Examination of skeletal age estimation methods and the influence of genetic background on cranial suture closure in adulthood

Ph.D. theses

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Budapest
2013

1. Introduction

Estimation the age of a person at the time of death is always one of the main purposes in anthropological investigations. Over the course of the past decades several age estimation methods were established to serve the needs of practitioners using known age and sex databases or during medicolegal autopsies. Meanwhile in the application of most of the methods all over the world, inter-population differences have been revealed. Therefore testing the adequacy of the used methods on local populations is highly recommended. According to some surveys, methods based on the morphological changes on the human pelvis and ribs are the most popular ones, when estimating the age of skeletal remains. Reliability of techniques based on cranial suture closure was always a matter of dispute. Practitioners have long been aware of the limitations associated with the use of cranial sutures as an indicator of age at death in the skeleton. Nowadays these methods are less preferred despite they have been used in age estimation for probably the longest time. Despite the many studies and findings on the behaviour of cranial sutures, their real nature is still unknown. Among the several factors that influence suture maintenance like the age of a person, muscular robostucity and activity, lifestyle and metabolism, genetic background, such as inheritable expression patterns of molecular factors that influence suture growth and patency can be a significant component of this complex process of regulation. The importance of certain genes in suture patency can be observed for example in syndromic craniosynostosis, where in some cases the alteration of only one single nucleotide can cause severe cranial manifestations in childhood.

2. Aims

1. Examination the reliability of Meindl & Lovejoy (1985) vault system based on ectocranial suture closure on a modern Hungarian sample, the mummies of Vác and the American Terry collection.

2. Examination of the endocranial suture closure component of the Nemeskéri et al. (1960) complex age estimation method on the Hungarian population and the American Terry collection.

3. Examination the adequacy of the age estimation method of Iscan et al. (1984, 1985) using the sternal end of the fourth ribs on a modern Hungarian sample, the mummies of Vác and the Terry collection.

4. Estimation the accuracy of the Iscan et al. (1984, 1985) method in case of the neighboring third and fifth ribs in modern Hungarians.

5. Comparison of the application of the methods in different sexes, races and samples.

6. Improve the accuracy of the tested methods in Hungarians.

7. Examination of some single nucleotide polymorphisms in targeted genes which encode factors that play an important role in cranial suture development and maintenance in relation to cranial suture obliteration status.

3. Materials and methods

Examination of three age estimation methods was conducted on different samples. Meindl & Lovejoy's (1985) cranial vault system, endocranial suture closure in relation to age according to Nemeskéri et al. (1960) and the Iscan et al. (1984, 1985) method were tested in the autopsy room of the Department of Forensic and Insurance Medicine at the Faculty of Medicine, Semmelweis University. After having obtained the permission of the local ethical committee, examination of the ossification of internal and external sutures on skulls and the alterations of sternal ends of the third, fourth and fifth ribs of a known sex and age was performed during medicolegal autopsies. After practitioners had opened the cranium, the skull was cleaned around the sutures properly. Obliteration status of ecto-, and endocranial suture sites were scored twice at least, first on the day of the autopsy and later, using photo-documentations. Sternal ends of the third, fourth and fifth ribs were collected and observed from autopsied individuals. The ribs were soaked in water for several weeks. During the putrification, the rib ends were cleaned sometimes under flowing water with a brush. Finally they were boiled cautiously in a hydrogen peroxide bath. All soft tissues were then removed, including the costal cartilage. In some cases, especially in older decedents, it was impossible to separate the entire cartilage, because it had become too hard due to calcification. Ectocranial suture closure was observed in 239, and endocranial suture closure in 238 individuals. Sternal end of the ribs was ascertained in 118 individuals during the research.

Ectocranial obliteration status and morphological status of the sternal end of the ribs were evaluated in the mummies of Vác at the Department of Anthropology of the Hungarian Natural History Museum. Observation of endocranial suture closure (and ectocranial obliteration status as well sometimes) was not possible due to the good preservation of the remains. Descendants of Vác were lying in peace for more than 150 years. During this time most of the individuals had been naturally mummified. Age estimation was conducted on 87 individuals however information about sex and age was available only in case of 47 of them. Examination of the Meindl & Lovejoy's (1985) vault system was conducted in 23 and the Iscan et al. (1984, 1985) method in 46 individuals.

The American Terry Collection contains the skeletal remains of almost 2000 white and black dissected individuals and it is housed by the Smithsonian Institution National Museum of Natural History in Washington, DC. Reliability of the Meindl & Lovejoy 81985) system was examined in the case of 383 skeletons, the Nemeskéri et al. (1960) method in 275 and the

Iscan et al. (1984, 1985) method in 369 individuals. General application of these techniques could be tested not only in different sexes but in different races as well.

Genomic DNA from 106 adults during medicolegal autopsies was obtained from whole, peripheral blood using the Chelex extraction process (Bio-Rad Laboratories, Munich, Germany). According to earlier findings, twelve SNPs in six candidate genes which seemed to be relevant in cranial suture formation and/or cranial suture synostosis were selected for further investigations. Genotyping of SNPs was conducted using competitive allele-specific PCR KASPar chemistry (KBiosciences Ltd, Hertfordshire, UK) according to the manufacturer's instructions at the Department of Genetics, Cell- and Immunobiology, Semmelweis University. Finally 101 samples were taken into consideration for statistical analysis.

Statistical analyses were carried out with R 2.14.0 software (2005). Kendall's correlation was used to test the expected correlation between the real age and the state of ectocranial suture closure. Inaccuracy and bias were defined as the average absolute error of age estimation and the mean over and under prediction. One-way ANOVA model and Tukey's post hoc pairwise tests were used to find significant differences between neighboring stages. Paired-samples Wilcoxon tests were performed to search for significant differences among assessed phase scores in an individual's different ribs. We applied analysis of covariance (ANCOVA) models in a general linear model framework to test whether SNP polymorphism of the investigated genes has a significant effect on the ectocranial suture synostosis of adults. The scores of suture closure were treated as a response variable, while known age and certain SNP polymorphisms were considered explanatory variables. The distributional criteria of general linear models were satisfied. All reported p-values are two-tailed.

4. Results

During this research no significant correlation was found between the ectocranial suture closure and the chronological age. The best result, 52% efficiency in age estimation was found in the case of the oldest and smallest group, the mummies of Vác. Effectiveness of the Meindl & Lovejoy (1985) vault system was 41% in the Terry collection and only 31% in case of the Hungarian autopsied sample. Between the two genders no significant differences were found in the extent of ectocranial suture closure. However accuracy was higher among the individuals of African origin compared to the individuals of European origin from the same sample.

Age estimation according to Nemeskeri et al. (1960) reached at least 70% accuracy in both samples, however the method can be used only to predict very broad and overlapping age stages. Efficiency in age estimation was 72% in Hungarians and 74% in the Terry collection. During this research an interesting observation was experienced, namely that the ossification of the endocranial sutures is a more general process and lacks the special suture closure patterns that can be seen in ectocranial suture obliteration. Similar to ectocranial suture closure, no significant differences between the two sexes were found in the samples.

All things considered, best results in age estimation of skeletonized individuals were found in the application of the Iscan et al. fourth rib method (1984; 1985). This system uses the narrowest and less overlapping intervals in age estimation. The age was predictable in Hungarians in 58%, 55% in the Terry collection and nearly 59% in the case of the mummies of Vác. Morphological changes of the sternal ends of the ribs seem to correlate the most with age, especially with slightly expanded age intervals, the estimation can reach a highly acceptable result.

In general, the Hungarians showed similar patterns like other populations before, that age estimation at time of death according to morphological indicators overages younger and underages older individuals. The most variable age group was the oldest decedents, which makes them the biggest challenge in age estimation.

In the genetic study, the assessment of the extent of cranial suture closure has been carried out according to Meindl and Lovejoy's (1985) vault system. Accuracy of age estimation according to ectocranial suture closure reached only 24% in this sample. Among the investigated genes and loci, the rs3821947 SNP polymorphism of the MSX1 gene had a robust significant effect on the state of ectocranial suture synostosis. Both known age and

genotypes affected cranial suture closure significantly. It has to be noticed, that this relationship was not driven by sampling bias (i.e., a given polymorphism group has higher ectocranial scores only because older people belong to that group), as the highest average ectocranial score was in the rs3821947AA genotype group, while the highest average age was in the rs3821947GG genotype.

Furthermore, a weaker association between the rs2761887 polymorphism of the BMP4 gene and the state of ectocranial suture closure was found. Taking into consideration that MSX1 and BMP4 are parts of the same signaling pathways and both are expressed in postnatal stages as well, this result strongly suggest their possible effect on cranial suture closure in adults.

5. Conclusions

1. Ectocranial suture closure has an extreme variability in different populations. Hungarians show no close correlation between real age and ectocranial suture obliteration.

2. The older the sample was, the better result in age estimation according to ectocranial suture fusion was found. The people of African origin showed better efficiency than individuals of European origin from the same sample.

3. Endocranial suture closure seems to associate more with age at time of death, but the method was useable only for rough age estimation. The age intervals are too large and overlapping, so the results are not sufficiently precise to be applicable solely, especially in forensic investigations.

4. Endocranial suture fusion is a more general process and lacks any closure patterns.

5. Morphological changes of the sternal ends of the ribs seem to correlate the most with age, but only with expanded age intervals, reached the estimation an acceptable rate.

6. Fourth rib ends did not always show the highest correlation with real age among the neighbouring ribs, so it is recommended to use more applicable rib ends during the process of age estimation of skeletonized individuals.

7. Age intervals are always expanded in forensic investigations, especially when only few morphological methods can be used in age assessment. To ascertain necessary changes for a better result, individual tests on the local population are required.

8. The more methods in the service of determining the age at death of skeletonized individuals can be used, the better accuracy in age estimation can be reached.

9. Due to the extreme variability and different factors that influence morphological changes in individuals, the potential in morphological methods can not be widened more. In some cases, techniques can be slightly modified according to local characteristics.

10. In archaeological context the usage of morphological age estimation methods are acceptable, while usually in demographic analyses wider age intervals are used.

11. In forensic investigations, application of more morphological and/or more precise molecular methods in the practise of age determination in skeletonized individuals is recommended.

12. Genetic background has a significant effect on the ectocranial suture closure in adults. Further investigations on independent, larger populations are required to explore the correlation between suture fusion and the participants of different signaling pathways involved in cranial suture mechanisms.

6. List of publications

I. Publications related to the Ph.D. thesis:

<u>Wolff K</u>, Vas Z, Sotonyi P, Magyar LG. 2012. Skeletal age estimation in Hungarian population of known age and sex. Forensic Sci Int 223(1-3):374 e371-378. **IF: 2.335**

<u>Wolff K</u>, Hadadi E, Vas Z. 2013. A novel multidisciplinary approach toward a better understanding of cranial suture closure: the first evidence of genetic effects in adulthood. Am J Hum Biol 25(6):835-843. **IF: 2.307**

II. Publications not related to Ph.D. thesis:

<u>Wolff K</u>, Evinger S, Hajdu T, Gyenis G. 2012. Anthropological examination of the chronologically separated groups of the 11th–13th century Zalavár-Chapel (Zalavár-Kápolna) cemetery from Hungary. Anthrop Anz 69(4):473-490. **IF: 0.676**

Evinger S, Bernert Zs, Fóthi E, <u>Wolff K</u>, Kővári I, Marcsik A, Donoghue HD, O'Grady J, Kiss KK, Hajdu T. 2011. New skeletal tuberculosis cases in past populations from Western Hungary (Transdanubia). Homo 62(3):165-183. **IF: 0.542**