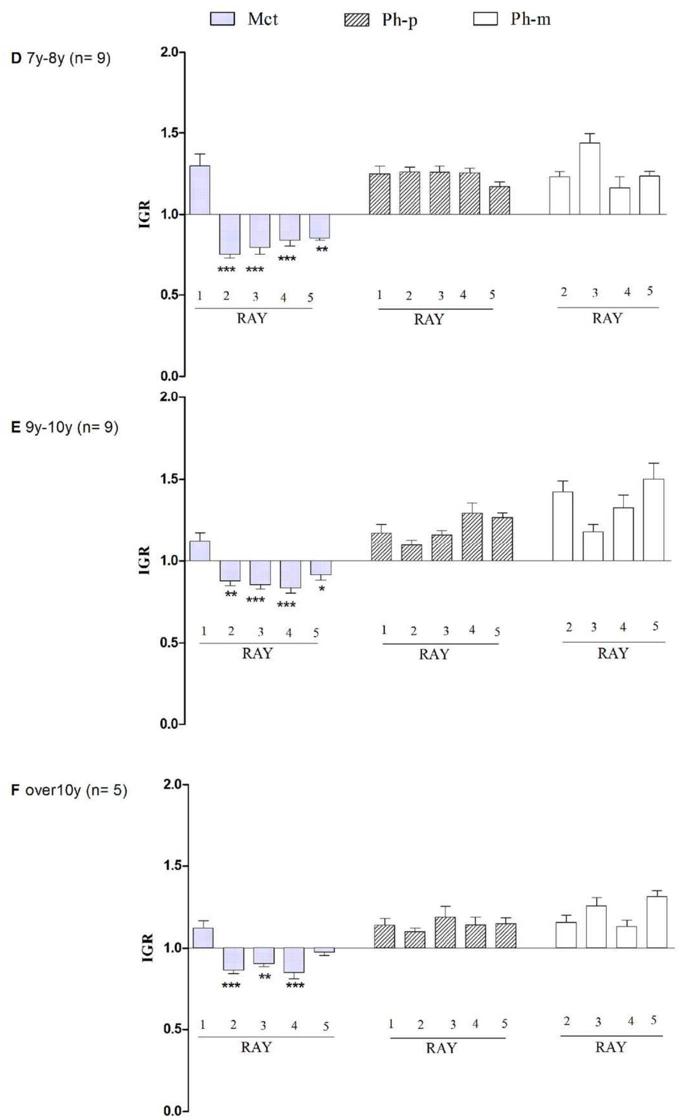

Fig. 7‼ ┼ Proximal-distal growth rate index (IGR) compared among R1-R5 metacarpals (Mtc), proximal phalanges (Ph-p) and mid phalanges (Ph-m) in age groups D – E. This parameter was not assessable in age groups A – C.‼ ┼ With reference to IGR ≅ 1 corresponding to symmetrical, bidirectional growth, the index was inverted at the passage from the 1st and the 2nd metacarpal with an evident relationship with the epiphyseal ossification center position (and later growth plate cartilage). Significant differences in proximal and mid phalanges (not reported in histograms), but without inversion.‼ ┼ (* $p<0.05$; ** $p<0.01$; *** $p<0.001$ versus R1 Mtc)‼ ┼

120x207mm (300 x 300 DPI)

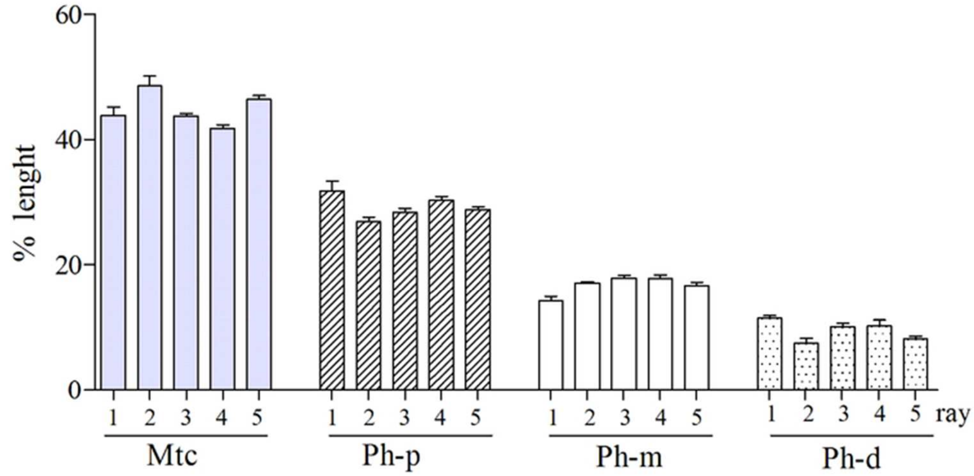

Fig. 8‼ ┼ Triphalangeal thumb metanalysis.‼ ┼ Comparison of the mean % length (measured on its own ray total length) 1st-5th ray metacarpals (Mtc), proximal (Ph-p), mid (Ph-m) and distal (Ph-d) phalanges of triphalangeal thumb series (mean ±SEM of 8 subjects). No significant difference when each segment type is considered in the transverse sequence R1-R5. The % length in all rays decreases from metacarpal to distal phalanges.‼ ┼

65x33mm (300 x 300 DPI)

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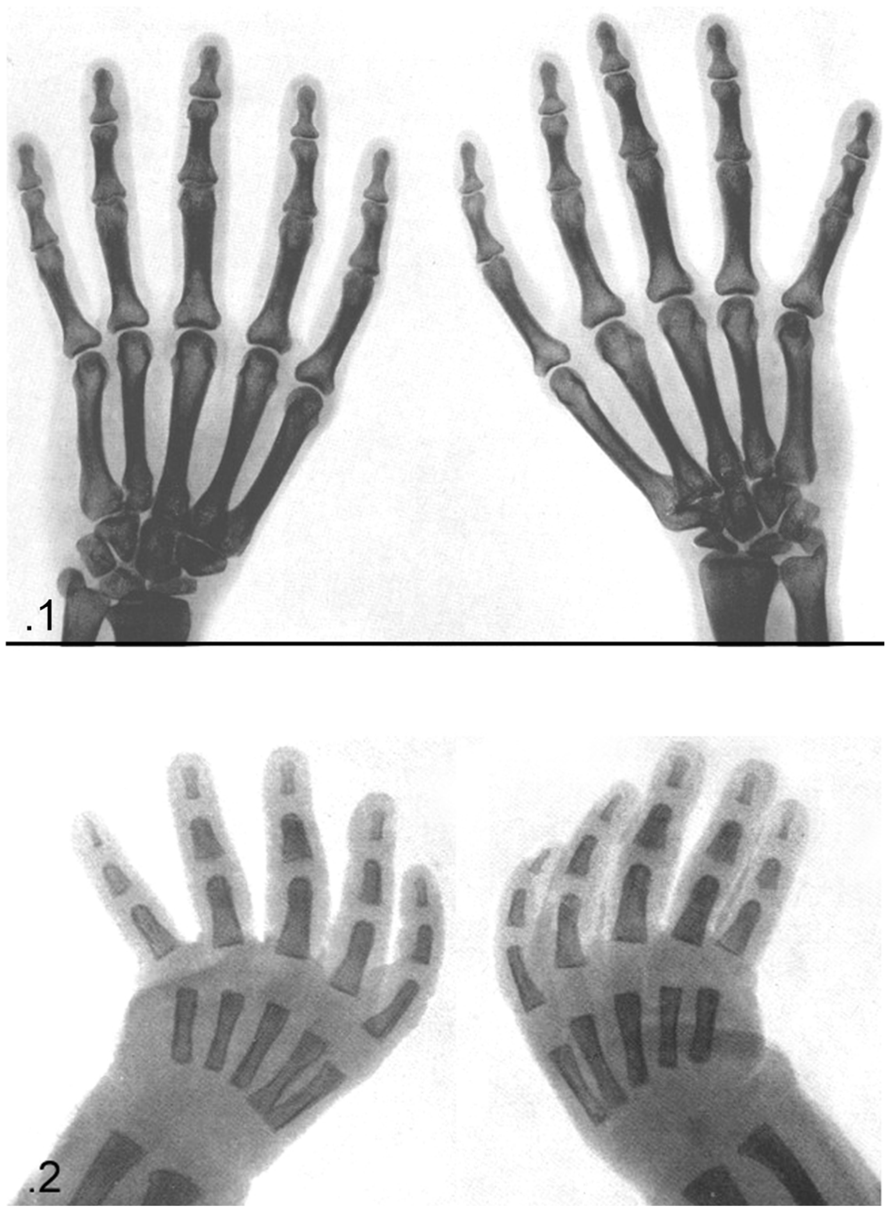
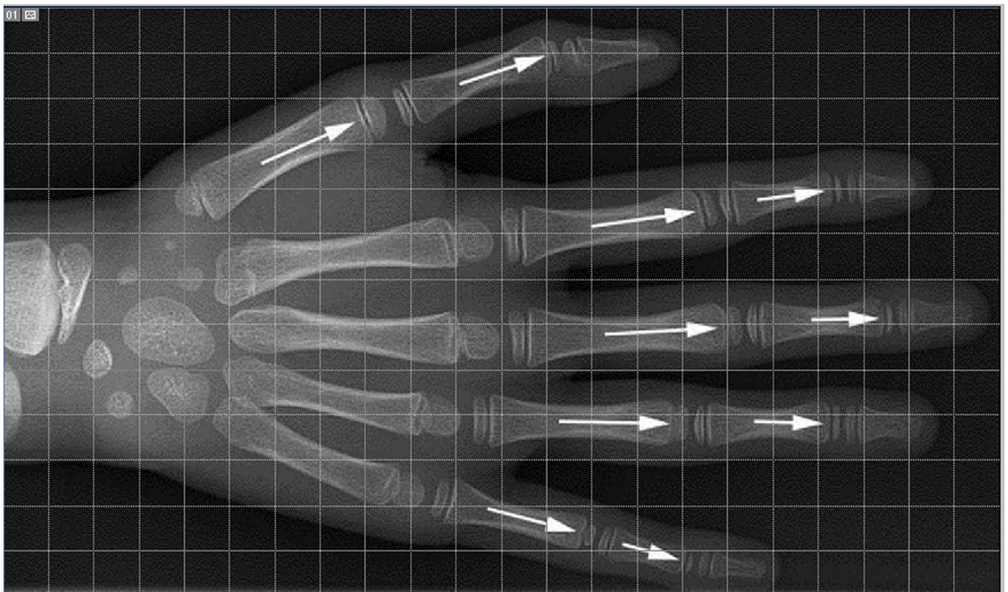


Fig. 9‼ ┼ Image of triphalangeal thumb of the right and left hand of the mother and her newborn reported by Heiss (1957) and reproduced from Zeitschrift fur Anatomie und Entwicklungsgeschicte with permission of Springer Nature (license n. 4334811065195).‼ ┼

395x540mm (72 x 72 DPI)



	1,4	1,3	[redacted]	1,5	R1
	0,77	1,2	1,6	2	R2
	0,75	1,1	1,5	1,8	R3
	0,72	1,3	1,5	1,8	R4
	0,75	1,3	1,6	1,7	R5
Carp	Mtc	Php	Phm	Phd	

Fig. 10‼ ┼ 1 Image of the hand with the widest distribution of proximal and distal, epiphyseal ossification centers, reported in two siblings by deJong et al (2014) and reproduced from The Journal of Hand Surgery with permission of Elsevier (license n. 4280070488758).‼ ┼ 2 The IGR was calculated in each hand segment.‼ ┼

119x96mm (300 x 300 DPI)

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Table 1. AUC values calculated by trapezoidal rule of % lenght of metacarpal (Mct) metacarpal (Mct) proximal (Ph-p) and mid phalanx (Ph-m) in different age groups (A-F). Thumb (R1) metacarpal and proximal phalanx % length was calculated on that of the 3rd ray of the same hand whereas 2nd – 5th (R2-R5) metacarpals and phalanges % length were calculated on its own ray *p<0,5,**p<0,01,***p<0,001 vs Mtc –Ray 1 group; °°p<0,01,°°°p<0,001 vs Ph-p Ray1 group

AUC (A-F age groups)					
Segment	Ray		Segment	Ray	
Mtc	1	140.2 ± 10.8	Ph-p	1	96.14 ± 0.71
Mtc	2	231.0 ± 1.3***	Ph-p	2	138.1 ± 0.9 °°
Mtc	3	206,3 ± 1**	Ph-p	3	147.3 ± 0.7 °°°
Mtc	4	196,3 ± 1.1*	Ph-p	4	149.1 ± 1 °°°
Mtc	5	213,2 ± 4.5**	Ph-p	5	139.3 ± 0.6°°
Ph-p	2	138.1 ± 0.9	Ph -m	2	78.78 ±0.86
Ph-p	3	147.3 ± 0.7	Ph -m	3	90.49 ± 0.78
Ph-p	4	149.1 ± 1	Ph -m	4	93.72 ± 0.63
Ph-p	5	139.6 ± 0.6	Ph -m	5	77.17 ± 0.4

Tab. 1‼ ┼ (Tab AUC) Quantitative assessment of differences among individual profiles was carried out and compared through the trapezoidal rule of AUC (Area Under Curve).‼ ┼ (Mtc R1 vs Ph-p R2-R5 * p<0.05; ** p<0.01; *** p<0.001)‼ ┼ (Ph-p R1 vs Ph-m R2-R5 ° p< 0.05; °° p<0.01; °°° p<0.001)‼ ┼

63x40mm (300 x 300 DPI)

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Triphalangeal thumb case reports with completely developed phalanges

REFERENCE	CASES NUMBER	HAND NUMBER	SUBJECT AGE	PARENTAGE
Hess H. (1957)	1	2	adult	mother
	1	2	newborn	son
Warm A. et al. (1988)	1	1	adult	father
	1	2	child	son
	1	2	infant	son
Zgurica J. Et al. (1997)	1	2	adult	
	1	1	adult	
Zuidam JM et al. (2009)	1	1	adult	
Wieczorek D. et al. (2009)	1	2	adult	
Quazi Q. & Kassner G. (1988)	1	2	adult	
	1	1	child	
Zuidam JM et al. (2010)	1	1	adult	
Zguricas J. Et al (1994)	1	2	adult	
Lamb DW et al. (1983)	1	2	infant	
Reynolds LR (2017)	1	2	unknown	

Tab. 2‼ ┼ Case reports used for metanalysis of triphalangeal thumbs.‼ ┼

68x47mm (300 x 300 DPI)

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Case reports of prox/distal ossification centers in the some hand segment

REFERENCE	CASES NUMBER	HAND NUMBERS	SUBJECT AGE	HAND SEGMENT
Milch H (1951) J Bone Joint Surg A.	2	2	3 5	1st Mtc 1st Mtc
Caffey J. (1973) Pediatric X-Ray diagnosis (book)	1	1	5	1st Mtc (dx/sx)
Nakashima T.& Furunowa H. (1997) Ann Anatomy	1	1	7	1st to 5th Mtc (dx/sx) 1st to 5th Mtc (dx/sx)
Limb D. & Loughenbury (2011) J Hand Surgery Eur	1 1	1 1	12 8	2nd Mtc 1st Mtc
De Jong TR et al (2014)J Hand Surgery Am	1 1	1 1	6 5	1st Mtc 1st to 5th Php 2nd to 5th Phm 2nd to 5th Php 1st Mtc 1st to 5th Php 1st to 5th Phm 1st to 5th Php

Tab. 3‼ ┼ Case reports used for metanalysis of double epiphyseal ossification centers and distribution in hand long bones segments.‼ ┼

64x42mm (300 x 300 DPI)

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