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Επιβλέπων Καθηγητής για την εκπόνηση της Μεταπτυχιακής Διπλωματικής Εργασίας κ. Τσολάκης Απόστολος

Τριμελής Επιτροπή για την Αξιολόγηση της Μεταπτυχιακής Διπλωματικής Εργασίας:

1. Χαλαζωνίτης Δημήτριος
2. Τσολάκης Απόστολος
3. Μπιτσάνης Ηλίας

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Περίληψη

Η αναλογία μήκους δείκτη προς μήκος παράμεσου δακτύλου (2Δ:4Δ) αναφέρεται ότι επηρεάζεται από τα επίπεδα των εμβρυϊκών ανδρογόνων. Μελέτες δείχνουν ότι αυτός ο λόγος παρουσιάζει διμορφισμό μεταξύ των 2 φύλων. Συγκεκριμένα, εμφανίζεται μικρότερος στα αγόρια σε σύγκριση με τα κορίτσια, το οποίο ενδεχομένως συσχετίζεται με μεγαλύτερη ενεργοποίηση των υποδοχέων των ανδρογόνων κατά την εμβρυϊκή περίοδο στα αγόρια, άρα και με άλλες διεργασίες διαφοροποίησης μορφολογικών χαρακτηριστικών του φύλου. Μία εξ' αυτών θεωρείται το σχήμα του κρσνιοπροσωπικού συμπλέγματος. Κατά την παιδική ηλικία, δεν παρατηρείται διαφορά στο σχήμα μεταξύ των δύο φύλων και ο διμορφισμός του φύλου φαίνεται να προκύπτει μετά την εφηβεία, κάτω από την επίδραση ορμονικών παραγόντων. Σκοπός της παρούσας εργασίας είναι η αξιολόγηση της συσχέτισης του σχήματος του κρανιοπροσωπικού συμπλέγματος με την αναλογία 2Δ:4Δ, προκειμένου να ανιχνεύσουμε τους πιθανούς μηχανισμούς που ενέχονται στις δύο αυτές διεργασίες. Για το σκοπό αυτό, ανατρέξαμε στο ιστορικό αρχείο του Burlington Growth Study Collection, προκειμένου να μελετήσουμε τη συσχέτιση αυτή σε δύο χρονικές στιγμές, πριν και μετά την εφηβεία, με τη μέθοδο της γεωμετρικής μορφομετρίας. Συγκεκριμένα, επιλέξαμε πλάγιες κεφαλομετρικές ακτινογραφίες και ακτινογραφίες άκρας χειρός 36 αγοριών, στην ηλικία των 10 (T1) και 18 (T2) ετών, καθώς και 34 κοριτσιών, στις ηλικίες των 10 (T1) και 17 (T2) ετών. Για τον υπολογισμό του μήκους των δακτύλων ιχνογραφήθηκαν 6 σημεία σε κάθε δάκτυλο, τα οποία ανά δύο αντιπροσωπεύουν το μήκος της κάθε φάλαγγας, οπότε αθροιστικά το συνολικό μήκος του δακτύλου. Το σχήμα του κρανιοπροσωπικού συμπλέγματος περιγράφηκε από 15 συνεχόμενες καμπύλες, αντίστοιχα με τις διάφορες κρανιοπροσωπικές ανατομικές δομές σε κάθε πλάγια κεφαλομετρική

ακτινογραφία.. Στη συνέχεια πραγματοποιήθηκε αλληλεπίθεση Προκρούστη και ανάλυση κύριων συνιστωσών (PCA) για την εκτίμηση της μεταβλητότητας του σχήματος του κρανιοπροσωπικού συμπλέγματος. Η πιθανή συσχέτιση του λόγου 2Δ:4Δ με το σχήμα του κρανιοπροσωπικού συμπλέγματος εξετάστηκε με ανάλυση παλινδρόμησης. Δεν βρέθηκε στατιστικά σημαντική διαφορά στην αναλογία των δακτύλων μεταξύ των δύο χρονικών στιγμών Τ1 και Τ2, για κάθε φύλο χωριστά (αγόρια: P=0,691, κορίτσια:P=0,379), οπότε εν συνεχεία υπολογίσαμε το μέσο όρο της αναλογίας των Τ1 και Τ2, για να συγκρίνουμε το λόγο μεταξύ των δύο φύλων. Βρέθηκε στατιστικά σημαντική διαφορά στην αναλογία 2Δ:4Δ μεταξύ των δύο φύλων και συγκεκριμένα μεγαλύτερος στα κορίτσια (2Δ:4Δ= 0,938945) συγκριτικά με τα αγόρια (2Δ: 4Δ= 0,924032)(P=0,002). Κατά την αξιολόγηση του σχήματος του κρανιοπροσωπικού συμπλέγματος, εν αντιθέσει με το λόγο των δακτύλων και σε συμφωνία με την υπάρχουσα βιβλιογραφία, δεν παρατηρήθηκε διμορφισμός φύλου πριν την εφηβεία(P=0,19), ο οποίος όμως διαπιστώθηκε μετά την εφηβεία, όπως ήταν αναμενόμενο. Οι πρώτες 11 κύριες συνιστώσες περιέγραφαν περίπου το 79% της μεταβλητότητας του σχήματος του κρανιοπροσωπικού συμπλέγματος. Η συσχέτιση μεταξύ του λόγου των δακτύλων και του σχήματος του κρανιοπροσωπικού συμπλέγματος έγινε για τις χρονικές στιγμές Τ1 και Τ2, για κάθε φύλο χωριστά. Συγκεκριμένα, δεν βρέθηκε συσχέτιση μεταξύ τους, ούτε στη χρονική στιγμή Τ1 (κορίτσια: P=0,20, αγόρια: P=0,32), αλλά ούτε και μετά την εφηβεία, στη χρονική στιγμή Τ2 (κορίτσια: P=0,78, αγόρια: P=0,80). Από τα ευρήματα της μελέτης μας, μπορούμε να συνάγουμε ότι πιθανώς διαφορετικοί βιολογικοί μηχανισμοί δράσης ενέχονται στη μορφολογική διαφοροποίηση της αναλογίας του μήκους των δακτύλων και του σχήματος του κρανιοπροσωπικού συμπλέγματος ή ίδιοι μηχανισμοί αλλά σε διαφορετικές χρονικές περιόδους για τα δύο αυτά μορφολογικά χαρακτηριστικά.

Abstract

2D:4D ratio follows a sexually dimorphic pattern; men tend to have a lower digit ratio than women, with overlap between male and female distribution. Lower digit ratio is associated with greater prenatal androgen stimulation in boys and characteristics more commonly displayed in males.2D:4D ratio has been suggested to be a marker of prenatal androgen levels in humans; this may affect the differentiation of many sex-dependent traits, such as the craniofacial shape, as well as the development of some behaviors and diseases. The aim of our study was to investigate if there is a correlation between this ratio and the craniofacial shape in pre- and post-pubertal subjects, using geometric morphometric analysis. Our sample was collected from the historic database of the Burlington Growth Study Collection and consisted of hand-wrist radiographs and cephalograms of 70 subjects at two time points; 36 boys at 10 (T1) and 18 (T2) years of age and 34 girls at 10(T1) and 17(T2) years of age. Digit ratio was estimated by digitized hand- wrist radiographs using computer software specifically configured, while the craniofacial shape was described by 15 continuous curves; Procrustes superimposition and PCA analysis described the shape variability of the craniofacial skeleton. The male digit ratio (2D:4D= 0,924032) was statistically significant smaller than the female ratio (2D:4D= 0,938945)(P=0,002). Craniofacial shape did not present sexual dimorphism before puberty and showed its sexually dimorphic pattern only post-pubertal. No statistically significant correlation was found between digit ratio and craniofacial shape at T1 for girls and boys (P=0,20 and P=0,32 respectively), neither at T2 (P=0.78 and P=0,80, respectively).

Introduction

The hypothesis of an association between 2D:4D digit ratio and prenatal androgen levels was first suggested more than 20 years ago (Manning et al., 1998), yet only eight human studies provide a direct evidence for this (Richards, 2017(a), (b); Manning and Fink, 2017).Testosterone and oestradiol measures were obtained from amniotic fluid during the second trimester (Lutchmaya et al., 2004; Ventura et al., 2013), from cord blood samples in newborn (Çetin et al., 2016; Hollier et al., 2015; Hickey et al., 2010; Whitehouse et al., 2015) and school-aged children (Mitsui et al., 2015, 2016), while in another two studies from maternal serum(Barona et al.,2015; Ventura et al., 2013). Five from the eight studies reported at least one significant effect between digit ratio and foetal androgen levels. Although there seems to be a trend towards a negative association between them, the results obtained from these papers are inconsistent, considering the limitations of each study.

Because of the difficulty in measuring hormonal levels during the first trimester of gestation, due to ethical constraints, most research has focused on diseases caused by unusual androgen exposure, as well as on sex-dependent behaviors, activities and features.

Indirect evidence that digit ratio is an indicator of prenatal sex hormone exposure comes from patients with adrenal congenital hyperplasia (CAH), a disorder attributed to excessive production of prenatal androgens. Subjects affected by CAH have lower digit ratios than the unaffected sex-matched controls, while high length ratios observed in males suffering from androgen insensitivity syndrome (AIS) (Berenbaum, 2009). AIS, a genetic disease caused by mutations in the androgen receptor gene, results in resistance to androgens in 46, XY individuals (McPhaul et al., 1993). Conditions related to high prenatal testosterone levels, such as autism and polycystic ovary syndrome, demonstrate a more masculinized finger length pattern than normal controls, while the opposite effect was noted in Klinefelter patients (XXY males) (Manning et al, 2013).However, results from twin studies (van Anders et al., 2006; Medland et al., 2008), as well as from animal studies are mixed (Abbott et al., 2012; Zheng and Cohn, 2011).

There is evidence that cancer development is influenced by intrauterine sex steroids (Folkerd and Dowsett, 2010;Madhunapantula et al., 2010), which are supposed to be negatively correlated with digit ratio. A systematic review and meta-analysis of the available literature assessed the relationship of 2D:4D with cancer; results demonstrated that prostate cancer and brain tumors were associated with low digit ratio, breast cancer and cervical dysplasia with high ratio, testicular, gastric and oral cancer were not related with 2D:4D, while higher levels of the digit ratio were associated with younger age of breast cancer and brain tumors presentation (Bunevicious 2018).

Digit ratio may also be related to violent behavior, as suggested by the evolutionary neuroandrogenic theory exposure to high levels of foetal testosterone influences the brain development leading to higher risk of aggression and criminal behavior later in life (Ellis 2003, 2005).A meta-analysis reported a statistically significant but weak correlation between digit ratio and violent outcomes (Turanovic et al.,2017). Results from other studies examining sex-typed behaviors related to prenatal androgens with digit ratio are inconsistent (Cohen-Bendahan et al., 2005, Wong and Hines, 2016), but the predicted negative correlation between handgrip strength and digit ratio in adolescent boys has been confirmed (Tomkinson, 2017).

The hypothesis of Neave et al. (Neave et al., 2003) that traits of face masculinity in adult males are attributed to the 'organizational' effects of high prenatal testosterone exposure, was also confirmed by Meindl et al., in a sample of young boys aged 4-11 years old (Meindl et al., 2012). The resulted significant association between digit ratio and face shape suggested a prepubertal 'organizational' impact onset. Until then, studies examining the relationship of digit ratio with face shape were referred to adults only and used frontal photographs to describe face shape (Burriss et al., 2007; Fink et al., 2005; Schaefer et al., 2005). One study later, using 3D surface images in order to capture the facial shape variability in a sample of 151 male adults, examined its relationship with digit length ratio. They found that the shape variance predicted by left digit ratio was 1,7%, a statistically significant correlation; lower digit ratio was related to a wider and shorter face shape in frontal view and to an increased protrusion of the mandible, nose and lips in profile (Weinberg et al., 2014). All the aforementioned studies have focused on the analysis of soft-tissue facial shape. Conversely, Valla and Halazonetis (2014)examined the skeletal craniofacial shape from lateral cephalograms in a sample of prepubertal children and found no statistically significant association between digit ratio and craniofacial shape; less than 1% of shape variation was accounted for by the digit ratio.

Few studies in the literature have evaluated the relationship between digit ratio and face shape; their sample consisted of adults, except from two studies which used a sample of pre-pubertal children (Meindl et al., 2012; Valla and Halazonetis, 2014).Taking into consideration that puberty is a crucial hormonal and growth time-point, which might affect both craniofacial shape and digit ratio, and therefore, their relationship, we would like to examine if this relationship changes from prepuberty to adulthood. For this, we needed longitudinal records at two time-points, one pre- and one post-puberty, but due to ethical considerations regarding radiation protection we could not obtain these. So, we used a historical sample of pre-pubertal children and adults, in order to investigate if the relationship between digit ratio and craniofacial shape changes during ontogeny.

Secondary, we will test whether digit ratio and craniofacial shape change from prepuberty to adulthood as well as if these two parameters differ between the sexes of our sample.

Methods and materials

The protocol of this study was approved by the Ethics and Research Committee of the School of Dentistry, National and Kapodistrian University of Athens.

In order to evaluate whether the correlation between craniofacial shape and digit ratio changes during growth, we sought for longitudinal records at pre- and post-pubertal time points. Due to ethical considerations regarding radiation protection, we sought our sample in the historical collections of the American Association of Orthodontists Foundation (AAOF). Craniofacial shape was assessed from lateral cephalograms, while digit ratio from hand-wrist radiographs, in order to avoid the bias and inaccuracy inherent in measuring directly on soft tissues (Honekopp and Watson, 2010; Manning et al, 2005).We included all subjects with lateral cephalograms and hand-wrist radiographs at 10 (T1) and 17 or 18 (T2) years of age, but excluded those with a record of, or indications of, orthodontic treatment, or low quality radiographs. The final sample comprised 70 subjects (36 boys and 34 girls) from the Burlington Growth Study, the only collection that includes hand-wrist radiographs (Tables

Table 1).

Cephalometric radiographs and measurements

Lateral cephalometric radiographs were digitized with Viewbox 4 software (dhal software, Kifissia, Greece). The craniofacial complex was described by 15 continuous curves (Figure 1); totally 156 landmarks were placed on the curves of each radiograph. Ten points representing local anatomic structures were considered as fixed, while the remaining 146 as semilandmarks.

The average shape of the 140 tracings was calculated and used as a reference. Then semilandmarks could slide along the curves to new positions, based on the reference configuration, to minimize bending energy. Their final position was obtained after three times of sliding.

The tracings of our 140 subjects were superimposed by Procrustes method and Procrustes coordinates were calculated for each subject. Then, principal component analysis (PCA) was applied on these coordinates for an estimation of the shape variability of our sample. All PCs represent the shape space of our sample.

Hand-wrist radiographs and measurements

Second and fourth digit lengths were obtained from the digital images of radiographs of the right hand only, using the Viewbox 4 software, specially configured for this research.

Radiographs of the right hand only were available from the Burlington Growth study. Some radiographic studies report differences in digit ratio between the right and left hand (Manning et al. 1998, 2004), others not (Paul et al., 2006; Robertson et al., 2008). A meta-analysis concluded that the right hand is a better indicator of prenatal androgen levels (Hönekopp and Watson, 2010).

Six landmarks were placed per digit; two landmarks at each phalanx, one at its distal and the other at its proximal end.

The length of each digit was measured in two ways: a) from the distal end of the distal phalanx to the proximal end of the proximal phalanx (direct length- DL), and b)as the sum of the following three segment lengths (phalanx sum- PS) (Figure 2, Figure 3):

a. from the distal end of the distal phalanx to the distal end of the medial phalanx
b. from the distal end of the medial phalanx to the distal end of the proximal phalanx
c. from the distal end of the proximal phalanx to the proximal end of the proximal phalanx

These measurements were made for the second and fourth digit of the whole sample at two time-points. Then, based on these two measurements for each digit, we calculate the second to fourth digit ratio (ratio direct and ratio sum). Then, we compared digit ratio with digit sum in order to detect if there is a difference between these two measurements.

Error estimation

In order to evaluate intra and inter-observer error, 20 lateral cephalograms and 20 hand-wrist radiographs of the same subjects were randomly selected and redigitize done month later, by the same and another investigator. Inter and intra- error for digit length ratio was estimated as the mean difference between the repeated measurements. Intra and inter-observer error for cephalograms was expressed as the Procrustes distance between repeated digitizations compared to the total variability of shape.

Statistical Analysis

We performed paired t-test and Bland-Altman analysis to evaluate intra and inter-observer error for digit length ratio. Paired t-test was also used to examine changes in digit ratio with age within each sex, and unpaired t-test to detect sexual dimorphism of digit ratio within the whole sample.

Permutation tests were applied in order to compare sexes in shape space at T1 and at T2 separately, as well as shape changes from T1 to T2 in either gender. Additionally, the relationship between digit ratio and craniofacial shape at two timepoints,for each sex separately, was calculated by permutation tests.

Results

Method error

Intra-observer error for digit ratio was assessed by paired t-test (P> 0,005) and was 9,81%. Bland-Altman analysis showed that the average of differences was -0,001 with limits of agreement (LoA) from -0,0079 to 0,0051. Paired t-test, which was also performed for inter-observer error, showed no systematic error (P>0,005) and was 9,6%. Bland-Altman analysis estimated that the average of differences was -0,0005 while LoA ranged from -0,0068 to 0,0058.

Intraobserver and interobserver error for the 20 repeated tracings was 7,26 per cent and 11,99 per cent of the total sample's variance, respectively.

Finger measurements

Two measurements were made per digit: digit ratio and digit sum (Table 2, Table 3). Paired t tests were performed in order to detect differences between digit ratio and digit sum; no statistically significant difference was found between them at either sex, neither at T1, nor at T2 (Table 4). So, we used digit sum, in order to compare digit ratios between the two time points.

Two-tailed paired t-test was performed for each gender separately, in order to detect any difference in digit ratio 2D:4D from T1 to T2. No difference in ratio was observed between the two timepoints for either sex (males: P= 0,69, females: P= 0,38, Table 5) so this ratio does not change during puberty (Figure 4,Figure 5). This pattern of relative stability of 2D:4D digit ratio from childhood to adulthood is consistent with the findings of other studies (McIntyre et al., 2005; Trivers et al., 2006). Because of this finding, we calculate the average ratios of two time-points for each sex separately and then an unpaired t-test was performed, in order to compare digit ratios between males and females. The mean average ratio for females was 0,938945 while for males 0,924032; the difference in digit ratio between sexes was statistically significant (P= 0,00272) (Table 6) and followed the sexually dimorphic pattern of being larger in females than males.

Cephalometric measurements

The first eleven principal components (PC1-PC11) of shape space described approximately 80 per cent of the sample's shape variability (Table 7). The first three PC accounted for 50,7% of the total variance of craniofacial shape. PC1 described the proportional relationship between cranial and facial structures, which followed the expected normal growth pattern; over time, there is a progressive reduction of the relative size of the cranium to the face. PC2 was related to the shape variability in the vertical direction, with hypo-divergent and hyper-divergent skeletal patterns at the two extremes. PC3 described shape variance in anteroposterior direction, differentiating between skeletal Class 2 and Class 3 subjects, while PC4 described variability in the gonial angle of the mandible.

Sexual dimorphism of the craniofacial shape was detected within the whole sample at two timepoints. The average craniofacial shape for either sex was calculated (Figure 6, Figure 7) and then the average shape of either sex at T1 was superimposed with the average shape at T2 (Figure 8, Figure 9);a significant shape change with growth, from T1 to T2, for each sex separately, was detected. Permutation test revealed no statistically significant difference between the two sexes at 10 years (P=0,19) (Figure 10), while shape sexual dimorphism was noted at 18 years (Figure 11).

Permutation tests were also performed in order to compare digit ratio 2D:4D with the craniofacial shape at each sex separately, at two timepoints. No statistically significant correlation was found between digit ratio and craniofacial shape at T1 for girls and boys (P=0,20 and P=0,32 respectively), neither at T2 (P=0.78 and P=0,80, respectively).

Discussion

In 1998 Manning and his colleagues proposed that the sexually dimorphic pattern of 2D:4D digit ratio is present from at least 2 years of age and is the result of prenatal testosterone action, through androgen receptors of fetal cartilaginous tissue (Manning et al, 1998; BenHur et al, 1997). Testosterone biosynthesis begins at 9 weeks of fetal life and sex variation in serum levels arises between 12 and 18 weeks gestational age (Grumbach et al, 2003); this may explain the fact that sex differences in digit ratio are present from 14 weeks gestational age (Galis et al, 2010; Malas et al, 2006).

Most studies investigating sex differences in digit length ratio are cross-sectional, consisting either of children before the pubertal growth spurt or adults after that. However, knowing that puberty is a crucial period characterized by increased amounts of gonadal steroids typical of adulthood, it is of interest to investigate if pubertal sex hormone levels affect digit ratio. The first study that used serial radiographs in order to evaluate the development of digit ratio from infancy to adulthood concluded that sex differences in digit ratio arise before puberty and are not strongly affected by this (McIntyre et al, 2005). Similarly, we used longitudinal data to investigate if puberty influences digit ratio; specifically, we searched for sex differences at 10 years of age and at adulthood. Our results showed sexual dimorphism to be present before puberty and maintain this pattern post-pubertal, in line with the findings of McIntyre et al. Results from these longitudinal data indicate thatthe secretion of sex hormones during puberty does not differentiate the developmental pattern of digit ratio between sexes; exposure to prenatal sex steroids seems to be too strong in order to determine the sex difference in digit ratio, so that the increased levels of pubertal hormones cannot alter it. We can conclude that sexual dimorphism is established in utero, not manifested during infancy and is present from childhood, not affected by puberty.

The above observation however is not consistent with other sexually dimorphic traits which present minor sex differences before puberty such as waist to hip ratio (Manning 2002) and shape of the craniofacial complex; it is known that craniofacial shape present age related differences from birth to adulthood; similar facial shape patterns before puberty and a sexually dimorphic pattern in adults, which probably arises at puberty (Bulygina et al, 2006; Gkantidis and Halazonetis, 2011; Coquerelle et al, 2011). Similarly, we found no difference in craniofacial shape between sexes before puberty (T1), but sexual dimorphism was apparent at adult stage (T2). Interestingly and in contrast to digit ratio, pubertal sex hormones influence craniofacial shape, such as this sexually dimorphic trait becomes apparent after puberty. There is a controversy between sexual dimorphism of digit ratio which is apparent before puberty and that of the craniofacial shape, which arise after that time-point. This indicates that these two sex dependent traits are influenced by different factors, explaining our primary outcome; no correlation was found between digit ratio and craniofacial shape for either sex at the two time-points.

The hypothesis that testosterone affects the development of digits in fetal life, does not explain if and how the production of testosterone after birth is responsible for the sex variability of 2D:4D, which is reported in adults (Manning et al, 1998). One study investigated if circulating testosterone levels, measured in saliva samples at 3 months post birth, is associated with 2D:4D. They found small and inconsistent sex differences in digit ratio in the first two years of life; more precisely, sexual dimorphism in digit ratio was only statistically significant at 2 weeks of age (Knickmeyer et al, 2011). Another two studies which focused on infants (Alexander et al, 2009) and on two year old children (Lutchmaya et al, 2004) showed similar results; no significant sex differences were recorded. This overlap in digit ratio between sexes during this period of infancy does not reflect the expected pattern observed in adults with males having significant lower 2D:4D ratios than females and is not compatible with the hypothesis of the effect of high testosterone exposure of newborn males, referred as neonatal surge (Fechner 2003). It is possible that digit ratio in infancy may not be strongly associated to prenatal androgen levels or its sexual dimorphism, although established in utero, has not yet become apparent. Another possible explanation for this high degree of overlap between sexes in this time period would be the ethnicity; Caucasian infants had higher ratios than African-American infants, with a small absolute size of difference on the order of 0,01 to 0,02 (Knickmeyer et al, 2011). Comparable results were reported between Caucasian and Afro-Caribbean children (Manning et al, 2004) as well as between white and black adults (Manning and Fink 2008);lower values were recorded in Blacks and Chinese compared to Caucasians and in African children compared to European children (McIntyre et al, 2006).This great variability in 2D:4D between ethnic groups may confound sex differences in digit ratio; so, we should keep in mind that results from ethnically heterogeneous studies are not comparable.

The suggestion that 2D:4D is a marker of prenatal androgen levels was first reported by Manning et al (1998); they measured digit lengths directly, using vernier calipers in a sample of 400 subjects from the Merseyside area aged from 2 years to 25 years. Since then a variety of measurements methods have been used; indirect measurements via photocopies, photographs and scans of the hands (Lutchmaya et al, 2004; Trivers et al, 2006), via hand wrist radiographs (Vehmas et al, 2006), as well as via visual classification from hand radiographs (Robertson et al, 2008). So it has been noticed that the way of measurement influences the magnitude of digit ratios; more precisely, lower digit ratios were obtained via indirect measurements (Manning et al, 2005) and direct measurements yielded smaller sex differences than indirect (Honekopp and Watson, 2010). This could be attributed to the finger's tip soft tissue distortion due to pressure of the hand on the glass plate. For our research, the need for serial data with almost 10 years difference, leaded us to search for these from historic databases; the only available database including both hand-wrist radiographs and lateral cephalograms was from Burlington Growth Centre. Due to the differences detected according to the way of measurement, we should notice that our results are able to be compared only with other radiographic studies. Another bias in finger length measurements could be osteoarthritis, common condition in middle aged persons; it leads to narrowing of joint spaces and shortening of fingers but it can be detected from radiographs. For this reason, one study investigated additionally metacarpal ratio to overcome interphalangeal joint problems and found a statistically significant lower ratio in males compared to females (Robertson et al, 2008).

There are some limitations in our study. We used hand-wrist radiographs obtained from the files of Burlington Growth Centre, which was initiated in 1952 by Dr. Robert Moyers. So the records were taken in the 1960s, almost 6 decades earlier. It is of interest that a higher digit ratio was recorded in a contemporary Lithuanian sample compared to a historical sample, collected in the 1880s. The researchers hypothesized that the increased exposure in endocrine disruptors in the modern population may contribute to the more feminized digit ratio (Voracek et al, 2007). In addition, there are other sex dependent traits that present a secular change; sperm concentrations as well as incidence of testicular cancer are related to the year of birth (Toppari et al, 2002).Environmental agents including endocrine disruptors have been suggested to be responsible for sexual developmental disorders and reproductive functions. The increased exposure to chemicals in modern population can disrupt the hormone dependent pathways; if this hypothesis is true, it is possible that digit ratio of our historical sample, as a proxy of prenatal androgen exposure, may differ from that found in a contemporary group and such a comparison could be judged as insecure. The predominant racial group of Burlington Growth Centre was Caucasian and mostly Anglo Saxon; Aboriginal peoples, Orientals and Blacks, although citizens of Ontario in that era, were not represented in the sample, as mentioned by Burlington Growth Centre. Knowing that digit ratio varies in different populations, being higher in Caucasians compared to Blacks and Chinese (McIntyre et al, 2006), the ethnic background of our study must be kept in mind, when our results are compared to other studies. We selected all the available radiographs from Burlington database, in the two time-points we were interested to; so there was no randomization in the selection of our sample, which means that it is not representative and our results cannot be applied to the general population. Another limitation is that we do not know if any standardized procedure has been followed during the irradiation of the hands; the way the fingers were placed affects digit measurements; this is one of the reasons we used two different digit measures; direct length and phalanx sum. Except from the aforementioned limitations, we should notice that digit measurements as well as tracing process of 20 radiographs were done by two investigators, increasing the precision of our results, although measurements errors cannot be avoided. Additionally, we used morphometric methods in order to capture the craniofacial shape and overcome the inherent problems of conventional cephalometric measurements; 15 continuous curves described the anatomic structures of the craniofacial complex instead of conventional landmarks. There is no study in the literature to correlate digit ratio with craniofacial complex using morphometrics.

Conclusions

- Digit ratio was sexually dimorphic at both pre- and post-pubertal time points (T1 and T2).
- Craniofacial shape was similar between sexes at T1 (pre-puberty) and sexual dimorphism arose only after puberty (T2).
- No correlation between digit ratio and shape of the craniofacial complex was detected for either sex, at either time point.

Acknowledgements

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Tables

Table 1.Descriptive statistics.

	Males (n=36)		Females (n=34)	
Age	initial age (years)	final age (years)	initial age (years)	final age (years)
Mean	10,03	18,12	10,01	17,08
SD	0,07	0,15	0,24	0,22
Range	9,8 to 10,3	17,9-18,5	9-10,9	16-17,4

Table 2. Hand measurements - ratio sum.

ratio sum	average	SD	range
males 10	0,923	0,021	0,88-0,97
males 18	0,924	0,021	0,87-0,95
females 10	0,938	0,019	0,90-0,97
females 17	0,939	0,020	0,89-0,96

Table 3. Hand measurements - ratio direct.

ratio direct	average	SD	range
males 10	0,923	0,021	0,87-0,97
males 18	0,924	0,021	0,86-0,95
females 10	0,938	0.019	0,90-0,97
females 17	0,939	0,020	0,89-0,97

Table 4. Comparison between ratio sum and ratio direct: paired t-tests.

	Difference:ratio direct- ratio sum				
	Average	SD	t	P	df
Males T1	0,0005	0,0022	-1,4711	0,1502	35
MalesT2	0,0003	0,0016	-1,2645	0,2144	35
Females T1	-0,0004	0,0015	1,5309	0,1353	33
Females T2	0,0003	0,0016	-1,2223	0,2302	33

Table 5. Comparison of ratio sum between T1 and T2: paired t- tests.

	Average	SD	Range	t	P	df
Males	0,0009	0,0146	-0,02- 0,03	-0,4002	0,6914	35
Females	0,001	0,009	-0,02- 0,01	-0,8905	0,3796	33

Table 6. Comparison of average digit ratio sum between males and females, independent t-test:

Average ratio sum	Average	SD	Range	t	P	df
Males	0,9240	0,020	0,881-0,967	-3,1116	0,0027	68
Females	0,9389	0,019	0,900-0,971			

Table 7. Variance described by the first 11 principal components of facial shape.

	%variance	%cumulative variance
PC 1:	27,5%	27,5%
PC 2:	15,6%	43,0%
PC 3:	7,6%	50,7%
PC 4:	6,4%	57,1%
PC 5:	4,9%	62,0%
PC 6:	4,0%	66,0%
PC 7:	3,3%	69,3%
PC 8:	3,0%	72,3%
PC 9:	2,8%	75,0%
PC 10:	2,4%	77,5%
PC 11:	1,9%	79,4%

Figures

Figure 1.

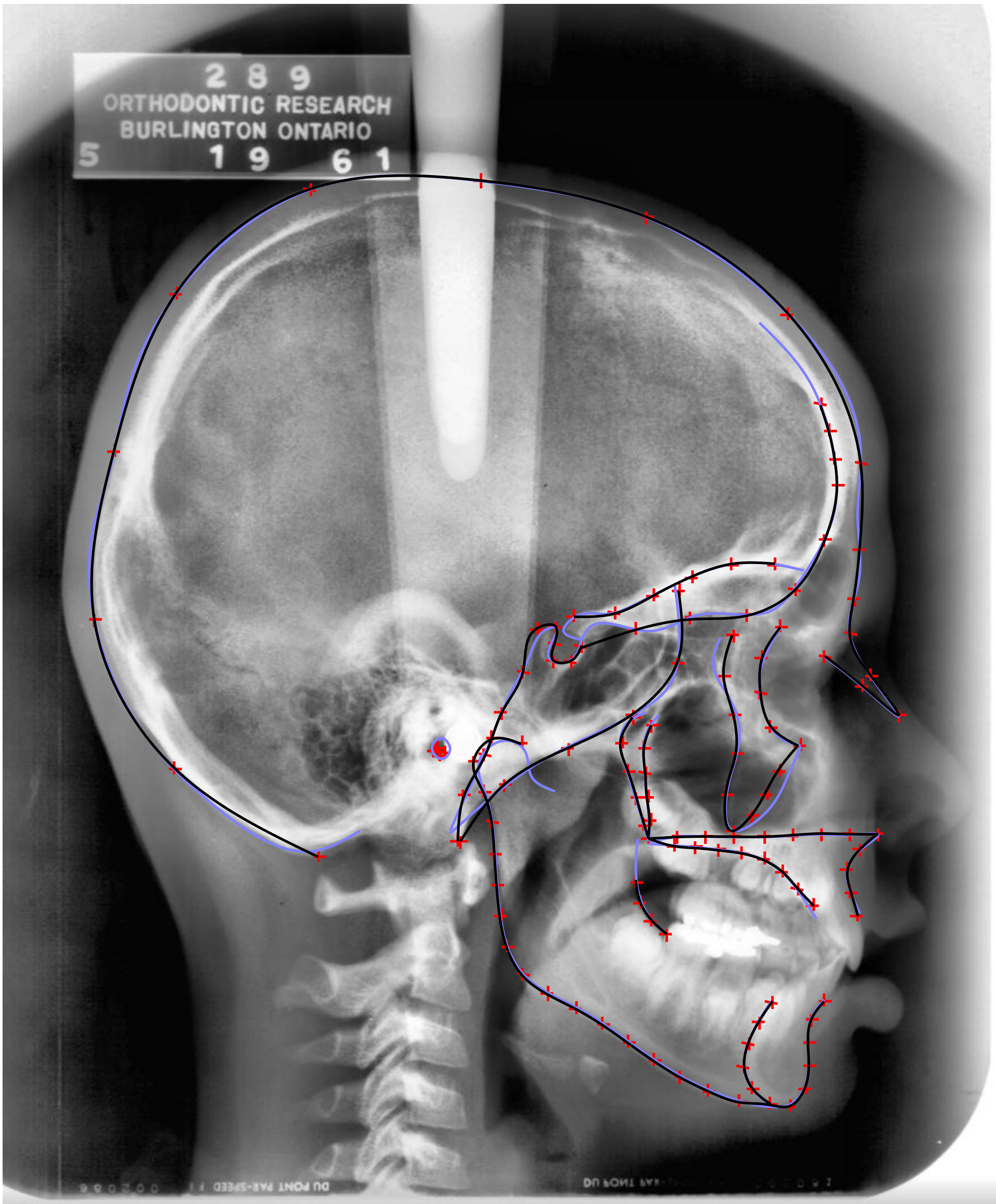

Figure 2.DL: direct length, D2P3: length of distal phalanx, D2P2: length of medial phalanx, D2P1: length of proximal phalanx.

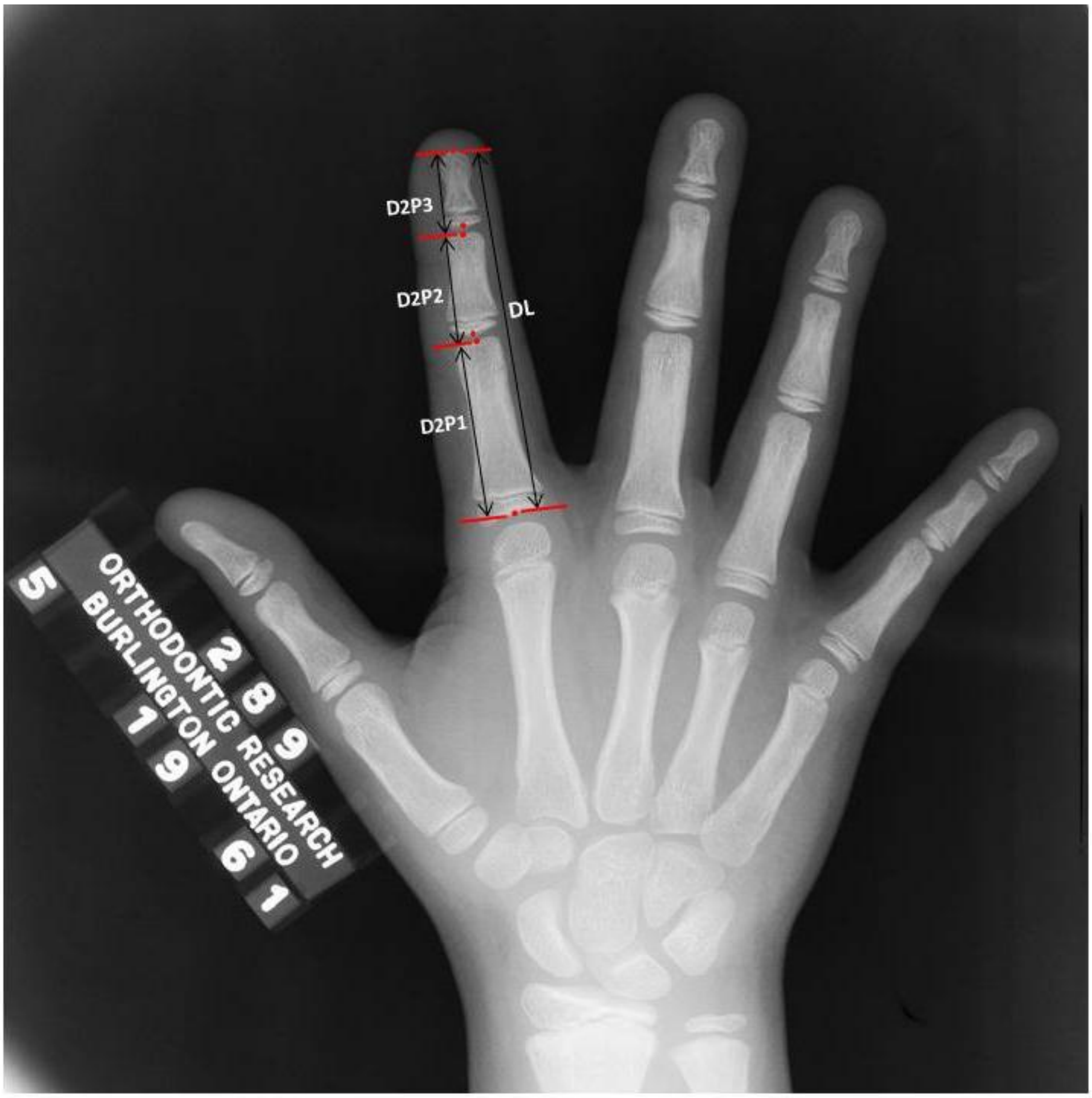

Figure 3.Close-up view of Figure 2.

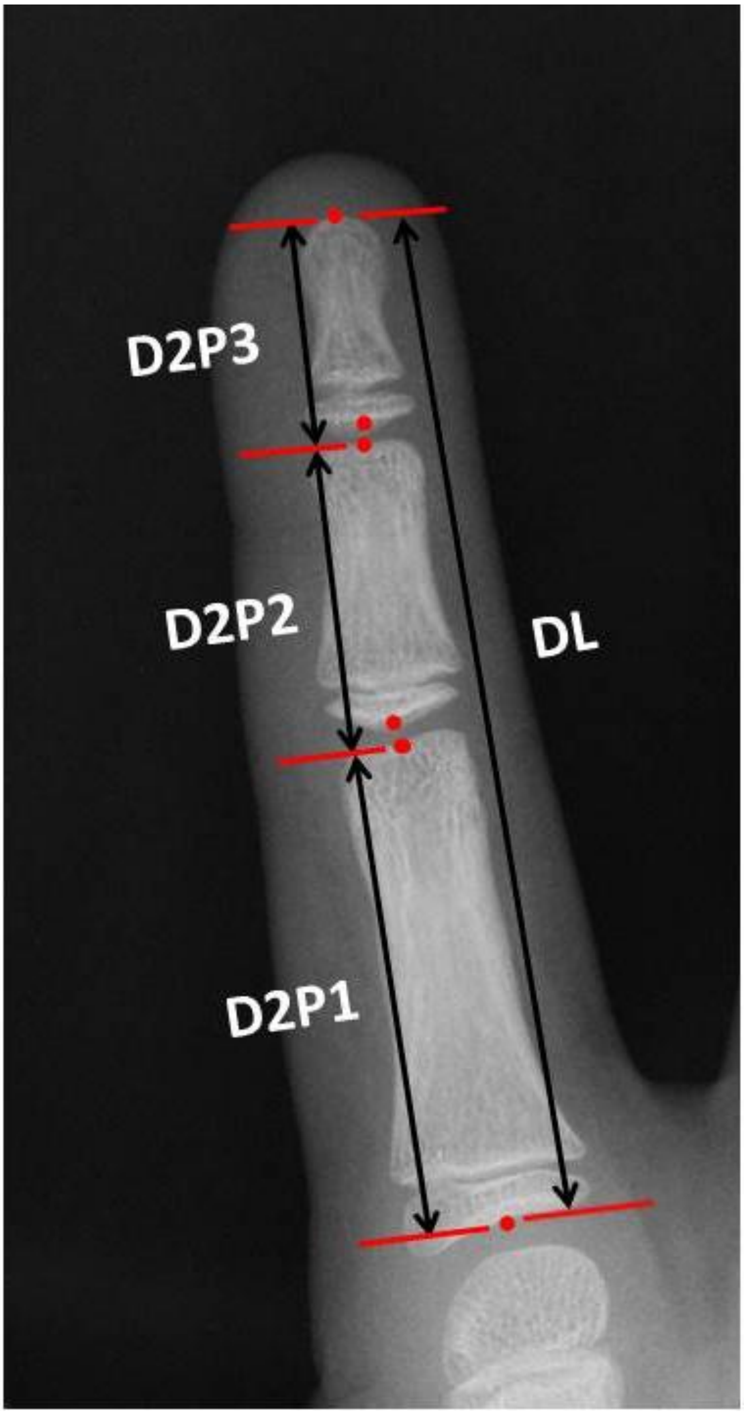

Figure 4.Digit ratio in males between T1 and T2.

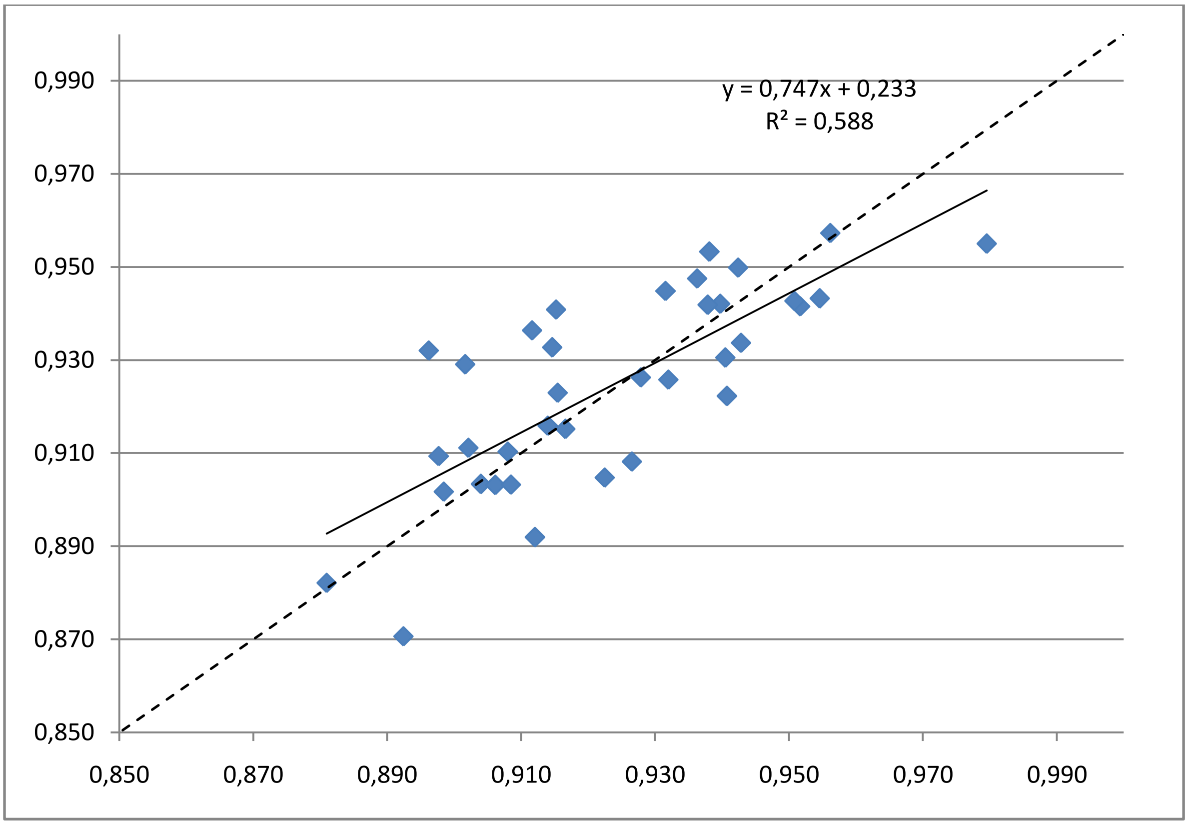

Figure 5.Digit ratio in females between T1 and T2.

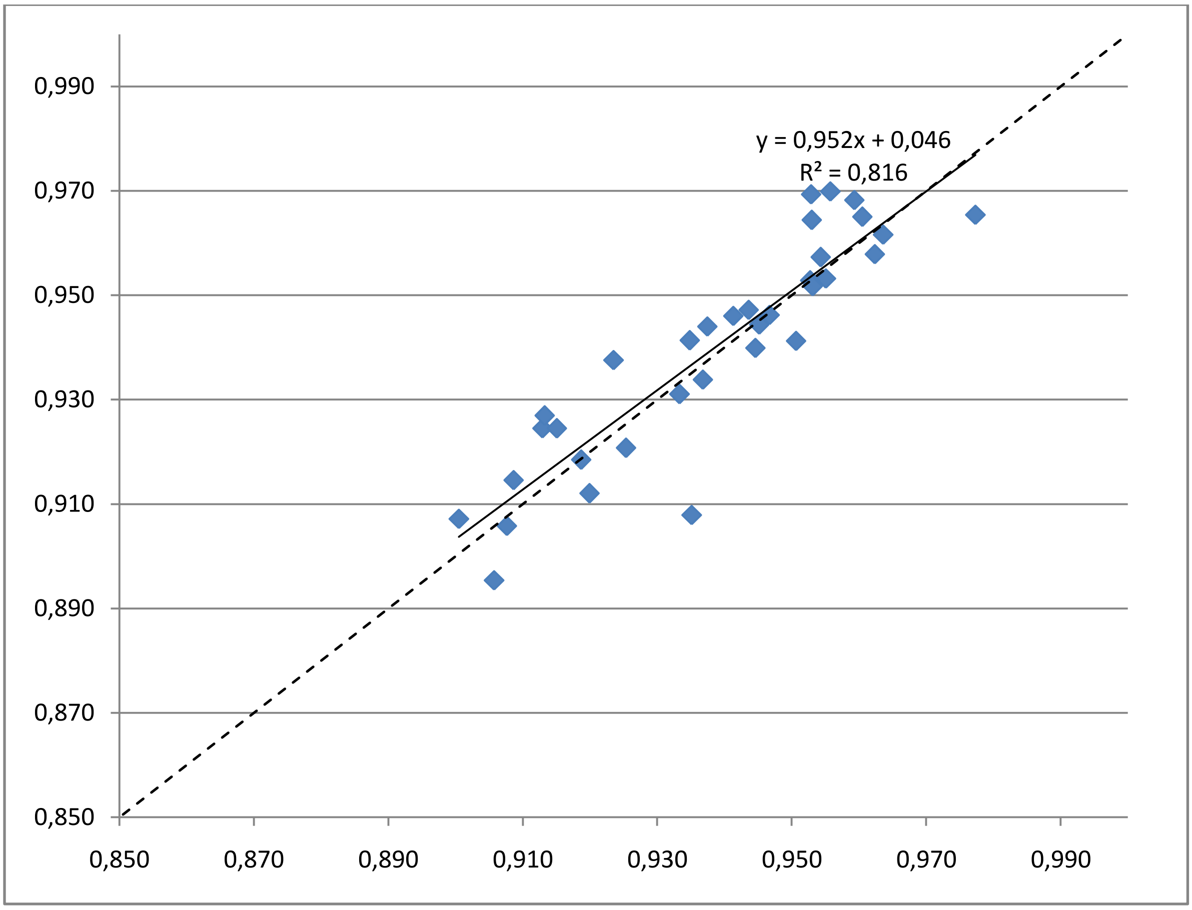

Figure 6.Average craniofacial shape-females.

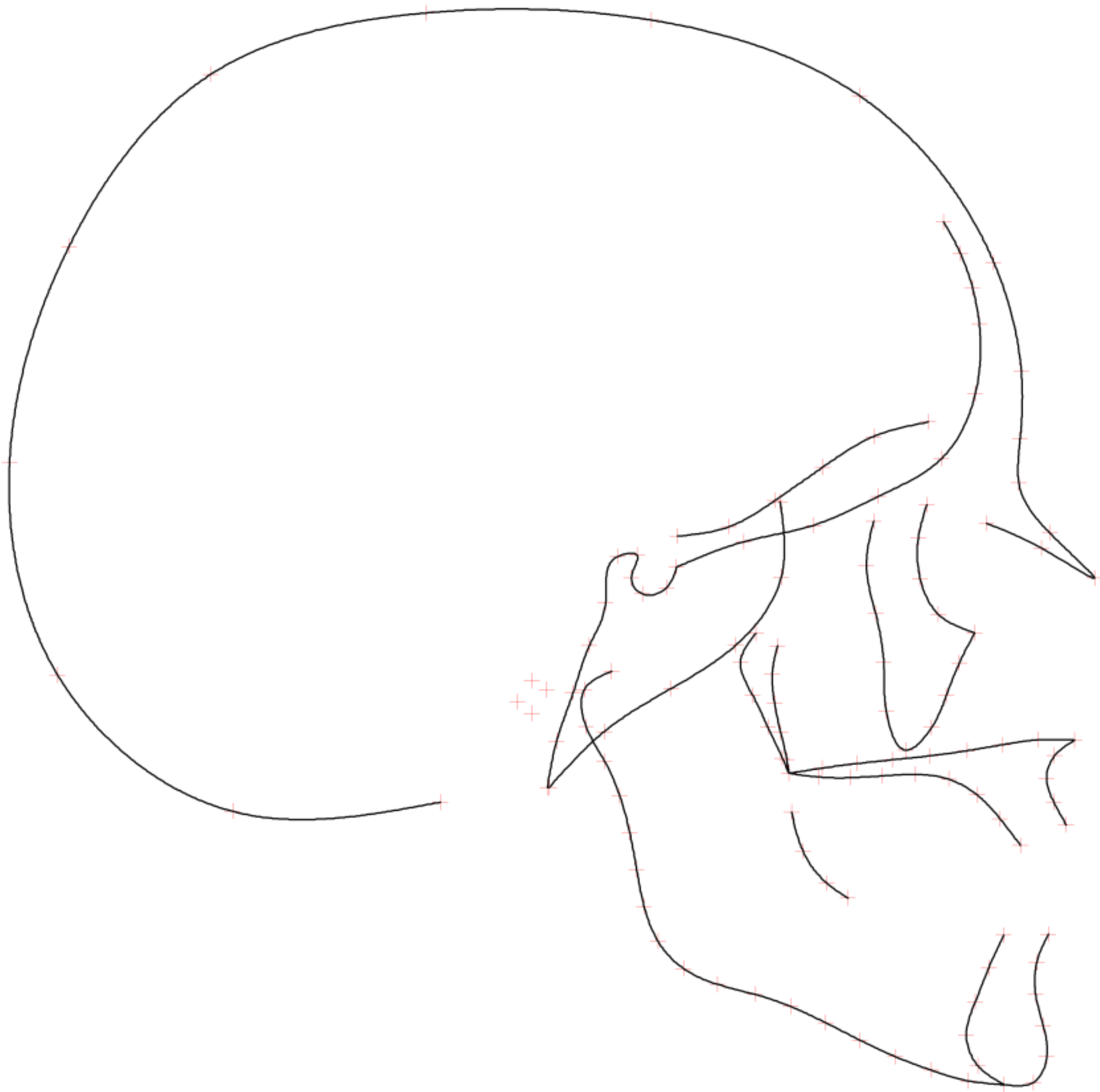

Figure 7.Average craniofacial shape- males.

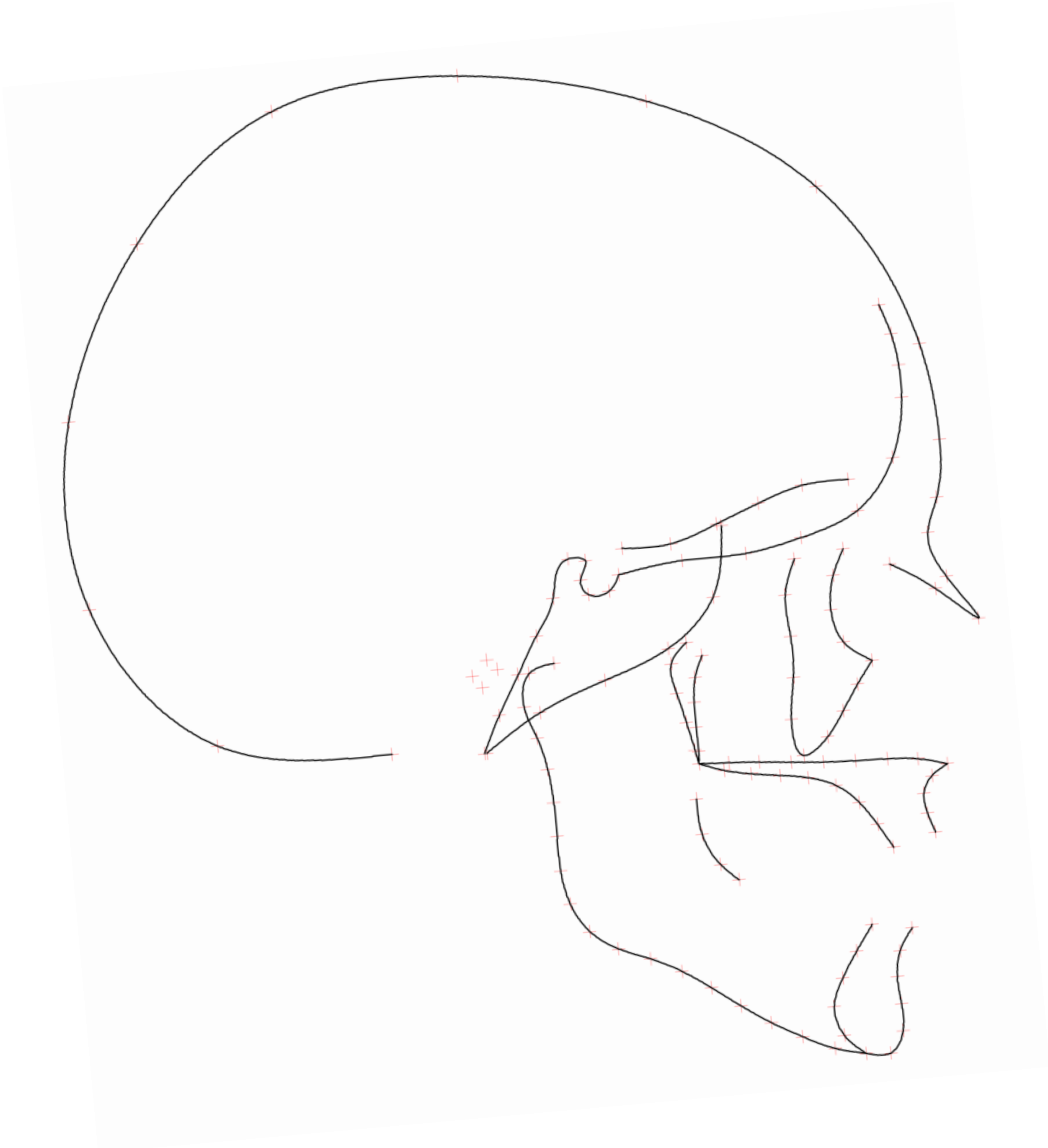

Figure 8. Procrustes superimposition of females T1 (red) and T2 (blue).

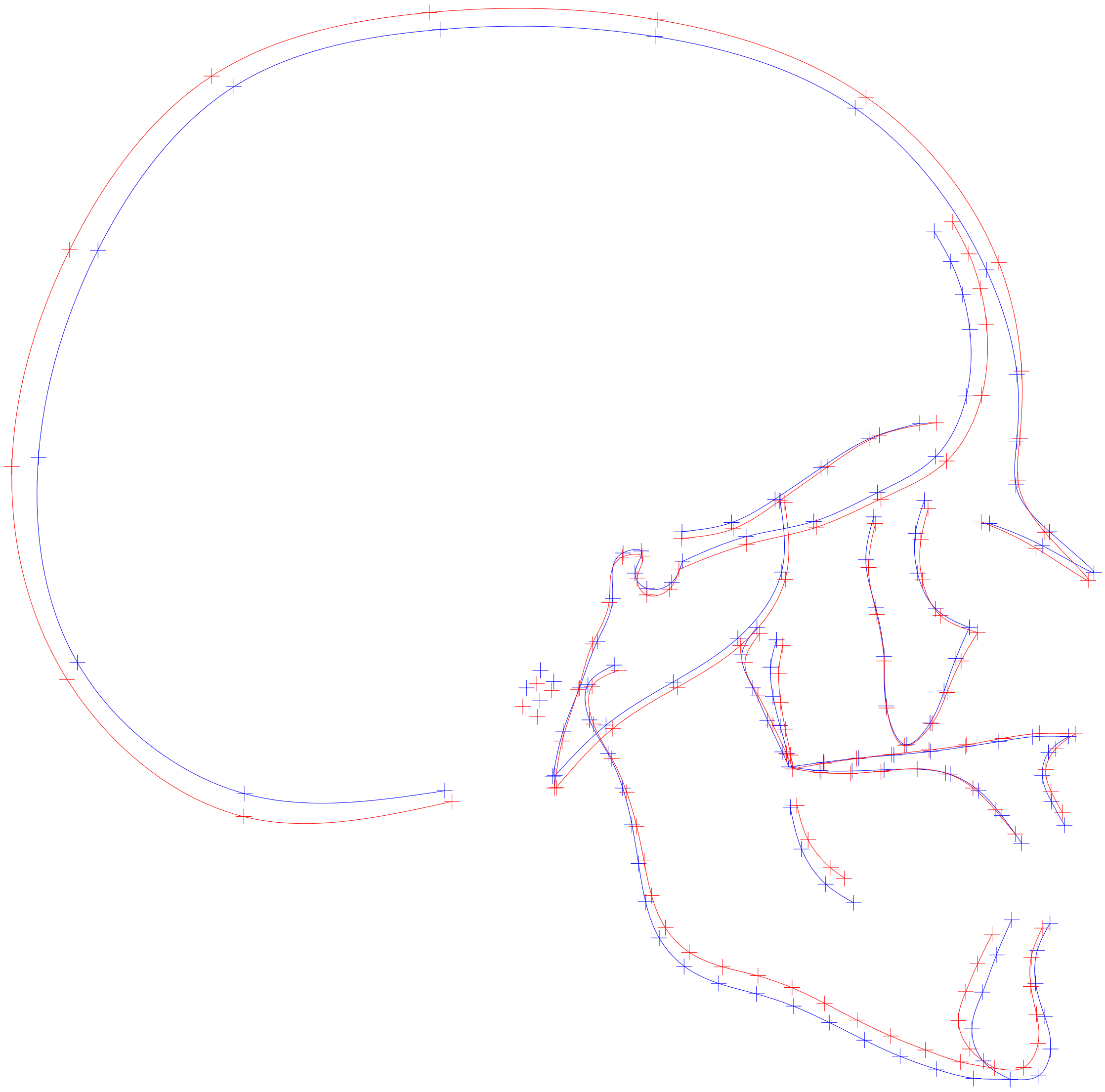

Figure 9.Procrustes superimposition of males T1 (red) and T2 (blue).

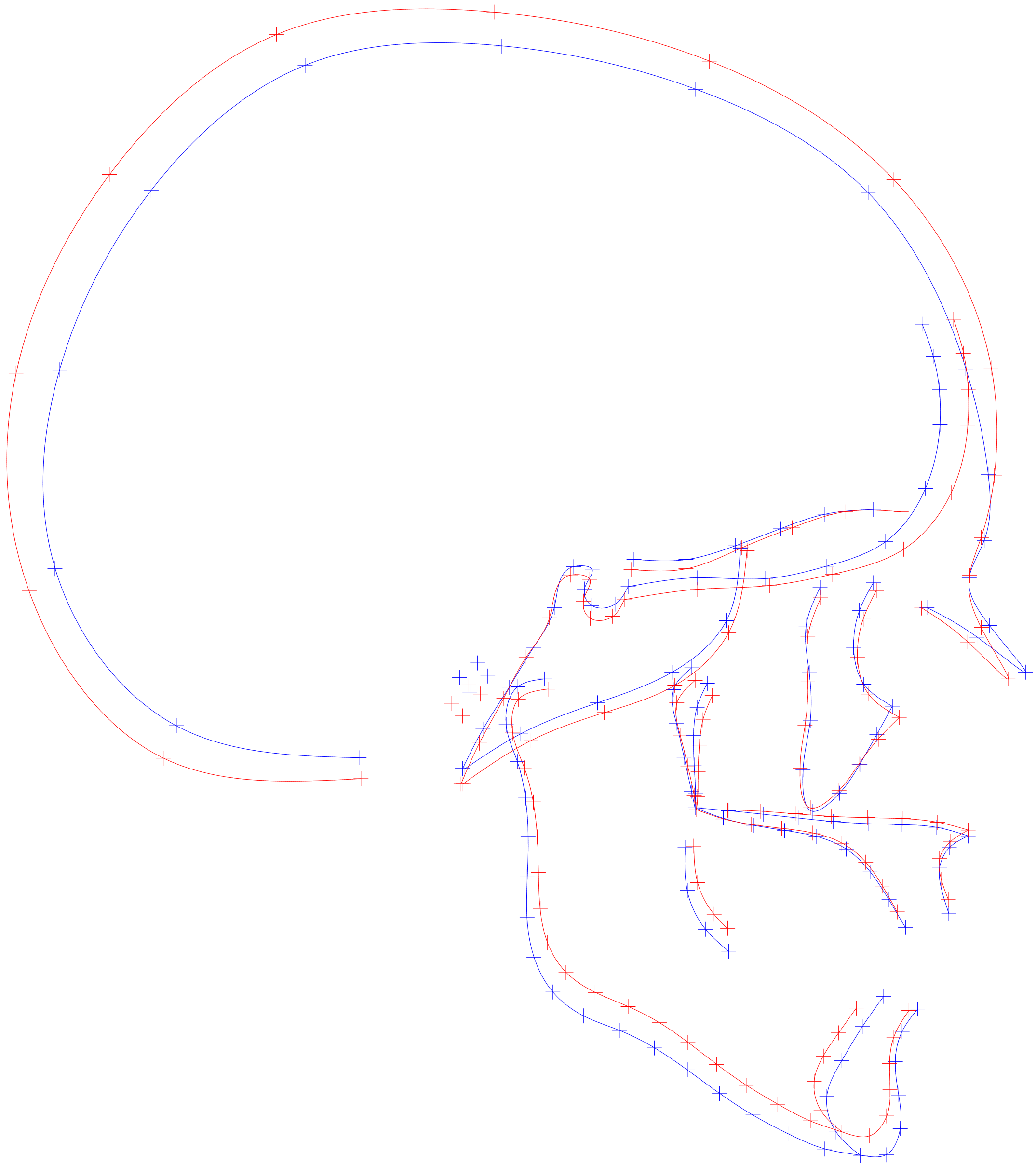

Figure 10.Procrustes superimposition of females (red) and males (blue) at T1.

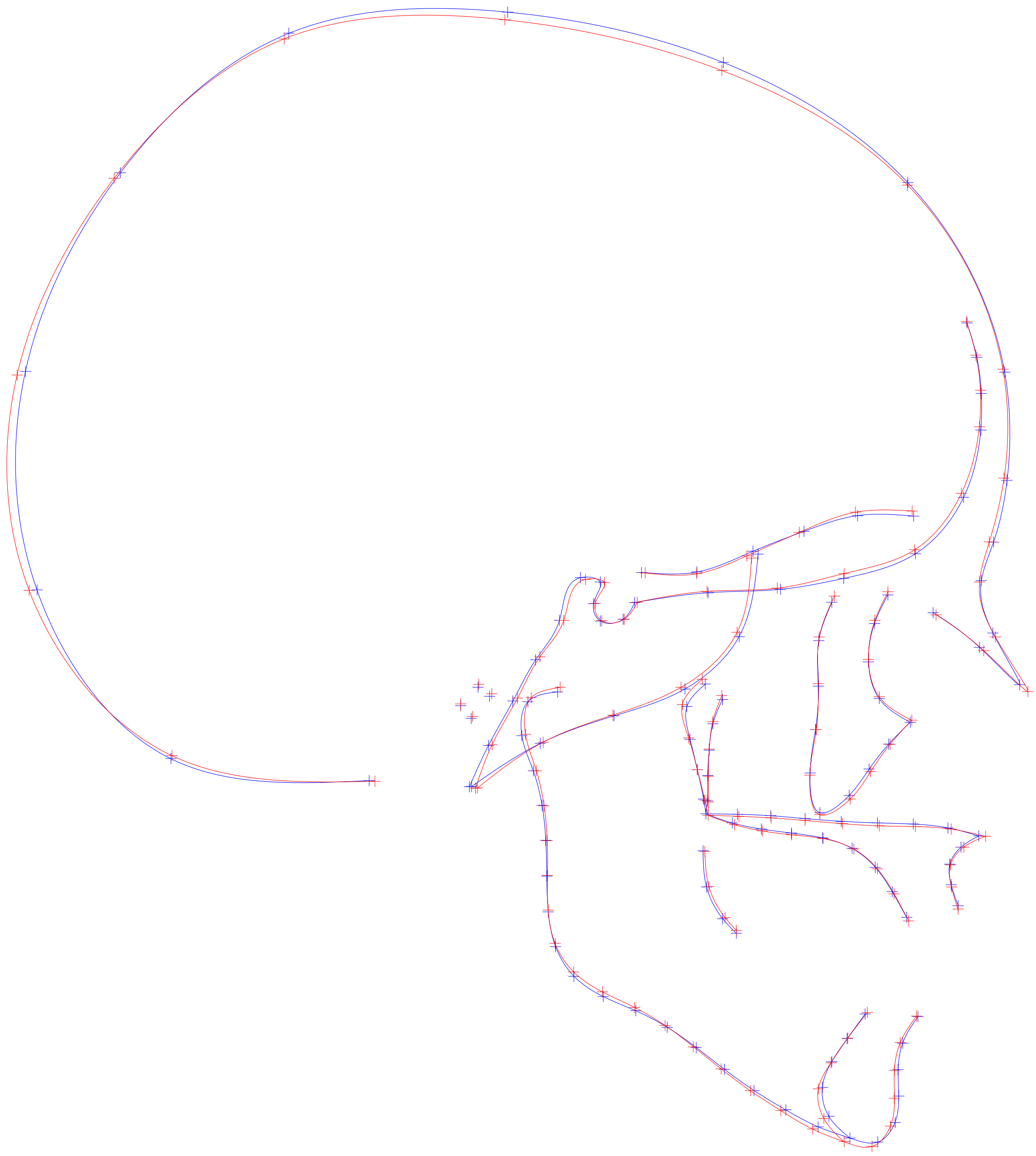

Figure 11.Procrustes superimposition of females (red) and males (blue) at T2.