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Abstract

This study examines the accuracy of the Pyle and Hoerr radiographic atlas technique in an effort to document the extent of normal variation associated with developmental timings in the knee for purposes of age estimation. The atlas has been previously tested; however, accuracy rates were produced from a dataset which spread in age from mostly 7 to 16 years. This study took a closer look at the younger age groups, examining radiographs from 297 children (147 females, 150 males) from birth to six years. Standard deviations representing the difference between skeletal age and chronological age were calculated according to two groupings. Each group represents episodes, or time periods, of differential developmental rates as expressed through the number of plates within the atlas dedicated to documenting each year of life. The beginning year of life is characterized by the most rapid of development as represented by the numerous image plates used to depict this time period. Individuals assigned to plates with a skeletal age between birth and one year were grouped collectively to document the variation associated with such rapidly changing morphology (SD=2.5 months in females; 2.3 months in males). Years one through to 3.8 years (females) and one through to 4.5 years (males) were represented by two or three images within the atlas and therefore individuals assigned to plates with a skeletal age falling within this range were placed within a second grouping (SD= 5.2 months in females; 7.0 months in males).   As expected, variation was observed to decrease as developmental processes accelerated in the younger children. The newly calculated standard deviations offer tighter predictions for estimating age in young children while at the same time maintaining an acceptable width that accounts for normal variation in developmental timings.

Key words: knee radiographs, age estimation, development, juvenile osteology

"In the study of any aspect of the growth and development of children, one is constantly bedeviled by their variability [1]." This statement holds true in every aspect of physical and cognitive growth and development including that of skeletal maturation [2-5]. While variability exists within the exact timing of developmental milestones associated with the skeleton, there is also a high degree of consistency in the overall time period in which these milestones occur [6-8]. Thus, it is possible to estimate the age of a child whose identity or date of birth is unknown based on the degree of maturity displayed by skeletal elements. Forensic practitioners requested to perform this task must balance modal developmental rates with normal variation to produce an age estimate that is wide enough to encompass variability in timing, yet narrow enough for the estimate to be meaningful [9,10].

Growth and development have been demonstrated to be influenced by numerous factors. Two of the more commonly cited factors include that of ancestry and nutritional status [4,5]. While both of these influences are typically discussed within the literature, ancestry has been shown to be less influential than nutritional status [11-15]. Poor nutrition leads to delayed development and shorter stature. It is important to keep in mind, however, that variation continues to exist even in a homogenous population

where nutritional levels and ancestry are equal [1,8].   Thus, intrinsic variation must be accounted for in any age estimate offered by a forensic practitioner even if population specific standards are utilized.

Age estimations based on the developing skeleton can potentially be derived from four events: bone measurements, the appearance of ossification centers, the fusion of the epiphyses, and the morphological changes that epiphyses undergo as they transform from a small non-descript nodule of bone into their adult shape [7].   Utilization of appearance and union times to form an age estimate tends to be limited to the early years or adolescent/post-adolescent time period, as this marks the general time frame in which these milestones occur [16,17]. Bone measurement also tends to be restricted in its utility in that overall height of an individual becomes increasingly variable as age progresses [18,19]. Height is also more strongly influenced by environmental stresses than is development, thus bone length has the potential to display more disparate results between underprivileged and well off individuals [12,20,21]. Morphological changes to the epiphyses occur throughout the whole extent of juvenility (from neonatal life through to maturity), and thus demonstrate greater potential for age estimation. Radiographic atlases that were published in the early to mid 1900's document morphological changes of epiphyses associated with the joint regions of the hand and wrist, elbow, knee and foot and ankle, and are a useful tool to facilitate age estimations [1,8,22-29]. When utilizing the atlas technique, practitioners are instructed to compare the radiograph being examined to a series of plates offered within the book that demonstrate progressive development of the joint region. The user then chooses the plate that most similarly reflects the appearance of the questioned radiograph.   A male age and female age is assigned to each plate in the atlas, and the age offered by the plate reflects the skeletal age of the child under question [1,8,24,25].

It must be stated that the original intent of these books was not to offer a tool by which one can estimate chronological age, but rather to document modal times in which developmental milestones occur, so that children whose skeletal maturation appeared to be outside these norms could be diagnosed as requiring clinical intervention [30,31]. The key understanding is that these atlases document modal development, not the extent of possible variation in the timing of different developmental events. Providing a single skeletal age as an estimate for chronological age is far too exclusive to successfully be used in forensic practice, and thus, an age range that better accounts for human variability is recommended [1,8-10].

Greulich and Pyle [1] address the issue of differential timing in their atlas of hand and wrist development by offering standard deviations demonstrating the extent of variation that was observed between skeletal age and chronological age [1,8]. Their report however was based on testing the Todd Atlas on radiographs from the Brush Foundation study rather than testing their own method. They state that most normal variation can be accounted for within two standard deviations of the mean skeletal age, and any difference of more than two standard deviations above or below the mean would make it highly probable that the child's skeleton is abnormally advanced or retarded [1].   While their intent still focused on identifying development that was outside the norm for clinical intervention, the intervals that their reported standard deviations provide could serve as a reasonable assessment of an individual's likely age. Unfortunately, the atlas of the hand and wrist is the only atlas that offers standard deviations so that variation associated with other joint regions was not well documented.

Hackman and Black have recently rectified this issue by documenting variation observed in the hand and wrist, knee and foot and ankle when utilizing radiographic atlas techniques [32-34].   They report standard deviations ranging from 9.86 months (female knees) to 14.97 months (female hand and wrist) [33]. If age estimations are to include plus and minus two standard deviations, the result is a predicted range that is nearly or over four years. While there is no doubt that the window of developmental variation is wide-ranging during the teenage years, can a tighter estimate be offered for infants and young children? The original standard deviations were calculated from a sample extremely limited in individuals younger than seven years, so does not reflect the more cohesive development that occurs during this time period [33].

The purpose of this study is to collect a large sample of radiographic images of the knee from children aged birth to six years to improve upon the original standard deviations offered by Hackman and Black [33]. This study aims to offer multiple standard deviations that are sex and general time period specific to allow increasingly narrow estimates as maturity levels decrease.

Materials and Methods

Radiographs utilized for analysis were obtained via online access to Query Patricia 0.36, a juvenile radiographic database created by Mercyhurst University. The database itself consists of radiographs collected from medical examiner's (ME) offices around the country as well as from clinical sources. Data regarding the ancestry of each individual within the database was recorded; however, that information was not publically available at the time of this writing. Ancestral representation of the database predominately includes, but is not limited to, White Americans, Black Americans and Hispanics [35]

For this study, radiographic images of the knee were selected from 297 individuals within the database, including 147 females and 150 males.   Ages within the sample ranged from birth to six years, and were divided into 11 age categories: 0-3 months, 3-6 months, 6-9 months, 9-12 months, 12-18 months, 18-24 months, 2 years, 3 years, 4 years, 5 years, and 6 years. Table 1 displays the frequency distribution according to sex and age.   Radiographs of children older than six years were not collected as it was believed that the sample of Hackman and Black adequately documented those from seven years onward.

Each individual was assigned a plate number according to the radiographic atlas developed by Pyle and Hoerr [25] based on the bony development displayed in the image. Only sex of the individual was known to the observer during the evaluation. The original Hoerr and Pyle technique was designed to be utilized with AP and lateral views of the knee. The vast majority of radiographs in this dataset were obtained from medical examiner's offices that did not require classic positioning of the child. Rather, the lower limb portion of the body was frequently imaged with the child lying on his or her back with their knees splayed out to varying degrees. Information was recorded on whichever knee displayed the least outward rotation (Figure 1).

A random subset of 60 individuals was selected from the sample to test the repeatability of plate assignment. The author responsible for the original plate assignment revisited the subset three months following her original observations to test for intra-observer error. A second assessor, new to the Pyle and Hoerr technique [25] was also selected to test for inter-observer error. Only sex of the individual was made available at the time of assessment. An intra class correlation was employed to analyze observed differences. All statistical tests within this study were performed utilizing SPSS version 21 software.

Chronological age was compared to skeletal age to gain an appreciation of how well the Hoerr and Pyle technique could be used to predict age utilizing images that displayed varying degrees of knee rotation. A Pearson Correlation calculated the closeness by which these variables were related. Once the strength of the association was established, variation in developmental timing accompanying each plate was documented. This was achieved by calculating the difference between chronological age and skeletal age, and providing descriptive statistics associated with each plate based on that difference.

In an effort to document variation present within specific periods of accelerated development rather than individual plates, data was combined into three groups based upon plate assignment. Each group represents episodes, or time periods, of differential developmental rates as expressed through the number of plates within the atlas dedicated to documenting each year of life (Tables 2 and 3). The majority of the atlas provides one plate per year, suggesting that little developmental change occurs from month to month. Younger ages however are represented by multiple plates, indicating that morphological change during this time period occurs at an accelerated pace. Development is most rapid during infancy (0-12 months), as demonstrated by the five (female) or six (male) plates utilized to represent this life stage. Development begins to slow within the next few years, as represented by the two to three plates assigned to ages one to three years (females) or one to four years (males). Following age four (females) and age five (males), a single plate is offered to represent each subsequent year.

Standard deviations were recalculated according to two categories, including individuals assigned to plates 2-7 (females) or plates 2-6 (males) and those assigned to plates 8-15 (females) or 7-14 (males). This grouping made it possible to collectively document the variation associated with two differential rates of development. Individuals who were assigned to plates 16 and higher (females) or plates 15 and higher (males), which included those in the third group, were not included in the analysis as it was believed that the standard deviations calculated by Hackman and Black adequately describe this developmental time period.

Once the parameters of variation were established according to each of the two plate groupings and sex, the efficacy of the predicted age ranges was tested on a second test sample. Supplemental radiographs comprising the test sample were obtained from the same Query Patricia database from which the original sample was obtained. It is important to note that the test sample was comprised of images from children who were not included in the original sample.   Table 4 provides a frequency distribution according to age category. Radiographs from the test population were assigned a plate and skeletal age according to the same principles as applied to the original sample. It was then noted whether the individual's true chronological age fell within the limits of the estimated age range.

<u>Results</u>

The results of the intra class correlation (ICC) indicated that there was excellent agreement between plate assignments made by the same observer (ICC=.99) as well as between that of two different observers (ICC=.98).   Out of a total of 60 cases, the original observer consistently plated 40 radiographs. Of the 20 cases that were not plated identically, assignments differed by only one plate.   The author's first observations were then compared to the second observer. Consistent scores were produced in 30 of the 60 cases. Of the 30 inconsistent classifications, all but one differed by only one plate number. The remaining case differed by two plates.

A Pearson correlation was utilized to assess the relationship between assigned skeletal age and actual chronological age (Figures 2 and 3). There was a strong correlation for both females (r= .979, p <.0005) and males (r= .969, p <.0005), demonstrating that skeletal age statistically explains 96% (females) and 94% (males) of the variation observed in chronological age. The results of this analysis suggest that the Hoerr and Pyle method can be appropriately adapted to apply to radiographs that do not display classic AP and lateral views of the knee.

Variability in developmental timing that could not be accounted for by plate assignment was calculated by subtracting skeletal age from chronological age. Descriptive statistics describing this difference are provided in Tables 5 and 6. The direction of the mean difference (i.e. either positive or negative) provides an indication as to whether individuals had the tendency to be over aged (negative mean) or under-aged (positive mean) according to each plate. The majority of plate assignments resulted in the under aging of individuals. The greatest mean difference was 5.3 months for females and 6.7 months for males. Interestingly, the highest means for both sexes were associated with plate 13 (skeletal age 38 months females; 48 months male). The maximum difference observed in the female sample was 15 months and occurred at plate 16 (skeletal age 56 months), while the male sample displayed its greatest difference of 20 months corresponding to plate 13 (skeletal age 48 months).

Standard deviations were recalculated to include all individuals who fell within specific plate groupings as defined by time periods marked by differential rates of development. Table 7 provides a summary of the standard deviations to be applied to Pyle and Hoerr's skeletal age according to sex and plate number. Males displayed both the least and greatest amount of variation. Development was most cohesive amongst males assigned to plate 2-6 (SD= 2.3); while males assigned to plates 7-14 demonstrated the greatest variation (SD=7.0).

Tables 8 and 9 provide the predicted age ranges for each plate and were calculated by adding and subtracting two standard deviations from the skeletal age assigned to each plate. The true chronological ages of individuals from the test sample are listed alongside the plate to which they were assigned. The predicted age ranges accommodate the correct chronological age in the complete female sample and in all but two individuals from the male sample. Thus, 48 out of 50 individuals or 96% of the sample were aged correctly.

Discussion

The key to successful forensic age estimation is to produce age intervals that are large enough to capture the majority of human variation, yet narrow enough for the estimates to be meaningful [9,10]. The extent of variability that occurs throughout different developmental periods is far from static. Thus, a single standard deviation is unlikely to adequately describe the expected variation seen from infancy through post-adolescence. Just as narrower intervals are offered when aging subadults as compared to adults, this study suggests that even tighter estimates can be offered for young children.

The intent of this study was to add to previous work by Hackman and Black [33] by documenting the extent of variation present in developmental timings of the knee in young children. With the addition of this data, standard deviations used to calculate an estimated age range associated with each plate were reduced from a generalized 9.86 months for females to 2.5 or 5.2 months depending on the developmental time period of the child, and from a generalized 10.75 months for males to 2.3 or 7.0 months. This reduction in standard deviation produced an overall age estimate that is 29 months (beginning year), or 19 months (years one to three) narrower in young female children and 34 months (beginning year) or 15 months (years one to four) narrower in young male children.

The sample utilized within this study presented an additional challenge in relation to plate assignment due to the rotated position of the lower limb in many individuals. The technique itself was developed using AP and lateral views of the knee, thus, it is suggested that both of these views are obtained for comparison. Standard views may not always be available, however, especially when applying this method to deceased children. Despite the challenges associated with the rotated views, this sample likely provides a more realistic example of the types of images one would receive if ageing a deceased child. Reassuringly, the relatively low differences between skeletal age and chronological age, suggest that the atlas is still applicable when classic views are not obtainable.

Despite the fact that skeletal age was found to be a good predictor of chronological age, the atlas technique had the tendency to underestimate age, as demonstrated by the vast majority of positive mean differences. This is surprising as the atlas itself was developed utilizing radiographs obtained from children as early as 1927. Many studies have argued that secular change has resulted in changes to the development timings of modern children [3,36-40]. The current study does not support evidence that modern children are developmentally precocious to that of historic children, at least not in relation to morphological development of the bony knee. The strong Pearson correlation between skeletal age and chronological age suggests that developmental rates remain similar. Practitioners however, should be aware of the demonstrated tendency to underestimate age using this method with this population.

The results of this study can be compared to additional research that investigates developmental changes of the knee for purposes of age estimation. MRI and radiographic studies considering developmental changes associated with the knee joint have been undertaken in recent years (Krämer et al. 2014a and b; Dedouit 2011; O'Connor et al 2008, 2012). Unfortunately, the youngest age that these studies report is nine years, thus a direct comparison is not possible. One can conclude however, that

less variation is present in our younger sample as demonstrated by their standard deviations that are generally somewhere within the 1 year range (12 to 24 months).

Comparison of our data with studies that investigate the accuracy of techniques utilized on children that are of a more similar age to those present within our study, i.e. long bone measurements, is not possible due to intrinsic incompatibility in research design. Both Maresh (1970) and Gindhart (1973) have produced impressive studies regarding the radiographic length of long bones in hundreds of children. Unfortunately, their standard deviations are reported in measurements of length (centimeters or millimeters) rather than time (months), and thus comparison between the two would not provide meaningful results.

Conclusion

One of the more challenging aspects of forensic age estimation is the balance between producing an estimate that is wide enough to cover the full extent of normal human variation, yet narrow enough for the estimate to be of value. While narrow estimates are optimum in theory, an interval that is too small leads to false exclusion of possible ages, which may potentially compromise identification efforts. This study examined the accuracy of the Pyle and Hoerr radiographic atlas technique in an effort to document the extent of normal variation associated with developmental timings in the knee from birth to 6 years of age [19]. Radiographs of 297 children (147 female, 150 male) were examined. Standard deviations representing the difference between skeletal age and chronological age were calculated according to two time periods characterized by distinct rates of developmental activity. This included the beginning year of life (SD= 2.5 months in females; 2.3 months in males) and years one to 3.8 in females (SD= 5.2 months) and years one to 4.5 in males (SD= 7.0 months). Plus and minus two standard deviations can be applied to skeletal age to transform the single age into an estimated age interval. The newly calculated standard deviations offer tighter predictions over those previously published, while at the same time maintaining an acceptable width that accounts for normal variation in developmental timings associated with two distinct time periods.

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Figure 1: Radiographic image of a male child displaying varying degrees of outward rotation of the knees as noted by the fibula partly overlaying the tibia. The right knee displays less exaggerated rotation and thus was utilized for assessment.   The image was assigned to plate 9, with a skeletal age of 24 months. The chronological age of this child was 30 months.

Figure 2: Chronological age plotted against skeletal age in the female sample.
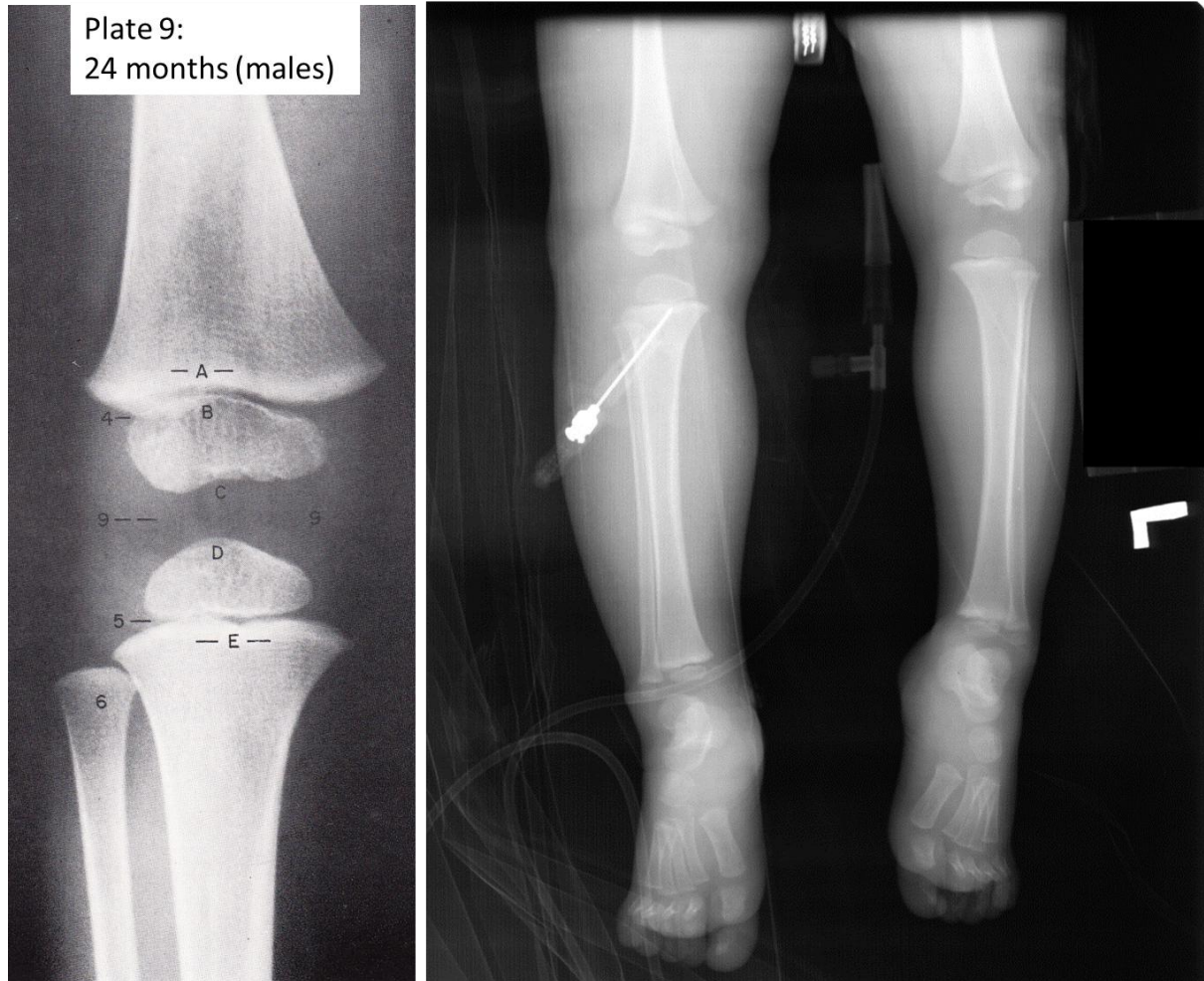
Figure 3: Chronological age plotted against skeletal age in the male sample.
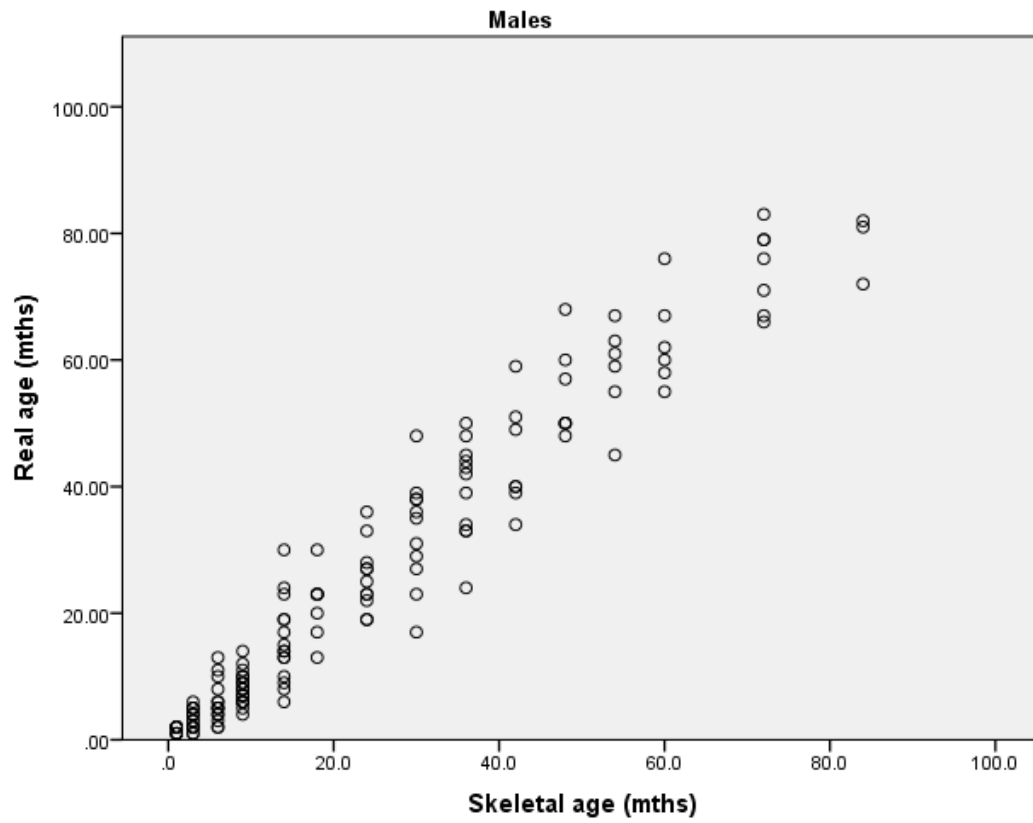
Table

Table 1: Frequency distribution of the sample according to age category and sex.

Age	Female N	Male N	Total
0-3m	14	17	31
3-6m	19	16	35
6-9m	14	16	30
9-12m	13	11	24
12-18m	14	13	27
18-24m	13	12	25
2y	18	17	35
3y	15	14	29
4y	10	16	26
5y	11	10	21
6y	6	8	14
Total	147	150	297

Table 2: The distribution of female skeletal ages assigned to each plate. Groups were created based on the number of plate images used to depict each year of life. The beginning year (year 0) utilizes the greatest number of plates and thus were collectively categorized into group one. Years one, two and three each utilize two to three images to describe developmental processes and thus were collectively organized into group two. Group three includes years in which a single plate was used to describe developmental changes.

Females

Plate	Skeletal age	Skeletal age (Yr)	Group
2	0-2 wks	0	1
3	0.5 mths	0	1
4	2.2 mths	0	1
5	5 mths	0	1
6	7.5 mths	0	1
7	11 mths	0	1
8	15 mths	1	2
9	19 mths	1	2
10	24 mths	2	2
11	28 mths	2	2
12	32 mths	2	2
13	38 mths	3	2
14	42 mths	3	2
15	46 mths	3	2
16	56 mths	4	3
17	64 mths	5	3
18	75 mths	6	3
19	84 mths	7	3

Table 3: The distribution of male skeletal ages assigned to each plate. Groups were created based on the number of plate images used to depict each year of life. The beginning year (year 0) utilizes the greatest number of plates and thus were collectively categorized into group one. Years one, two, three and four each utilize two images to describe developmental processes and thus were collectively organized into group two. Group three includes years in which a single plate was used to describe developmental changes.

Males

Plate	Skeletal age	Skeletal age (Yr)	Group
2	0-2 wks	0	1
3	1 mth	0	1
4	3 mths	0	1
5	6 mths	0	1
6	9 mths	0	1
7	14 mths	1	2
8	18 mths	1	2
9	24 mths	2	2
10	30 mths	2	2
11	36 mths	3	2
12	42 mths	3	2
13	48 mths	4	2
14	54 mths	4	2
15	60 mths	5	3
16	72 mths	6	3
17	84 mths	7	3

Table 4: Frequency distribution of the test sample according to age category and sex.

Age	Female N	Male N
0-3m	2	2
3-6m	2	2
6-9m	2	2
9-12m	2	2
12-18m	2	2
18-24m	3	3
2y	4	4
3y	3	3
4y	2	2
5y	3	3
Total	25	25

Table 5: Descriptive statistics describing the difference between skeletal age and chronological age according to each plate for females (reported in months).

Females

Plate	N	Min	Max	Mean	SD
2	2	0	0	0.0	0.0
3	9	1	5	1.8	1.4
4	13	-1	3	0.4	1.2
5	11	-3	6	0.4	3.3
6	17	-3	9	0.9	3.0
7	8	-4	2	-1.6	1.9
8	9	-5	6	-0.1	3.7
9	11	-8	8	-0.4	4.5
10	9	-6	4	-1.1	3.4
11	5	-8	5	-1.4	4.8
12	12	-4	14	2.2	5.3
13	7	-4	14	5.3	7.0
14	7	-5	9	1.6	6.1
15	4	-8	1	-3.3	4.4
16	10	-8	15	1.3	8.4
17	9	-2	10	3.3	4.5
18	3	2	5	4.0	1.7
19	1	-9	NA	NA	NA

NA= no calculations were made

Table 6: Descriptive statistics describing the difference between skeletal age and chronological age according to each plate for males (reported in months).

Males

Plate	N	Min	Max	Mean	SD
2	0	NA	NA	NA	NA
3	9	0	1	0.6	0.5
4	15	-2	3	0.3	1.6
5	14	-4	7	0	3.4
6	21	-5	5	-0.8	2.4
7	15	-8	16	1.6	6.6
8	7	-5	12	3.3	5.4
9	11	-5	12	1.6	5.4
10	11	-13	18	2.8	8.6
11	11	-12	14	3.5	7.8
12	7	-8	17	2.6	8.7
13	7	0	20	6.7	7.3
14	6	-9	13	4.3	7.7
15	6	-5	16	3	7.5
16	7	-6	11	2.4	6.5
17	3	-12	-2	-5.7	5.5

Table 7: Recalculated standard deviations to include plate groupings (reported in months).

Group	Plates	skeletal age	N	SD
Females				
1	2-7	0 yrs	60	2.5
2	8-15	1-3 yrs	64	5.2
Males				
1	2-6	0 yrs	59	2.3
2	7-14	1-4 yrs	75	7.0

Table 8: Chronological ages (CA) of female individuals within the test sample assigned to each plate. Predicted age range was calculated by adding and subtracting 2 SD from each skeletal age (all ages are reported in months).

Females

Plate	Skeletal age	SD	Predicted age range	CA of test sample
2	0-2 wks	2.5	0-5 mths	
3	0.5 mths	2.5	0-5.5 mths	0.5
4	2.2 mths	2.5	0-7.2 mths	4, 2, 4
5	5 mths	2.5	0-10 mths	7
6	7.5 mths	2.5	2.5-12.5 mths	7, 9
7	11 mths	2.5	6-16 mths	11, 11, 11
8	15 mths	5.2	4.6-25.4 mths	11, 14, 16
9	19 mths	5.2	8.6-29.4 mths	18, 20, 23, 25,
10	24 mths	5.2	13.6-34.4 mths	21, 25, 26, 30
11	28 mths	5.2	17.6-38.4 mths	21
12	32 mths	5.2	21.6-42.4 mths	23
13	38 mths	5.2	27.6-48.4 mths	30
14	42 mths	5.2	31.6-52.4 mths	43
15	46 mths	5.2	35.6-56.4 mths	

N= 25
Misclassified=0

Table 9: Chronological ages of male individuals within the Scottish and American test samples assigned to each plate. Predicted age range was calculated by adding and subtracting 2 SD from each skeletal age (all ages are reported in months).

Males Plate	Skeletal age	SD	Predicted age range	CA of test sample
2	0-2 wks	2.3	0-4.6 mths	
3	1 mth	2.3	0-5.6 mths	
4	3 mths	2.3	0-7.6 mths	1, 2
5	6 mths	2.3	1.4-10.6 mths	3, 5, 7, 10
6	9 mths	2.3	4.4-13.6 mths	8, 9, 15*
7	14 mths	7.0	0-28 mths	17
8	18 mths	7.0	4-32 mths	11, 23
9	24 mths	7.0	10-38 mths	19, 21, 26
10	30 mths	7.0	16-44 mths	30, 30, 31
11	36 mths	7.0	22-50 mths	
12	42 mths	7.0	28-56 mths	41, 42, 44, 53
13	48 mths	7.0	34-62 mths	49
14	54 mths	7.0	40-68 mths	61, 69*

N= 25
Misclassified= 2

* Individuals whose chronological age did not fall within the predicted age range