*Age estimation combining radiographic information of two dental and four skeletal predictors in children
and subadults*

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Introduction

In forensics, age estimation is mainly performed in the context of human identification and to clarify legal or social ramifications of the concerned individual [1]. Many methods for age estimation have been established and their application in a considered case depends on the available evidence. Usually, in children and adolescents, the radiographic methods based on the development of the teeth [1-6] and skeleton [7-11] are used. In addition, the scientific literature indicates that more accurate age estimation outcomes are obtained combining different age predictors [5, 12-14].

In previous studies dental age estimation in children was performed based on the radiographically observed development of the permanent teeth [1-3, 5, 6] and third molars [4, 12]. The most commonly used age estimation method based on the development of the permanent teeth was developed by Demirjian et al. [3]. This method was based on an own established staging technique which comprises 8 developmental stages for each of the seven mandibular left permanent teeth. Willems et al. [15] adapted this method and it was validated in several country-specific populations [16-19]. Köhler's 10 points staging technique [4] was frequently used to classify third molars development. This technique was previously used in regression models aiming to combine the age information of all present third molars [20-22]. To avoid the drawbacks of regression analysis for age estimation Bayesian approaches were established [20]. Because in children,

early third molar(s) development overlaps the development of the other permanent teeth, both dental age predictors can be combined [16, 17, 19].

Age information can be retrieved from diverse skeletal parameters. Gilli [23] compared the hand and wrist-based age estimation methods of Greulich and Pyle [7], Tanner [24], and the FELS method described by Roche and coworkers [25]. It was concluded that hopefully the development of a computerized method of assessment will lead to greater accuracy in the determination of skeletal maturity. The development of the cervical vertebrae registered on lateral cephalometric radiographs was also employed in forensic age estimation. Different registration techniques were described in the scientific literature such as the assessment of the vertebrae C2, C3, and C4 by Baccetti et al. [26], and Hassel et al. [10] and only C3 by Seedat et al. [27]. In infancy facial width seems to show a higher age related growth rate than facial depth or height, furthermore, the growth increments are different between cranial width and mandibular width [28] implementing age prediction potentials.

Dental and skeletal parameters were combined by several authors and in diverse settings. Thevissen et al. [29] combined the techniques of Baccetti et al. [26], Seedat et al. [27], and Köhler et al. [4] to pool age related information from cervical vertebrae and third molars. They observed that the age estimates drastically improved, especially during the period of early third molar development. Cameriere et al. [30]

compared the age prediction accuracy of methods based on the open apices of the tooth roots, carpal bone areas, and the ossification level of the epiphyses of the ulna and radius. The combined method was more accurate for age prediction than the methods using separately teeth or hand and wrist parameters.

In order to establish an optimal age estimation method, dental and skeletal developmental parameters could be combined. Currently, these developmental data are mainly observed using ionizing medical imaging techniques. Therefore, they are associated with the ethical issues related to the biological effects of exposure to radiation. In fact, forensic professionals must be aware of the risks involved with multiple radiation exposures for age estimation in the living [31].

The aim of the present study was to validate the age estimation performances combining the dental development of the permanent teeth and third molars with the skeletal development of cervical vertebrae, hand and wrist bones and craniofacial bones.

Materials and method

Panoramic radiographs (PR), lateral cephalometric radiographs (LCR), frontal cephalometric radiographs (FCR) and left hand and wrist bone radiographs (HWR) were retrospectively collected from 256 subjects (females: 135, males: 121), totaling 1024 images. The subjects were of Japanese nationality

and were aged between 4 and 20 years old (Table 1). All radiographs were digitally captured in the same day for each subject at the Iwate Medical University, Iwate, Japan. Ethical approval was provided by the Ethical Committee of the School of Dentistry at Iwate Medical University under reference No. 01237 according to the Declaration of Helsinki regarding human experimentation. Radiographs showing hypodontia or gross dental and cranial pathologies or treatments were excluded.

In the PR, the development of the first seven mandibular left permanent teeth was assessed and registered according to the staging technique of Demirjian et al. [3]. Additionally, the development of all the present third molars was registered according to the staging technique described by Köhler et al. [4]. In the LCR, the development of the third cervical vertebral body (C3) was registered according to the staging technique of Hassel and Farman. [10]. In the FCR, the craniofacial growth was registered using the maximal cranial width and the width at the level of the mandibular angles (Fig. 1). The first was defined as the width of the cranium between the most lateral points on the cranium parallel to the connection of the most superior point of both orbits. The latter was defined as the distance between the most lateral margins of the left and right mandibular angles. The measures were performed using Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, CA) software package, and expressed in the Adobe Photoshop CS6 unit "points". One millimeter (mm) contained 2.67 "points". Because the radiographs had no magnification

factor, the obtained "point" measures can be expressed for clinical relevance in mm, by dividing them by 2.67.

The development of the hand and wrist bones was registered according to the method of Greulich and Pyle [7]. This classification system contains 31 and 27 standards for females and males, respectively. However, in the current study the number of standards was condensed to 4 stages in females and 3 stages in males, based on standard thresholds that exhibited the same degree of hand and wrist development in females and males. The condensed stages grouped in females included the standards <19, 19-22, 23-26, and >26, while in males it included the standards <23, 23-27, and 28-31.

The age estimation was separately performed for females and males. The age estimation procedure was based on the application of Bayes' rule to a multivariate continuation ratio (CR) model (with a forward formulation) for the distribution of the stages and their related scores [32]. To circumvent the calculation of correlations, an ad hoc procedure was used [33]. To handle over fitting, the age predictions were obtained after the application of 10-fold cross-validation. Scores with a frequency of less than 10 were combined into a single category. To stay within the framework of combining multiple ordinal age indicators, the cranial width and mandibular width measurements were each divided into four categories. Used cut points for cranial width were 410, 425, and 440 for females, and 420, 435, and 450 for males. For the mandibular

width, the thresholds between stages of development were set at 245, 260, and 275 for females, and 260, 275, and 290 for males. Given the relatively small number of subjects in the dataset compared with the number of age indicators, linearity for age (on the logit scale) and the proportionality of its effects on all logits were assumed. Spearman's rank correlation coefficient (Rho) was calculated to test the association between CA and each dental and skeletal parameters. The added value of the 4 variables was verified by extending the CR model with each of these measurements separately as an extra variable and by combining all variables. Root means squared error (RMSE) values were analyzed, and the 5% trimmed mean; i.e., the mean for the 95% prediction interval, was used as the predicted value. The Wilcoxon signed rank test was used to compare the mean error (ME) (error = chronological age minus estimated age) and mean absolute error (MAE) between the established models. All analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA) software package. The bulk of the calculations was done using PROC NLMIXED as core to fit the continuation-ratio model and obtain the likelihood evaluations of each score and age combination.

Results

Fig. 2 and 3 show the scatter plots between the chronological age (CA) and each of the skeletal parameter used, sex-specifically. Strong correlations with age were found between hand and wrist bones (Rho=0.89 in females, Rho=0.84 in males), mandibular width (Rho=0.68 in females, Rho=0.62 in males), cervical vertebrae (Rho=0.77 in females, Rho=0.82 in males) development and CA. However, the correlation between cranial width and CA was weak (Rho=0.19 in females, Rho=0.24 in males).

In table 2 the ME, MAE and RMSE values obtained from the multivariate CR models were reported. In females, the RMSE was significantly reduced by adding hand and wrist bones, mandibular width, or cervical vertebrae development to the dental development (based on MAE). Note, however, that the reduction in RMSE and in MAE was not very large. In males, only the reductions in the RMSE observed after adding the hand and wrist bones or cervical vertebrae development were significant. For females as well as for males, the simultaneous addition of the four skeletal variables significantly reduced the RMSE (from 1.25 years to 1.14 years in females, $p<0.0001$ and from 1.35 years to 1.19 years in males, $p=0.005$).

In table 3 the parameter estimates from the multivariate CR model were reported sex specific.

Discussion

Combining the dental and all skeletal parameters maximally improved the age prediction

performances in both sexes. Combining the dental and each of the four studied skeletal variables revealed that the hand and wrist bones parameter was the best performing single added skeletal parameter. The difference in age prediction performance compared to all skeletal parameters added was only 0.05 years RMSE, -0.02 years ME and 0.03 years MAE for females and 0.03 years RMSE, -0.02 years ME and 0.03 years MAE for males (Table 2). In forensic practice most age estimation examinations in children and subadults are requested in the context of asylum procedures, migration or criminal liability, implicating that living individuals are examined. Therefore, the bioethical principles of beneficence and non-maleficence, which assure the examined person the best outcomes with the least damage possible should be applied [34]. Each medical examiner should balance the advantage of information obtained from radiographs against the possible effect of increased exposure to radiation of the examined individual [35]. In fact the principle of As Low As Reasonably Achievable (ALARA) should be taken into account (<https://eu-alara.net>). Applied to the current study results, the combination of dental and hand and wrist bone parameters provide the best age prediction performances with the lowest radiation exposure.

Magnetic Resonance Imaging (MRI) may be considered as an alternative to reduce the exposure to ionizing radiation. It was already studied for skeletal age estimation based on ossification stages of the clavicular epiphyses [36, 37], hand and/or wrist bone ossifications [38-41] and the

development of third molars [42-44]. However future research is necessary to investigate the age prediction performances combining skeletal and dental age estimation parameters obtained from MRI.

In the present study, the age prediction performances combining dental parameters and hand and wrist bone parameters resulted in 1.19 years RMSE, -0.06 years ME and 0.94 years MAE for females and 1.22 years RMSE, -0.06 years ME and 0.98 years MAE for males (Table 2). These results improved the age prediction performances using only dental parameters. Cameriere et al. [30] also detected better age prediction outcomes combining dental and hand and wrist parameters. Specifically, the obtained ME was 0.07 years for the combined parameters, 0.11 years for the dental parameters alone and -0.09 years for hand and wrist parameters alone. Although the considered dental and skeletal development registration differed in both studies, comparable age prediction result (ME difference of 0.01 years) combining dental and hand and wrist bone parameters were obtained. In fact, in the present study the dental parameter was registered as developmental stages of the third molars, in the Cameriere et al. study [30] the dental parameter was registered as measures of the open apices of the permanent tooth roots, normalized on tooth length. For the assessment of the hand and wrist parameter, the present study considered the ossification level of the hand and wrist bone according to the Greulich and Pyle standards, while the study of Cameriere et al. [30] calculated ratio's of the total area of the carpal bones and the area of the epiphyses of ulna and radius.

Combining dental and cervical vertebrae parameters, the present study revealed 1.20 years RMSE, -0.06 years ME and 0.96 years MAE for females, and 1.28 years RMSE, -0.07 years ME and 1.02 years MAE for males (Table 2). Thevissen et al. [29] combined dental and cervical vertebrae parameters for age estimation and reported 1.75 years RMSE for females and 1.22 years RMSE for males. Comparing both study results disclosed similar RMSE values for males and a RMSE value of 0.55 years less for females in the present study. In an integrated pilot set up Thevissen et al. [29] detected different age prediction performances depending on the registration technique for cervical vertebrae development used. In the current study a cervical vertebrae development registration technique (Hassel and Farman [10]) different from the Thevissen et al. study (Baccetti [26] and Seedat [27] combined) was applied. Moreover, both studies had different sample sizes and age distributions per sexes.

To the authors' knowledge no skeletal age related variables were studied on frontal cephalometric radiographs before. Although, in the age range of the studied sample (between 4 and 20 years) a considerable growth of the craniofacial bones was expected, the study results provided divergent correlations between both craniofacial parameters and CA. A strong correlation between CA and mandibular width was found in males ($Rho=0.68$ in females, $Rho=0.62$), while in females a weak correlation was found between CA and cranial width ($Rho=0.19$ in females, $Rho=0.24$). Further on, scarce

differences in age prediction performances were obtained in both sexes comparing the combination of dental parameters and the cranial width parameter and the combination of dental parameters and mandibular width parameter. Adding one of both studied frontal cephalometric radiograph parameters to the dental parameters provided the least improvement in age prediction performances when compared with all other studied skeletal parameters added. A consequence in forensic age estimation practice is that both frontal cephalometric parameters should not be considered when dental parameters are available.

A limitation inherent to the present study concerned the relatively small and heterogeneous distribution in age and sex of the studied sample (Table 1). This limitation was especially observed around the 18 year old threshold. Therefore the results of the current study didn't allow to draw reliable conclusions related to the age of legal majority (in most countries set at 18 years) when combining the studied parameters. The limitations were due to the fact that a retrospective collection of individuals from which in the same day 4 different types of radiographs were taken was hard to establish - especially because all radiographs had to be taken for dental/medical treatment purposes in order to comply with ethical standards [34]. The studied sample was collected retrospectively from the Iwate Medical University orthodontic patient files.

The present study outcomes proved that combining developmental parameters enhanced the age prediction performances. This is in agreement with multiple studies combining diverse developmental parameters. Degalp et al. [45] combined clinical measurements from the head circumference and the teeth (tooth and crown height, mesio-distal and labiolingual crown width) in fetuses and infants. The reported age estimation standard error using the head circumference was ± 1.13 weeks ($r^2=0.97$). Combining it with the tooth measurements decreased the age estimation standard error to less than 1 week ($r^2=0.99$) [45]. Shi et al. [46] combined 5 DNA methylation markers in blood with dental (Willems method [15]) and hand and wrist (Tanner and Whitehouse method version 3 on radius-ulna [24] in boys and Greulich and Pyle atlas method [7] in girls) parameters for age estimation. In boys and girls the dental age estimation performance was quantified with mean absolute errors of 0.63 and 0.54 years, respectively. Combining the dental and skeletal age estimations improved the mean absolute errors by 0.12 and 0.10 years in boys and girls, respectively. Additionally adding the DNA methylation markers to the dental and skeletal model reduced the mean absolute errors to 0.47 and 0.33 years in boys and girls respectively [46]. Bassed et al. [47] screened the correlation of age and third molars, the medial clavicular epiphysis and the spheno-occipital synchondrosis. Initially, the authors tested each parameter separately in regression models and observed a weak correlation between the spheno-occipital synchondrosis and age (adjusted r^2 of 5.5% in females and

21.3% in males). Based on this outcome the spheno-occipital synchondrosis was excluded from the combined models. The age estimation performances were quantified using standard errors of the estimate (SEE). The obtained SEE values were for third molars and the medial clavicular epiphysis separately 2.13 (F), 2.25 (M) and 1.85 (F), 1.49(M) years, respectively. Combining both parameters reduced the SEE to 1.29 (F) and 1.38 (M) years. Based on the cited and present study outcomes, it can be concluded that combining specific age estimation parameters ameliorates the age prediction performances. In the context of forensic practice it has to be evaluated at the cost of what a specific prediction improvement was obtained (e.g. in the Shi et al. study [46], the methylation reduces in boys the MAE only 0.04 years, using a complex, time consuming and expensive method). As a result of this evaluation, the current study revealed discrete improvement in age estimation performance adding the cervical vertebral body development and both craniofacial growth parameters which, in forensic practice doesn't weigh against the improved radiation exposure taking the LCR and FCR and the time and work necessary to register these parameters.

Conclusions

The age estimation performances in children and sub-adults increased maximally, in females and males, adding simultaneous the four skeletal parameters to the considered dental parameters. In

forensic practice, bioethical aspects related to the use of ionizing medical imaging techniques in the living need to be taken into account. Therefore, the best balance between obtaining optimal age estimation performances and using low radiation doses was achieved, combining only dental and hand and wrist bone parameters.

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Captions of figures

Fig. 1 – Measurements of the cranial width and mandibular angle width

Caption: Cranial width (1) was measured between the most lateral points of the cranium parallel to the line connecting the most superior points of both orbits. The mandibular angle width (2) was measured between the most lateral points of right and left mandibular angles.

Fig. 2 – Scatter plots relating chronological age and each skeletal parameter analyzed, in females

Caption: Rho: Spearman's rank correlation coefficient; N: number of subjects. Width measures are expressed in Adobe Photoshop CS6 unit "points".

Fig. 3 – Scatter plots relating chronological age and each skeletal parameter analyzed, in males

Caption: Rho: Spearman's rank correlation coefficient; N: number of subjects. Width measures are expressed in Adobe Photoshop CS6 unit "points".

Table 1: Sample distribution on age and sex

Age	F	M	F + M
4	1	0	1
5	1	1	2
6	3	4	7
7	3	5	8
8	9	7	16
9	13	5	18
10	12	17	29
11	19	12	31
12	16	21	37
13	13	11	24
14	13	8	21
15	14	12	26
16	6	11	17
17	4	5	9
18	6	1	7
19	2	0	2
20	0	1	1
Total	135	121	256

F: female; M: male; Age expressed in years.

Table 2: Age estimation outcomes distributed on age predictors and sex

	Female			Male		
	RMSE	Error	Absolute error	RMSE	Error	Absolute error
T	1.25	-0.07	1.00	1.35	-0.09	1.07
T + CV	1.20	-0.06	0.96	1.28	-0.07	1.02
T + CW	1.25	-0.07	1.00	1.35	-0.08	1.06
T + MW	1.24	-0.06	0.97	1.34	-0.07	1.06
T + HW	1.19	-0.06	0.94	1.22	-0.06	0.98
T + ALL	1.14	-0.04	0.91	1.19	-0.04	0.95

T: teeth; CV: cervical vertebrae; CW: cranial width; MW: mandibular width; HW: hand and wrist; ALL: CV + CW + MW + HW; RMSE: root mean squared error

Table 3: Parameter estimates from the continuation-ratio model (assuming proportional odds and linearity in the effect age) for each of the considered age indicators, males and females separately.

	Parameters	t18	t28	t38	t48	t31	t32	t33	t34	t35	t36	t37	CW	MW	HW	CV
Females	score=0	-7.32	-7.44	-6.79	-6.67											
	score=1	-6.31	-6.80	-6.19	-6.56								0.27	-3.91	-18.91	
	score=2	-8.00	-7.86	-7.47	-7.60								-0.74	-5.68	-22.21	-5.91
	score=3	-8.49	-9.16	-8.44	-8.81								-1.41	-7.10		-7.90
	score=4	-10.50	-10.76	-8.37	-9.18				-8.89	-10.91		-11.87				-9.71
	score=5	-10.18	-11.30	-9.71	-9.15			-6.72	-11.80	-14.15		-15.77				
	score=6	-11.75	-11.66			-20.40	-12.73	-9.68	-14.64	-17.66	-14.82	-18.67				
	score=7					-28.26	-17.86	-13.08	-16.86	-19.93	-20.48	-23.89				
	age	0.70	0.72	0.59	0.60	2.93	1.75	1.00	1.35	1.48	2.08	1.53	0.09	0.49	1.73	0.62
Males	score=0	-6.27	-6.31	-5.95	-6.22											
	score=1	-5.41	-5.24	-5.12	-5.51								0.51	-3.33	-19.14	
	score=2	-5.08	-5.82	-5.66	-6.07								-0.24	-4.39	-21.66	-9.08
	score=3	-6.19	-6.25	-6.49	-7.11								-2.05	-6.15		-11.44
	score=4	-7.24	-7.94	-7.37	-7.89				-8.37	-8.91		-10.30				-12.88
	score=5	-8.85	-8.67	-8.32	-8.97			-12.50	-11.68	-10.06		-11.92				
	score=6	-8.97	-9.74			-11.44	-9.38	-16.42	-14.41	-13.27	-13.29	-14.11				
	score=7					-17.63	-12.45	-20.14	-16.58	-14.88	-18.78	-17.75				
	age	0.54	0.57	0.51	0.54	1.83	1.22	1.45	1.27	1.10	1.89	1.16	0.11	0.40	1.48	0.80

F: females; M: males; t: tooth position (third molars: 18-48; seven lower left permanent teeth: 31-37); CW: cranial-width; MW: mandibular width; HW: hand and wrist bones; CV: cervical vertebrae; : non-existing categories. For an age indicator with K levels, the following K-1 logits were defined: $\log \left\{ \frac{\pi_1}{\pi_2 + \pi_3 + \dots + \pi_K} \right\} = \alpha_1 + \beta * \text{age}$, $\log \left\{ \frac{\pi_2}{\pi_3 + \dots + \pi_K} \right\} = \alpha_2 + \beta * \text{age}$, ..., $\log \left\{ \frac{\pi_{K-1}}{\pi_K} \right\} = \alpha_{K-1} + \beta * \text{age}$. The number of parameters depends on the number of ordinal categories used for the specific age indicator. Scores with too low prevalence in the dataset were combined neighboring higher or lower score. No parameter estimates are available for the K-th score level, for non-existing levels and for levels which are due to a too low prevalence (<10 cases) combined with the neighboring higher or lower score level. For AW and CW the used score levels were 1-4 corresponding to the categorization based on the used cutoff points.

Figure 1

Figure 2

Figure 3