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ORIGINAL ARTICLE

The incidence of asymmetrical left/right skeletal and dental development in an Australian population and the effect of this on forensic age estimations

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Abstract The prevalence of developmental asymmetry between left and right sides of the body in the third molar tooth and medial clavicular epiphysis is examined in a contemporary Australian population (92% Caucasian). The contention that differences between left and right side developmental timing is statistically insignificant, and can therefore be ignored in forensic age estimation procedures, is questioned. It was found that of a population sample of 604 individuals, 177 displayed asymmetrical timing in development between antimeres of the third molar, the medial clavicle or both. There was no correlation found between the third molar tooth and medial clavicular epiphysis in terms of left/right synchronicity. For those individuals differing in development by two or more developmental stages in either age marker or one stage in both age markers, the effect upon the accuracy of forensic age estimations can be significant. Differences in age estimates for each side were as much as 3.1 years. Age estimations based on one side only may not provide the best estimate for an individual, and more accurate results can be achieved if both sides are taken into consideration. A protocol for dealing with asymmetrical development is discussed with reference to the multifactorial age estimation method proposed by the same authors in previous research.

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Introduction

The development of the body's hard tissues, the skeleton and the dentition, and the changes associated with increasing chronological age, is a tool utilised in forensic practice for age determination of unknown individuals, both living and deceased. Much productive research has been conducted in this field. Accepted standards have been developed [1–6], and these standards are continually being refined and improved to make them applicable to a wider range of populations and situations [7–15].

In recent years, increased attention has been devoted to the development of the medial clavicular epiphysis and the third molar tooth, specifically to improve forensic age estimations in the hitherto problematic age cohort of the late teenage years and early adulthood (15–25 years) [9, 11, 13, 16]. This age group is difficult to assess as developing skeletal and dental sites beyond the age of 14–15 years are few. Research into skeletal and dental development of this age cohort has taken on increasing importance due to the requirement by judicial authorities to assess, in living individuals, the likelihood of having reached legal adulthood. This is especially relevant in cases of asylum seekers crossing international borders without supporting identification documentation [11, 16–19] and also in disaster victim identification where accurate age estimation can significantly aid the identification process [20].

Constructing an age estimate for an unknown individual or set of human remains involves assessing the degree of development of the age marker of interest, comparing this with published standards, preferably those having been derived from the same or similar population as the target individual, and determining an approximate age based upon these standards. Sources of error in age estimation can be many and include utilising population standards which are not applicable to the target individual, the presence of unrecognised developmental anomalies which confound the age estimate, and finally, individual genetic variation.

A constant theme running through the literature is the oft stated conclusion that left/right developmental asymmetry is not statistically significant within a population sample and can therefore be ignored [21–31]. This leads to the conclusion that it is only necessary to evaluate one side of the body to assign an age estimate to a particular individual [3, 32–39]. Several authors have attempted to address the issue of right/left asymmetry by stating that both left and right third molars and/or clavicles need to be assessed, and either all data included into an age estimate, or either the least or most developed side used, depending upon the judicially conservative point of view relevant for that particular case [11, 13, 17, 40].

Asymmetrical development between contralateral dental and skeletal elements, also known as fluctuating asymmetry, has been recognised for many years as a normal feature of human growth, and population-based development studies have reported upon its general characteristics [41–45]. This asymmetry may result from factors such as random local genetic variation or external environmental disturbances [46]. Fluctuating asymmetry does not favour one side over the other, but can be described in terms of a normal distribution fluctuating about a mean of zero. The normally distributed nature of left/right skeletal asymmetry means that when statistical inferences are made about the development of teeth or bones on a population basis, then asymmetry becomes statistically insignificant, despite the fact that there may well be many individuals within that population who have markedly differing development timing between left and right skeletal or dental antimeres.

To date, no studies have assessed the effect of left/right developmental asymmetry upon forensic age estimations. This paper attempts to address this issue, extending previous work by the same authors on age estimation using the medial clavicular epiphysis and the third molar tooth [47–49]. The same population data used in the previous studies, based upon a post-mortem sample of the modern Australian population, were used in the current study.

Materials and methods

The individuals comprising this study were a sample of the modern Australian population, which is predominantly Caucasian (92%). The sample consisted of post-mortem high-resolution multi-detector computed tomography scans of 604 individuals in the age range of 15–25 years, gathered during medicolegal death investigation procedures carried out at the Victorian Institute of Forensic Medicine, Melbourne, Australia. The scans were conducted between January 2006 and September 2009.

The imaging system utilised was a Toshiba Aquilion 16® multi-detector scanner, which captures 1-mm thick slices of the head and neck and 2-mm slices of the remainder of the body, which are then viewed using the TeraRecon Inc Aquarius-Net® software package. This imaging protocol results in clavicular epiphyses being captured with either a 2- or 1-mm slice scan, depending upon the position of the body during the scanning process. Third molar teeth were imaged using the 1-mm protocol. Clavicles were examined using the bone algorithm, both axial and coronal slices were evaluated, and the slice displaying the greatest degree of development was scored. Third molar teeth were viewed using the Maximum Intensity Projection 3D reconstruction algorithm in the bone window, which provides what is in effect a 3D

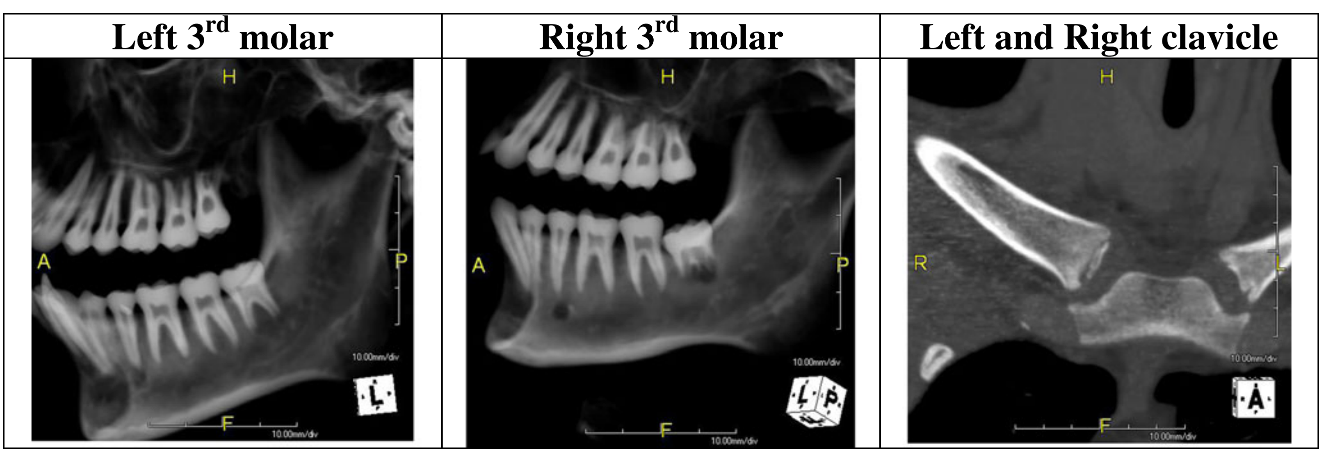

Fig. 1 Examples of L/R developmental asymmetry in the same individual

Table 1 Numbers of cases exhibiting asymmetric left/right development

Sex	Total sample	Asymmetric molar development	Asymmetric clavicle development	Total asymmetry cases
Male	420	48 (11.4%)	84 (20%)	130 (31%)
Female	184	27 (14.6%)	24 (13%)	47 (25.5%)
Total	604	75 (12.4%)	108 (17.9%)	177 (29.3%)

The final column displaying the total number of individuals with asymmetrical development is not a sum of the molar and clavicle columns, as six individuals display asymmetry in both third molar and medial clavicle

radiograph which can be rotated in space to clearly expose the long axis of the developing tooth root.

Only those individuals who possessed both left and right antimeres for third molar teeth and medial clavicular epiphyses were included in the study. Images were examined and numerically scored based on the root lengths of the third molar teeth and the appearance and fusion status of the medial clavicular epiphysis. Third molars were scored according to the protocol developed by Demirjian [1], with the letters A–H being converted to the numbers 1–8 for ease of statistical analysis, and clavicles were scored according to Schulz et al. [29].

The sample was assessed to determine the number of cases displaying discrepant development between left and right sides, and the identified asymmetric cases were extracted and form the basis of this work. Development scores were converted into age ranges separately for left and right sides using the multiple regression formulae developed by the same authors [49]. The formulae used for males are:

$$\begin{aligned}\text{Lower age limit} &= 9.91 + (0.74 \times \text{molar score}) \\ &\quad + (1.19 \times \text{clavicle score}) \\ \text{Upper age limit} &= 11.55 + (0.99 \times \text{molar score}) \\ &\quad + (1.41 \times \text{clavicle score})\end{aligned}$$

and for females:

$$\begin{aligned}\text{Lower age limit} &= 10.90 + (0.35 \times \text{molar score}) \\ &\quad + (1.51 \times \text{clavicle score}) \\ \text{Upper age limit} &= 12.62 + (0.67 \times \text{molar score}) \\ &\quad + (1.85 \times \text{clavicle score}).\end{aligned}$$

The mean of each age range was then calculated and used as the basis for comparing differences between left and right sides in each individual. Males and females were treated as separate groups.

Statistical analysis was performed to quantify the difference between each side and to assess how different third molar and medial clavicular development needed to be in order to have a significant effect upon calculated age estimates for individual cases. Statistical analysis was conducted using SPSS 17® for Windows, IBM, Chicago, IL.

Results

The sample of deceased individuals in the age range of 15–25 years consisted of 420 males and 184 females. An example of CT images depicting asymmetric development in both third molar and medial clavicle is depicted in Fig. 1.

The developmental asymmetry of the sample was assessed by independently scoring the left and right sides for both clavicle and the third molar, and then determining the degree of asymmetric development in either or both age markers for each individual. One hundred seventy-seven individuals (130 males and 47 females) displayed asymmetrical development in either or both third molar and clavicle (Table 1). The sample was further divided into those individuals differing in development by one stage only for either third molar or medial clavicle, those differing by more than one stage in either age markers, or those differing in development score in both age markers (Table 2). The mean difference in age estimate between left and right sides for the 177 asymmetric cases, males and females combined, was 1.23 years.

The difference between left and right sides in all asymmetric cases was assessed using a paired samples t test, males and females being assessed separately. The results of this test showed that for males, the developmental difference between left and right sides was statistically

Table 2 Breakdown of asymmetric cases by site and degree

Sex/no	1-stage difference third molar	1-stage difference clavicle	2-stage difference third molar	2-stage difference clavicle	Both sites asymmetric
Male (130)	45 (34.6%)	75 (57.7%)	4 (3.1%)	9 (6.9%)	3 (2.3%)
Female (47)	25 (53.2%)	25 (53.2%)	2 (4.2%)	0	3 (6.4%)
Total (177)	70 (39.5%)	100 (56.5%)	6 (3.4%)	9 (5.1%)	6 (3.4%)

Table 3 Typical examples of age ranges and degree of left/right discrepancy

Stage discrepancy	Sex	Age range (left)	Average (left)	Age range (right)	Average (right)	L/R discrepancy (years)
1 stage (molar)	M	15.54–18.9	17.2	14.8–17.9	16.3	0.9
	F	15.3–19.00	17.1	14.9–18.3	16.6	0.5
2 stage (molar)	M	20.6–25.1	22.8	19.1–23.1	21.1	1.7
	F	15.7–19.7	17.7	16.4–21.0	18.7	1.0
1 stage (clavicle)	M	16.3–19.9	18.1	17.5–21.3	19.4	1.3
	F	16.0–20.3	18.1	14.5–18.5	16.5	1.6
2 stage (clavicle)	M	19.4–23.7	21.5	16.0–20.9	18.4	3.1
1 stage (both)	M	18.7–22.7	20.7	16.7–20.3	18.5	2.2
	F	17.5–22.2	19.8	15.7–19.7	17.7	2.1

There were no female cases in the sample with a two-stage discrepancy between left and right medial clavicular epiphyses. Age ranges calculated using the method outlined by Bassed et al. [49]

significant (t=2.08, p<0.05), with a mean paired difference for the sample of 0.25 years. For females, the difference was not significant (p>0.05), and the mean paired difference for the sample was only 0.1 years.

An example of calculated age ranges (at 95% confidence interval), average ages and the differences noted between left and right sides is displayed in Table 3. It can be seen that when there is only one-stage difference in the development for either third molar or clavicle, then the resultant average discrepancy between the left and right sides is relatively minor, whereas a two-stage difference, or a difference in both molar and clavicle in the same individual, results in a much larger discrepancy. There were 21 individuals who displayed a greater than one-stage difference in third molar and/or medial clavicular development, and this accounts for 3.4% of the entire sample of 604 individuals. The differences in age estimates between the left and right sides for these individuals range from 1.0 year to a maximum of 3.1 years. The order of the effect of asymmetry in each age marker on the variation in age estimate, from greatest to least, is as follows:

1. Two-stage difference in L/R medial clavicle,
2. One-stage difference in both L/R third molar and L/R medial clavicle,
3. Two-stage difference in L/R third molar,
4. One-stage difference in L/R medial clavicle, and
5. One-stage difference in L/R third molar.

For those cases exhibiting a two-stage difference in either third molar or clavicular development, or a one-stage difference in each age marker, separate age range estimates were calculated for each side, then averaged, and the average age range compared to the actual age. An example of these cases is displayed in Table 4. Of the ten cases shown, only one has a calculated average age range that does not include the actual age of the individual concerned, being 3 months younger than the estimated lower age limit.

There was no correlation found between the third molar tooth and medial clavicular epiphysis in terms of left/right synchronicity. That is, if a left third molar was less developed than its enantiomer, there was no corresponding tendency for the medial clavicle to follow the same developmental trend.

Discussion

In forensic age estimation, practitioners attempt to derive the approximate age of an individual by applying skeletal and dental developmental data obtained from an analysis of

Table 4 L/R estimates and average age ranges compared to actual age (in years)

Gender	Age range (L)	Age range (R)	Average range	Actual age
F	17.0–21.2	15.7–19.7	16.3–20.4	16.0
M	17.5–21.3	16.0–19.3	16.7–20.3	18.2
F	15.7–19.7	16.4–21.0	16.0–20.3	18.5
F	17.5–22.2	15.7–19.7	16.6–20.9	18.7
F	16.4–21.0	18.2–23.5	17.3–22.2	18.9
M	18.7–22.7	16.7–20.3	17.7–21.5	19.7
M	16.7–20.3	18.2–22.3	17.4–21.3	20.5
M	19.4–23.7	16.0–20.9	17.7–22.3	20.9
M	16.0–20.9	17.9–21.7	16.9–21.3	21.3
F	20.9–26.6	19.7–25.4	20.3–26.0	24.3

Age ranges calculated using the method outlined by Bassed et al. [49]

a large sample of the population from which that individual comes. Inherent genetic and/or environmental variation will necessarily make any age estimation subject to error, hence the need to include an error term with each estimate. In the interest of scientific precision, this error term should be made as small as is scientifically reasonable. This is achieved by defining as accurately as possible the population parameters of the target individual and applying population data which fits most accurately with that individual. The techniques to refine population data include separating samples on the basis of gender, socioeconomic status, ancestry and geographic location [50–56]. For many unknown individuals, especially in the case of severely decomposed or skeletonised remains, this narrowing of population parameters will be practically impossible, but where it can be done with some certainty it should be attempted. It is our contention that the presence of right/left developmental asymmetry also needs to be assessed and dealt with in order to present the most accurate estimate possible with the lowest realistic error.

Fluctuating asymmetry has no bias toward either side for both medial clavicle and the third molar, and therefore any asymmetry becomes statistically invisible when a large enough sample is assessed. When all of the identified asymmetric cases are extracted and assessed separately, however, it is seen that asymmetric development is an issue on an individual basis. The significance of calculated discrepancies of up to 3.1 years between left and right sides in certain individuals implies that age estimations based on one side only may not provide the best estimate for an individual, and more accurate results can be achieved if both sides are taken into consideration.

This study has demonstrated that when there is a discrepancy of more than one developmental stage between antimeres in either the third molar or medial clavicular epiphysis, then the difference in age estimate for left and right sides can be significant. Discrepancies in clavicular development have a greater effect on the resultant age estimate than third molar teeth, with the greatest effect seen in those individuals differing by two developmental stages for left and right clavicular epiphyses. Although the percentage of individuals with greater than one-stage difference between left and right sides is small (3.4%), the fact remains that in order for age estimates to be as precise and as accurate as possible for the greatest number of individuals, then it is necessary to develop a protocol to deal with this fluctuating asymmetry.

It is proposed that, where possible, both left and right sides be assessed for any anatomical age marker being examined. If it is found that there is one stage or less discrepancy, then the age estimate can be calculated using whichever side is the most judicially conservative. In these cases, the discrepancy in the age range for each side will not be significant enough to result in any meaningful inaccuracy. If, however, there is greater than one developmental stage discrepancy between antimeres, then it is recommended that age ranges be calculated individually for each side and the average of these calculations used as the final age range. An example of this is depicted in Table 4, which shows that in nine out of the ten cases presented, the actual age of the individual was within the calculated average age range, with the remaining case being 3 months younger than the age range would suggest. If the single side age ranges were to be used, then in three of the cases the actual age would have fallen outside the calculated range, in one case by as much as 1 year.

In summary, this research has shown that whilst fluctuating asymmetry occurring in development timing of the medial clavicular epiphysis and the third molar tooth may be insignificant on a population-wide basis, when assessing individual forensic cases, these developmental left/right discrepancies need to be considered where the difference in developmental staging is greater than one stage. A method of averaging the age ranges for each side is presented which should improve the accuracy of forensic age estimation procedures.

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