Analysis the craniofacial morphometrics of mice: determination the deformations caused by Down Syndrome
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To cite this version:
Xueke Bai. Analysis the craniofacial morphometrics of mice: determination the deformations caused by Down Syndrome. Methodology [stat.ME]. 2014. <dumas-01059882>

HAL Id: dumas-01059882
https://dumas.ccsd.cnrs.fr/dumas-01059882
Submitted on 2 Sep 2014

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Analysis the Craniofacial Morphometrics of Mice

Determination the Deformations Caused by Down Syndrome

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Acknowledgments

First, I would like express my special thanks of gratitude to my supervisor Dr. Yann Hérault for giving me the opportunity to accept me in his team and help me a lot patiently in statistics and biology. It is a great honor to work with such a intelligent people.

I would like to thank all my colleagues for help me in explication biology and dataset. It is nice to meet these friendly people. I also would like to thank my best friend Jing Hou, without your help I wouldn’t be able to make it.

I would like to thank my tutor Dr. Nicolas Poulin, for his kindness, for his advice to help me finish my final report.

Thanks once again.
Abstract

Down Syndrome is the most common chromosome abnormality in humans [11]. It is associated with mental retardation or intellectual disabilities and manifestations of variable severity (e.g., heart anomalies, reduced growth, shortened life-span). Craniofacial dysmorphology and dental anomalies are consistently observed in all people with Down syndrome [16]. Mouse models are useful for studying the effects of Down syndrome.

Scientists use mouse models of Down Syndrome to determine the relationship between genotype and phenotype. Because mice display a number of phenotypes that are directly comparable to those in humans with trisomy 21. With the help of new technology, such as X-ray, Topography, μCT we get the three-dimensional images are available to make the evaluation of morphometric changes more accurate.

Exploring the craniofacial changes in Down Syndrome mouse models could be done to study how the genetic change the skull and mandibular so that we can know about Down Syndrome people. We used a Down Syndrome mouse models zoo, because different trisomic models stand for a region of human chromosome 21. We separated every genotype of the mice into two groups: control group and mutant group. In each group, there are about 10 mice. We locate 39 points in every mouse for the analysis of the skull and 22 points for mandibular. The target of my internship was to determine the difference between those two groups and also the difference deformation area.

In my report, I use the skull as example (39 points) and put the mandibular (22 points) in the Appendix A.
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1 Introduction

1.1 Host organization presentation

Institute of Genetics and Molecular and Cellular Biology (IGBMC) is one of the most important figures in biomedical research. There are two infrastructures in IGBMC: Centre of Integrative Biology and Mouse Clinical Institute(MCI). I did my internship at MCI which is a mouse research infrastructure for translational research and functional genomics. Founded in 2002 by Pierre Chambon, operated by Inserm, CNRS and the University of Strasbourg and supervised by GIE-CERBM, it provides a comprehensive set of specialized services to academic and industrial users and is a major role in the European post-genomics era programs. ICS consists in 3 departments: Genetic Engineering, Mouse Supporting and Phenotyping which generate and characterize more than 200 genetically modified mice per year. The services of the ICS will ultimately help the scientific community to use the mouse to develop a complete functional annotation of the human genome and to employ this to better understand human diseases and their underlying physiological and pathological basis.

During my internship, I worked on one ongoing project about Down Syndrome in Dr.Yann Héral’s team at IGBMC. Our team use mice as model to observe the relationship between genotype and phenotype. My internship supervisor Dr.Yann Héral, is one of the Sisley-Jérôme Lejeune International Awards winners and publish article in Nature for his research about Down Syndrome.

1.2 Subject of Internship

My internship was a part of the Down Syndrome project, I tried to find some statistical methods in order to determine the deformation caused by this disease. First of all, it was the deformation between the mutant and control group, secondly, using the new dataset in order to determine the confidence interval and then compared the deformation.
1.3 The Internship Plan

First, I learned how the laboratory got the dataset from mice, what the project was and tried to understand the method Euclidean Distance Matrix Analysis (EDMA) which was introduced by Lele and Richtsmeier in order to achieve the first goal: analysis the difference between two groups.

Then applying this method on software $R$ to get a new dataset. I used this new dataset to analyze the deformation of mouse, I could distinguish the regions of human chromosome 21 which causes the deformation (more explanation in Paragraph 2 Mouse Model).

During my internship, I use R version 3.0.2 "Frisbee Sailing".
2 Some Biological Definitions

Phenotype

A phenotype is the composite of an organism’s observable or measurable characteristics or traits, such as its morphology, development, biochemical or physiological properties, phenology, behavior, and products of behavior (such as a bird’s nest) [3].

In my report, the phenotype implies the morphometrics of mice, the distance between every Landmark.

Genotype

The genotype of an organism is the inherited instructions it carries within its genetic code [8].

A phenotype results from the expression of an organism’s genes as well as the influence of environmental factors and the interactions between the two [8]. The phenotype of the typical form of a species as it occurs in nature is called wild type (shorted as "wt"). We consider a control group consists of wild type, and a mutant group consists of genetic modified mice.

In the laboratory, researchers control the environmental factors and use the different genetic mouse model to find which genetic model alter the phenotype of mice.

Down Syndrome

Down Syndrome is named after John Langdon Down, the British doctor who fully described the syndrome in 1866. It is the most common chromosome abnormality and the most common disease causes intellectual disabilities in humans, occurring in about 1 per 1000 babies born in each year [4].

Human cells have 23 pairs of chromosomes (22 pairs of autosomes and one pair of sex chromosomes), people normally have two copies of each chromosome, giving a total of 46 per cell. Chromosome 21 is one of the 23 pairs of chromosome in humans, when there is a genetic disorder caused by the presence of all or a third copy of this chromosome, rather than the usual two [12].
People with Down syndrome may have some or all of the following physical characteristics: a small chin, slanted eyes, poor muscle tone, a flat nasal bridge, a single crease of the palm, and a protruding tongue due to a small mouth and large tongue. Other common features include: a flat and wide face, a short neck, excessive joint flexibility, extra space between big toe and second toe, abnormal patterns on the fingertips and short fingers [5].

**Mouse Model**

There are several reasons for using mouse models, among many advantages the most important is their striking similarity to humans in anatomy, physiology, and genetics. Over 95% of the mouse genome is similar to our own, making mouse genetic research particularly applicable to human disease. Many of the affected biological systems can not be directly tested in humans, but mouse models can help us do. Besides, mice are small, have a short generation time and an accelerated lifespan (one mouse year equals about 30 human years), keeping the costs, space, and time required to perform research manageable [9].

Genetically engineered mouse models are useful for elucidating the effects of gene-dosage imbalance on development and contribute to therapies that ameliorate the effects of trisomy 21 [16]. In the early 1990s, the generation of a genetic mouse model for Down Syndrome by Muriel Davison provided the basis for demonstrating that trisomy for the same genes has some closely
related structural and functional outcomes in mouse and human [13]. The mouse orthologs of genes on human chromosome 21 are found on mouse chromosome 10, chromosome 16 and chromosome 17. A number of Down Syndrome mouse models have (for instance Ts1Cje, Ts3Yah, Ts65Dn, . . .) segmental trisomy for portions of these chromosomes that have conserved synteny with human chromosome 21 [16].

Figure 2: Comparison of human chromosome 21 with mouse models

Figure 2 shows the different genetic mouse stand for different regions of human chromosome 21. If we observe a deformation on mouse model Ts65Dn, we know this genetic region of human influence the form of carnioc-facial.

**Euclidean distance**

In Cartesian coordinates, if \( p = (p_1, p_2, \ldots, p_n) \) and \( q = (q_1, q_2, \ldots, q_n) \) are two points in Euclidean \( n \)-space, then the distance from \( p \) to \( q \), or from \( q \) to \( p \) is given by:

\[
d(p, q) = d(q, p) = \sqrt{(q_1 - p_1)^2 + (q_2 - p_2)^2 + \cdots + (q_n - p_n)^2}
\]

\[
= \sqrt{\sum_{i=1}^{n} (q_i - p_i)^2}
\]
Here is the three-dimensional Euclidean space:

\[ d(p, q) = \sqrt{(q_1 - p_1)^2 + (q_2 - p_2)^2 + (q_3 - p_3)^2} \]

**Landmark**

In many biological investigations, the most effective way to analyze the forms of whole biological organs or organisms is by recording geometric location of *landmark points*. These are loci that have names (“bridge of the nose”, ”tips of the chin”) as well as Cartesian coordinates. The names are intended to imply true homology (biological correspondence) from form to form. That is, landmark points not only have their own locations but also have the ”same” locations in every other form of the study. The most basic requirement of a landmark is that it can be easily identified and located with accuracy and precision [10] [14].

![Figure 3: The 39 Landmarks of mouse skull](image)
Figure 3 shows the landmarks of mouse skull that is studied by laboratory. The researchers put Landmark to the same place of every mouse. Some of these Landmarks were chosen symmetrically.
3 Data Collection

After we scan a mouse, we have the 3D image with .ply format. A .ply file consists of a header followed by a list of vertices and a list of polygons. The header specifies how many vertices and polygons are in the file, and the (x,y,z) coordinates of each vertex. The polygon faces are simply lists of indices into the vertex list, and each face begins with a count of the number of elements in each list.

The software LandmarkEditor which was developed by IDAV( Institute for Data Analysis and Visualization) and the University of California, Davis, helps us manipulate the .ply file.

![Landmark](Image)

Figure 4: The software for capture three-dimensional coordinate of landmarks

Loading a 3D mouse image, then placing landmark as Figure 3, finally we have the three-dimensional coordinate of these landmarks(Figure 5). In this file, we can see these 39 landmarks’ X, Y and Z axis values.

Then gathering all mice of one model in one file, as Figure 6 showing, we put the 39 landmarks’ information of all the mice who have the genotype $Ts2Yah - Ts65Dn$ – double in a .txt file, and considering it as a mutant group. We have a similar file who gathered the wild type of genotype $Ts2Yah - Ts65Dn$ and considering it as a control group. I started analysis from these two files.
Figure 5: The output of software LandmarkEditor

Figure 6: The final result for the genotype $Ts2Yah - Ts65Dn$
4 Methods

We have the basic knowledge to start the analysis of morphometrics of mice. Now I will introduce the method Euclidean Distance Matrix Analysis (EDMA) which is shown by Lele and Richtsmeier [17]. There are some related concepts and a testing procedure for shape differences.

After archiving landmark locations of a three dimensional form, let $X_i$ be this matrix of landmark coordinates with 39 rows and 3 columns (Figure 5): the $i^{th}$ row consists of the 3 coordinates of the $i^{th}$ landmark. We can calculate Euclidean distances between all possible pairs of landmarks, let $F(X_i)$ denote the form matrix corresponding to the object with landmark coordinates matrix $X_i$ and $d(i,j)$ the Euclidean distance between two points $i,j$, $i,j=1,2,...,39$. $F(X_i)$ is a symmetric distance matrix of dimension 39*39 with the form

$$F(X_i) = \begin{pmatrix}
    d(1,1) & d(1,2) & \ldots & d(1,j) & \ldots & d(1,39) \\
    d(2,1) & d(2,2) & \ldots & d(2,j) & \ldots & d(2,39) \\
    \vdots & \vdots & \ddots & \vdots & \ldots & \vdots \\
    d(i,1) & d(i,2) & \ldots & d(i,j) & \ldots & d(i,39) \\
    \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\
    d(39,1) & d(39,2) & \ldots & d(39,j) & \ldots & d(39,39)
\end{pmatrix}$$

We assume that $X$ is some matrix valued random variable. The random variable $X$ consists of the independent $X_1,X_2,...,X_n$.

$$X = (X_1,X_2,...,X_n)$$
We define equality of form and equality of shape in terms of the matrix valued random variables $X$ and $Y$, where $Y$ is a matrix of landmark coordinates for form $Y = (Y_1, Y_2, \ldots, Y_m)$. We use "random variable" as shortened "form of matrix valued random variable".

**Definition 1.** Two random variables $X$ and $Y$ are said to have the same form if after proper rotation and translation $X$ and $Y$ are identically distributed. That is:

$$X \overset{d}{=} YP + 1_k t^T$$

for some orthogonal matrix $P$ and a vector $t$. By identically distributed we mean that the probability distribution functions for $X$ and $Y$ are the same, although particular observations could and would be different.

**Definition 2.** Two random variables $X$ and $Y$ are said to have the same shape if after proper translation, rotation and scaling $X$ and $Y$ are identically distributed. That is

$$X \overset{d}{=} bYP + 1_k t^T$$

for some scalar $b > 0$, $P$ and $t$ as above. The corresponding definitions in terms of the form matrix are:

**Definition 3.** Two random variables $X$ and $Y$ are said to have the same shape if

$$F(X) \overset{d}{=} cF(Y)$$

for some scalar $c > 0$. If $c=1$, then they have the same form.
In practice it is difficult to test hypotheses of two distributions \((X \equiv Y)\), especially for matrix valued random variables in reason of the sparing data. We give simplified versions of equality of form and shape below in terms of mean form and mean shape. Let \(E(\cdot)\) denote the expectation operator. For example, \(E(X)\) denotes the average of the random variable \(X\), or the average form representing a sample of forms.

**Definition 4.** We say that random matrices \(X\) and \(Y\) are equal in mean form if and only if
\[
E(X) = E(Y)P + 1_k t^T
\]
for some orthogonal matrix \(P\) and a vector \(t\), i.e., after translation and rotation the means of \(X\) and \(Y\) are equal.

**Definition 5.** We say that random matrices \(X\) and \(Y\) are equal in mean shape if and only if
\[
E(X) = bE(Y)P + 1_k t^T
\]
for some scalar \(b > 0\), \(P\) and \(t\) as above, i.e., after translation, rotation, and scaling, the means of \(X\) and \(Y\) are equal.

Here we are dealing with form matrices \(F(X_i)\) and \(F(Y_i)\) which are invariant under rotation and translation and are therefore identically distributed.

We now give the same definitions in terms of form matrices.

**Definition 6.** Given two random variables \(X\) and \(Y\) we say that they are equal in mean shape if and only if
\[
F[E(X)] = cF[E(Y)]
\]
for some scalar \(c > 0\). By this we mean that two mean forms have the same shape if one form is a scaled version of the other. If \(c=1\) then they are equal in mean shape.

To examine the differences between two average forms we use a matrix of ratios of corresponding linear distances measured on \(X\) and \(Y\). We call this matrix the average form difference matrix.

**Definition 7.** Given two random variables \(X\) and \(Y\), we define the average form difference matrix by
\[
D[E(X), E(Y)] = \frac{F_{ij}[E(X)]}{F_{ij}[E(Y)]}
\]
where \(j > i, i=1,2,\ldots,39\)
when this matrix is a matrix of 1s, we say that the two random variables are equal in mean form.
4.1 Testing for Equality of Average Shapes

Suppose there are two populations whose shapes we want to compare. Let $X_1, X_2, \ldots, X_n$ be a random sample of forms from Population $X$ and $Y_1, Y_2, \ldots, Y_m$ be a random sample from Population $Y$. The null hypothesis is that the average shapes of the two populations are equal, which can be expressed using Definition 6, as follows:

$$H_0 : F[E(X)] = cF[E(Y)]$$ for some $c > 0$

$$H_1 : F[E(X)] \neq cF[E(Y)]$$ for some $c > 0$

A natural way to test this hypothesis would be to estimate $F[E(X)]$ and $F[E(Y)]$ from the data, calculate the estimate of average form difference matrix $D[E(X), E(Y)]$ using these estimates, and then test whether or not this matrix is "almost" a matrix of constants or not.

4.2 Estimating the Form Difference Matrix

We will try to find the estimating $F[E(X)]$ and $F[E(Y)]$. The most natural way to estimate $F[E(X)]$ would be to estimate the average coordinates of $X, E(X)$, and then calculate its form matrix. We use generalized procrustes analysis (GPA) \[2\]. Given $X_1, X_2, \ldots, X_n$ we apply GPA to get $\bar{X}$. This $\bar{X}$ is a consistent estimator of $E(X)$. Similarly one can estimate $E(Y)$ by $\bar{Y}$. Here $\bar{X}$ and $\bar{Y}$ are coordinewise averages of $X_1, X_2, \ldots, X_n$ and $Y_1, Y_2, \ldots, Y_m$. $F[E(X)]$ and $F[E(Y)]$ can be estimated by using $F(\bar{X})$ and $F(\bar{Y})$.

$$F_{ij}(\bar{X}) = \frac{\sum_{k=1}^{n} F_{ij}(X_k)}{n}$$ (9)

where $j > i, i=1,2,\ldots,39$

$F(\bar{X})$ is a symmetry matrix of dimension $39 \times 39$.

According to Definition 6, the Form Difference Matrix (FDM) is obtained by:

$$FDM = \frac{F(\bar{X})}{F(\bar{Y})}$$ (10)
4.3 Bootstrap Procedure

In the following we introduce a bootstrap procedure for estimating the null distribution of the test statistic $T$. This is based on the permutation test procedure coupled with Bootstrap methodology to reduce the computational burden.

For one genotype, let $X_1, X_2, \ldots, X_n$ be the sample of mutant group and $Y_1, Y_2, \ldots, Y_m$ be the control group.

**Step 1** Select $X_i^*, i=1,2,\ldots,n$ from $X$ and $Y_i^*, i=1,2,\ldots,m$ from $Y$ randomly and with replacement. We have two new samples $X^* = (X_1^*, X_2^*, \ldots, X_n^*)$, $Y^* = (Y_1^*, Y_2^*, \ldots, Y_m^*)$.

**Step 2** Use the formula (9) to find the average form $F(\bar{X}^*)$ and $F(\bar{Y}^*)$ of $X^*$ and $Y^*$ respectively.

**Step 3** Calculate $T^*$ by form difference matrix between $F(\bar{X}^*)$ and $F(\bar{Y}^*)$,

$$T^* = \frac{F(\bar{X}^*)}{F(\bar{Y}^*)}$$

the division of the two average form.

**Step 4** Repeat Steps 1-3 $B$ times. A histogram of $T_j^*, j=1,2,\ldots,B$ estimates the null distribution of $T$, when $H_0$ is true.

4.4 Testing Procedure

If the observed value of $T$, i.e., the value calculated with original sample $X$ and $Y$ is in the extreme right-hand tail of the null distribution, we reject $H_0$ at the appropriate level of significance and consider $X$ and $Y$ have difference form.

Let us see a study introduced by Subhash LeLe [17] of morphological differences between normal children and those affected with a disease.

When repeat 100 times the bootstrap procedure we have a distribution of $T$ for the comparison of normal boys and those affected with the disease (Figure 7). $T_{obs}$ is equal to 1.58 and 10% of the bootstrapped $T_s$ exceed $T_{obs}$.
4.4 Testing Procedure

In my case we do not want to know the morphometrics of all landmarks but every landmark, so instead of the histogram of $T$, we plot another graph. Once obtained $T^*_i, i = 1, 2, \ldots, B$, calculating $E(T^*)$ the average form difference matrix of $T^*_i, i = 1, 2, \ldots, B$. $E(T^*)$ is a symmetry matrix of dimension $39 \times 39$.

$$E_{ij}(T^*) = \frac{1}{B} \sum_{k=1}^{B} T^*_k(i, j)$$

where $j > i, i = 1, 2, \ldots, 39$ (11)

Because we consider every column of matrix $E(T^*)$ as an indication of a landmark, drawing up all points $(j, E_{ij}(T^*))$ for $i, j = 1, 2, \ldots, 39$, there are 38 values (there is a 0 for the reason the distance between the point itself is 0) for the landmark $j$. In this graph, we can see how these 38 values scatter around axis $y = 1$. We also compare the distribution among landmarks.

$$
\begin{pmatrix}
1 & 2 & \ldots & 39 \\
0 & E_{1,2}(T^*) & \ldots & E_{1,39}(T^*) \\
E_{2,1}(T^*) & 0 & \ldots & E_{2,39}(T^*) \\
\vdots & \vdots & \ddots & \vdots \\
E_{39,1}(T^*) & E_{39,2}(T^*) & \ldots & 0
\end{pmatrix}
$$

In order to observe which landmarks have more serious deformation, we are trying to define a confidence interval then compare how many values are
out of the confidence interval. The more values there are, the more serious deformation observed.

4.5 Confidence Interval

Let us find the confidence interval by using control groups of all genotypes. Supposing in total $p$ different genotypes $Y_1, Y_2, \ldots, Y_p$ constitute a set of Control denoted $\mathcal{Y}$, $\mathcal{Y}=(Y_1, Y_2, \ldots, Y_p)$. We define the genotype $i$ which consisting of $m_i$ observations $Y_{i1}, Y_{i2}, \ldots, Y_{im_i}$, $Y_i=(Y_{i1}, Y_{i2}, \ldots, Y_{im_i})$.

We define a confidence interval of all kinds of genotypes in the way below:

Step 1 Pick one of $Y_{ij}$ for $i=1,2,\ldots, p$, $j=1,2,\ldots,m_i$ and treat the Euclidean Matrix $F(Y_{ij})$ as a reference.

Step 2 Select $Y_{k*l}$, for $l=1,2,\ldots,m_k$ from $Y_k$, where $k \neq i$ randomly and with replacement, calculate $F(Y_{k*})$ and divide the matrix reference,

$$V^* = \frac{F(Y_{k*})}{F(Y_{ij})}$$

repeat this procedure for $B$ times, determine the average matrix of $V_{q}^*$, $q=1,2,\ldots,B$.

Step 3 Apply Step 2 to all $Y_{k*}$ for $k \neq i,k=1,2,\ldots,p$. We obtain a set of matrix when matrix $Y_{ij}$ is a reference. Find the average of these values, note $a_{ij}$.

Step 4 Apply Step 1-3 to all observed mice, and obtain a group of $a_{ij}$, for $i=1,2,\ldots,p$, $j=1,2,\ldots,m_i$.

Step 5 Observe the distribution of the $a_{ij}$ for $i=1,2,\ldots,p$, $j=1,2,\ldots,m_i$, then find the 95% interval confidence of all mice.

Another confidence interval is defined for a genotype, it’s similar as we do above in the part Bootstrap Procedure, mixed permutation test and Bootstrap methodology:

In $\mathcal{Y}=(Y_1,Y_2,\ldots,Y_p)$, we want to calculate the confidence interval of group genotype $Y_i$, where $i=1,2,\ldots,p$.

Step 1 Choose one of control groups $Y_j$, for $j \neq i$, $j=1,2,\ldots,p$ different from $Y_i$. 

20
Step 2 Select $Y_{ik}^*$, $k=1,2,\ldots,m_i$ from $Y_i$, and $Y_{jl}^*$, $l=1,2,\ldots,m_j$ from $Y_j$ for $j \neq i$ randomly and with replacement.

Step 3 Use the formula (9) to find the average form $F(\bar{Y}_i^*)$ and $F(\bar{Y}_j^*)$ of $Y_i^*$ and $Y_j^*$.

Step 4 Calculate $U^*$ with the form difference matrix,

$$U^* = \frac{F(\bar{Y}_j^*)}{F(\bar{Y}_i^*)}$$

the division of the two average form.

Step 5 Repeat Steps 1-4 $B$ times. We can obtain a set of $U_q^*$, $q=1,2,\ldots,B$, calculating $E(U^*)$ the average matrix of $U_q^*$, $q=1,2,\ldots,B$.

Step 6 Apply Step 1-5 for all the rest of control groups, collect all these values together and find the 95% interval confidence of them.

Here we apply average of all values instead of matrix, because when we divide one matrix by another, we standardize the distance between two landmarks, then all values are ratio around 1 with the same unit.

### 4.6 Student’s $t$-test

#### 4.6.1 One-Sample $t$-test

**Assumptions** $X_1, X_2, \ldots, X_n$ are from sample $X \sim N(\mu, \sigma^2)$, $\mu$ and $\sigma^2$ are unknown.

**Hypothesis**

$$H_0 : \mu = \mu_0$$

$$H_1 : \mu \neq \mu_0$$

**Test Statistic**

$$t_{\text{obs}} = \frac{\bar{X} - \mu_0}{S \sqrt{n}} \sim t_{n-1}$$

where $\bar{X} = \frac{1}{n} \sum_{i=1}^{n} X_i$, $S = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (X_i - \bar{X})^2}$

**Critical Region**

If $|t_{\text{obs}}| > t_{\alpha/2,n-1}$, we reject $H_0$, hence $\mu \neq \mu_0$
4.6.2 Two-sample $t$-test

Assumptions

The two samples $X_1, X_2, \ldots, X_n$ and $Y_1, Y_2, \ldots, Y_n$ have the same variance.

Hypothesis

$$H_0 : \mu_1 = \mu_2$$
$$H_1 : \mu_1 \neq \mu_2$$

Test Statistic

$$t_{\text{obs}} = \frac{(\bar{X} - \bar{Y})}{S_w \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \sim t_{n_1+n_2-2}$$

where $S_w^2 = \frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2}$, $\bar{X} = \frac{1}{n_1} \sum_{i=1}^{n_1} X_i$, $\bar{Y} = \frac{1}{n_2} \sum_{i=1}^{n_2} Y_i$ stand for the mean of two samples, $s_1 = \sqrt{\frac{1}{n_1-1} \sum_{i=1}^{n_1} (X_i - \bar{X})^2}$, $s_2 = \sqrt{\frac{1}{n_2-1} \sum_{i=1}^{n_2} (Y_i - \bar{Y})^2}$ are the standard deviation of two samples.

Critical Region

If $|t_{\text{obs}}| > t_{\alpha/2, n_1+n_2-2}$, we reject $H_0$, hence, the two samples have different means.

4.7 Correlation and Simple Linear Regression

The purpose of correlation analysis is exploring the relationships between variables. Two commonly coefficients, the Pearson correlation coefficient and the Spearman for measuring linear and nonlinear relationship respectively [15].

If we have a series of $n$ measurement of $X$ and $Y$ written as $X_i$ and $Y_i$ where $i = 1, 2, \ldots, n$, then the Pearson correlation coefficient $r$ between $X$ and $Y$ is:
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\[ r = \frac{\sum_{i=1}^{n} (X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{n} (X_i - \bar{X})^2 \sum_{i=1}^{n} (Y_i - \bar{Y})^2}} \]

where \( \bar{X} \) and \( \bar{Y} \) are the sample average of the \( X \) and \( Y \) respectively.

Both correlation coefficients have values between -1 and +1, ranging from being negatively correlated (-1) to uncorrelated (0) to positively correlated (+1). The sign of the correlation coefficient (positive or negative) defines the direction of the relationship. The absolute value indicates the strength of the correlation.

The purpose of simple regression analysis is to evaluate the relative impact of a predictor variable on a particular outcome [15].

A simple regression model contains only one independent variable \( X_i \), for \( i = 1, 2, \ldots, n \) subjects, and is linear with respect to both the regression parameters and the dependent variable. The model is:

\[ Y_i = a + bX_i + e_i \]

where the regression parameter \( a \) is the intercept, and the regression parameter \( b \) is the slope of the regression line. The random error term \( e_i \) is assumed to be uncorrelated, with a mean of 0 and constant variance.

It is meaningful to introduce the value of the Pearson correlation coefficient \( r \) by squaring it; It is the term \( R \)-square \( (R^2) \) or coefficient of determination. This measure is the fraction of the variability in \( Y \) that can be explained by the variability in \( X \) though their linear relationship.

\[ R^2 = \frac{SS_{regression}}{SS_{total}} = \frac{\sum_{i=1}^{n} (Y_i - \bar{Y})^2}{\sum_{i=1}^{n} (f_i - \bar{Y})^2} \]

where \( SS \) stands for the sum of squares, \( f_i = a + bX_i \), and \( \bar{Y} \) is the average of the observed data.

4.8 Effect Size (Cohen’s d)

We do the test by calculating a \( p \) value, which indicates the probability of the null hypothesis being correct. This probability goes down as the size
of the effect goes up and as the size of the sample goes up. However, given a sufficiently large sample size, a statistical comparison will always show a significant difference. So here is the idea of effect size.

The most common effect-size measure, as the correlation/regression coefficients $r$ (As we talk about before) is actually measures of effect size. The coefficient $r$ covers the whole range of relationship strengths, from no relationship whatsoever (zero) to a perfect relationship (1, or -1), it is telling us exactly how large the relationship really is between the variables we study and is independent of the size.

As we want do a $t$-test to compare two means, another common measure of effect size is $d$, sometimes known as Cohen’s $d$. This is simply the difference in the two groups’ means divided by the average of their standard deviations. $X_1, X_2, \ldots, X_{n_1}$ and $Y_1, Y_2, \ldots, Y_{n_2}$ are two groups:

$$d = \frac{\bar{X} - \bar{Y}}{s}$$  \hspace{1cm} (12)

where $s = \sqrt{\frac{(n_1-1)s^2_X + (n_2-1)s^2_Y}{n_1+n_2-2}}$ and $s_X = \frac{1}{n_1-1} \sum_{i=1}^{n_1} (X_i - \bar{X})^2$

If $n_1=n_2=n$

$$d = \frac{\bar{X} - \bar{Y}}{\sqrt{(s^2_X + s^2_Y)/2}}$$  \hspace{1cm} (13)

![Figure 8: The indication of cohen’s $d$](image)

If we see a $d$ of 0.5, we know that the two groups’ means differ by half a
standard deviation; a $d$ of 1 tells us that the two groups’ means differ by one standard deviation and so on.

### 4.9 Software R

R was created by Ross Ihaka and Robert Gentleman at the University of Auckland, New Zealand. Because of the two ”R” fathers first name begins with R, so it is called R \cite{7}. R is free, but it works as well as the other software, it provides a series of statistical analyses and drawing tools, now it widely used by statisticians, engineers and scientists and become one of the mainstream statistical software.
5 Result

5.1 Result of EDMA

We took the mouse model \(Ts2Yah - Ts65Dn\) as an example. \(Ts2Yah - Ts65Dn\) is a mouse model, \(Ts2Yah - Ts65Dn\) is one kind of its mutant group. Two groups are compared in this study: a mutant group(\(Ts2Yah - Ts65Dn\) – double) with sample size \(n\) and a control group of size \(m\). We followed the Bootstrap Procedure, repeating 500 times to resample the mutant and control groups. We calculated the means of pairwise distance between landmarks for each group, which allowed the generation of the form difference matrix between the two groups(Paragraph 4.3).

As described in Paragraph 4.4, we calculated the mean of these 500 form difference matrices (because I resampled for 500 times)by formula (11). This procedure allowed us to generate, a symmetry matrix of dimension 39 \(\times\) 39 (Figure 9), which is essential to analyze the morphometrics.

![Figure 9: The form difference matrix of the mouse model \(Ts2Yah - Ts65Dn\) – double](image-url)

To obtain useful information from this matrix, we plot it as described in Paragraph 4.4. In fact, values in every column as represent the average distance from a given landmark relative to every other landmark studied. Every column in the matrix is represented as scattered points, as an indication of the spatial arrangements of any given landmark. For axis \(x = i\), we can see the distance relationship from Landmark \(i\) for \(i = 1, 2, \ldots, 39\) to all the other
landmarks. In this way, the effect in every landmark can be observed.

![Graph showing the difference matrix of the mouse model Ts2Yah – Ts65Dn – double](image)

**Figure 10:** The graph form difference matrix of the mouse model *Ts2Yah – Ts65Dn – double*

In this graph (Figure 10), we can see clearly how these 39 landmarks scatter around horizontal axis $Y = 1$. The value inferior to 1 indicates the mutant mice are smaller on average than the control mice, otherwise, a value superior than 1 indicates the mutant mice has a larger form.

Using this method, we average the spatial position of each landmark in relation to all the others. Deviation of values from 1 indicates a potential shift of the relative position of the landmark.

To determine the confidence interval, we used a combined dataset from control groups of different models of mice (Paragraph 4.5). As the overall shape of one model differs from another, we incorporated the variance models to the determination of the confidence interval, which allows for eliminating the variations due to the mice model (false positive) from the deformation caused by genotypic mutations (true positive). The Table 1 shows the control groups and their number of mouse.

As Table 2 showing, we took the mouse 3940wt *Ts5Yah* as a reference,
### 5.1 Result of EDMA

<table>
<thead>
<tr>
<th>mouse model</th>
<th>number of mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ts5Yah</td>
<td>10</td>
</tr>
<tr>
<td>2 Ts3Yah</td>
<td>12</td>
</tr>
<tr>
<td>3 Ts2Yah</td>
<td>10</td>
</tr>
<tr>
<td>4 Ts2Yah-Ts65Dn</td>
<td>8</td>
</tr>
<tr>
<td>5 Ts2Yah-Ts1Cje</td>
<td>10</td>
</tr>
<tr>
<td>6 Ts1Yah-Ts65Dn</td>
<td>14</td>
</tr>
<tr>
<td>7 Ts1Yah-Ts65Dn-F</td>
<td>7</td>
</tr>
<tr>
<td>8 Ts1Yah-Ts65Dn-M</td>
<td>7</td>
</tr>
<tr>
<td>9 Ts1Rhr</td>
<td>10</td>
</tr>
<tr>
<td>10 Tc1-Ms4Yah</td>
<td>10</td>
</tr>
<tr>
<td>11 Ms5Yah</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 1: Every control group’s number of mouse

#### Ts5Yah

<table>
<thead>
<tr>
<th>mouse</th>
<th>3940wt</th>
<th>3906wt</th>
<th>3900wt</th>
<th>3935wt</th>
<th>3902wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>1.0182</td>
<td>1.0133</td>
<td>1.0141</td>
<td>1.0145</td>
<td>1.0043</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>mouse</th>
<th>3936wt</th>
<th>3917wt</th>
<th>3918wt</th>
<th>3895wt</th>
<th>3946wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>1.0330</td>
<td>1.0329</td>
<td>1.0271</td>
<td>1.0210</td>
<td>1.0114</td>
</tr>
</tbody>
</table>

#### Ts3Yah

<table>
<thead>
<tr>
<th>mouse</th>
<th>27wt</th>
<th>72wt</th>
<th>73wt</th>
<th>74wt</th>
<th>79wt</th>
<th>80wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>0.9643</td>
<td>0.9729</td>
<td>0.9610</td>
<td>0.9617</td>
<td>0.9441</td>
<td>0.9620</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>mouse</th>
<th>82wt</th>
<th>89wt</th>
<th>94wt</th>
<th>103wt</th>
<th>110wt</th>
<th>111wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>0.9590</td>
<td>0.9752</td>
<td>0.9532</td>
<td>0.9709</td>
<td>0.9653</td>
<td>0.9555</td>
</tr>
</tbody>
</table>

#### Ts2Yah

<table>
<thead>
<tr>
<th>mouse</th>
<th>87799wt</th>
<th>87800wt</th>
<th>87801wt</th>
<th>...</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>1.0195</td>
<td>1.0191</td>
<td>1.0463</td>
<td>...</td>
</tr>
</tbody>
</table>

Table 2: The average of every mouse defined by form difference matrix
Using this dataset, we first resampled the group $Ts3Yah$, then the average distance matrix of group $Ts3Yah$ dividing the distance matrix of 3930wt, repeating this procedure for hundreds of times and obtaining a average form difference matrix; next resampling $Ts2Yah$ dividing the distance matrix of 3930wt. After applying to all mouse models, calculating the average of all these values, we got 1.0182 (Table 2) when 3940wt as reference. We restarted to take 3906wt and then 3900wt of genotype $Ts5Yah$ until the last mouse of the last genotype. We do combinations between one mouse and all the other mouse models, so that we get all the possible values among the mouse models. Finally the list of average could help us find the final confidence interval $[0.95,1.06]$. Figure 12 red line shows this interval’s location of $Ts2Yah - Ts65Dn - double$.

There is another example shown in Appendix A(Figure 22), there are seldom values are located out of this interval, the comparison of these two genotype show that the genetic region of human chromosome 21 which $Ts2Yah - Ts65Dn - double$ stand for will effect deformation.

For any given column in the form difference matrix, values which are not in the interval indicate the degree of deformation around this landmark. There are many values out of this interval, we use the number of values which is not in the interval to see how serious the deformation caused by this landmark(Figure 12, the purple numbers below indicate how many values are beyond the confidence interval ). For example the Landmark 9, 38 values are out of confidence interval, au contrary, Landmark 3 has only 16. So the deformation in Landmark 9 is serious than 3.

We took control group of $Ts2Yah - Ts65Dn$ as an example to see how we define the confidence interval for it.

Resampling the control group $Ts2Yah - Ts65Dn$ and calculating the average matrix; then finding another control group for instance $Ts5Yah$, resampling and calculating its average matrix; the form difference matrix is defined by these two matrices. Repeating this procedure in order to determine the average of form difference matrix, applying all these steps to the rest of mouse model $Ts2Yah$, $Ts3Yah$ and so on. At last we pick 95% values among them.

By excluding the impact of the models, we could observe the real effect of a mutation genotype on the deformation. We calculated the confidence
5.1 Result of EDMA

<table>
<thead>
<tr>
<th>mouse model</th>
<th>Ts5Yah</th>
<th>Ts3Yah</th>
<th>Ts2Yah</th>
<th>Ts2Yah-Ts65Dn</th>
<th>Ts2Yah-Ts1Cje</th>
<th>Ts1Yah-Ts65Dn</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI(2.5%)</td>
<td>0.9131</td>
<td>0.8648</td>
<td>0.9528</td>
<td>0.9356</td>
<td>0.9349</td>
<td>0.9007</td>
</tr>
<tr>
<td>CI(97.5%)</td>
<td>1.1299</td>
<td>1.0550</td>
<td>1.1410</td>
<td>1.1003</td>
<td>1.1131</td>
<td>1.0932</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>mouse model</th>
<th>Ts1Yah-Ts65Dn-F</th>
<th>Ts1Yah-Ts65Dn-M</th>
<th>Ts1Rhr</th>
<th>Tc1-Ms4Yah</th>
<th>Ms5Yah</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI(2.5%)</td>
<td>0.9102</td>
<td>0.8913</td>
<td>0.9396</td>
<td>0.9062</td>
<td>0.8767</td>
</tr>
<tr>
<td>CI(97.5%)</td>
<td>1.1063</td>
<td>1.0870</td>
<td>1.1358</td>
<td>1.10722</td>
<td>1.0499</td>
</tr>
</tbody>
</table>

Table 3: The confidence intervals of every control group

Figure 11: The bar chart of every control group correspondent Table 3

Figure 12: The confidence interval of all control group (red line) and $Ts2Yah - Ts65Dn$ (black line)
5.2 Result of Correlation and Simple Linear Regression

Table 4: The correlation coefficient of the symmetry right and left landmarks

<table>
<thead>
<tr>
<th>landmark</th>
<th>8,10</th>
<th>9,17</th>
<th>10,18</th>
<th>11,19</th>
<th>12,20</th>
<th>13,21</th>
<th>23,31</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3878</td>
<td>0.3955</td>
<td>0.5487</td>
<td>0.2887</td>
<td>0.7667</td>
<td>0.7658</td>
<td>0.5553</td>
</tr>
<tr>
<td>landmark</td>
<td>24,32</td>
<td>25,33</td>
<td>26,34</td>
<td>27,35</td>
<td>28,36</td>
<td>29,37</td>
<td>30,38</td>
</tr>
<tr>
<td></td>
<td>0.7973</td>
<td>0.5117</td>
<td>0.6537</td>
<td>0.6666</td>
<td>0.7647</td>
<td>0.7456</td>
<td>0.6825</td>
</tr>
</tbody>
</table>

intervals for different mouse models (Table 3) and their variation (Figure 11). Here we take example of a control group: $[0.9356, 1.1003]$ is the confidence interval for the model $Ts2Yah - Ts65Dn$ (Figure 12 blue line).

5.2 Result of Correlation and Simple Linear Regression

To examine the deformation symmetry between right and left sides. I used correlation coefficients between landmarks that are located symmetrically (Figure 3).

The correlation coefficients were calculated between seven pairs of symmetrical landmark (Table 4).

Figure 13: The linear regression between Landmark 24 and 32
5.3 Result of t-test and Effect Size

Among these pairs, one pair (Landmark 24 and 32) has the highest correlation coefficient, indicating that the variations between these two landmarks are positively correlated (Figure 13).

5.3 Result of t-test and Effect Size

Using the average form different matrix in Figure 9 (still the sample Ts2YahTs65Dn), I performed a one sample t-test by comparing the control group and mutant group. The average equals to 1 indicates that there is no significant differences between the two groups.

Testing $H_0 : \mu = 1$ versus $H_1 : \mu \neq 1$

One sample t-test

date: Ts2Ts65Dn
t = -29.1765, df = 1520, p-value < 2.2e-16
alternative hypothesis: true mean is not equal to 1
95 percent confidence interval:
0.0.964244 0.9113978
sample estimates:
mean of x
0.00389209

Figure 14: The t-test result Ts2Yah − Ts65Dn

The result is summarized in Figure 14 $H_0$ is rejected, meaning that there is a difference between the control and mutant group.

Next I applied a two sample t-test to assess if the deformation caused by mutation is more significant than the deformation results from model variations. To find the confidence interval of Ts2Yah − Ts65Dn(Paragraph 4.5 and Paragraph 5.1), we resampled the control group of Ts2Yah − Ts65Dn, then divided its mean Euclidean matrix by another resampling control group, this was repeated for hundreds of times until a mean form matrix was generated. The procedure was applied to all the other control groups. By doing this, we obtained a mean form matrix for each control group, which allowed for the calculation of a final mean form matrix for all control groups, denoted $M_a$. To test if the mean of $M_a$ is equal to the mean form difference matrix of Ts2Yah − Ts65Dn—Double(Figure 9), I used the following:

Testing $H_0 : \mu_1 = \mu_2$ versus $H_1 : \mu_1 \neq \mu_2$
5.3 Result of t-test and Effect Size

Welch Two Sample t-test

data: Ts2Yah and Ts65Dn

t = -2.8173, df = 1870.084, p-value < 2.2e-16

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

-0.1178663 -0.1027029

sample estimates:

mean of x mean of y

0.9039108 1.0519295

Figure 15: The t-test result of Ts2Yah – Ts65Dn and the mean form difference matrix

As shown in Figure 15, the test is significant in level of 5%, which means the influence of the mutant group Ts2Yah – Ts65Dn is more significant than the variation observed between the mouse models. Nevertheless, as the dataset is relatively large, it is always possible to a false significant result. Using the formula (12) and (13), I obtained a value of 3.9763 for cohen’s $d$, indicating that the two matrix’s means differ by almost four standard deviations.

I also calculated the cohen’s $d$ of these two matrices by column in order to observed how these landmarks affect the deformation.

Figure 16: The 39 landmarks’ Effect Size result of Ts2Yah – Ts65Dn

Comparing Figure 12 with Figure 16, for cohen’s $d$, the Landmark 15 is large and many values surpass the confidence interval. By contrast, the Landmark 4 has less number of outlined values that are compact distributed below the interval. Therefore We can consider that with the genotype Ts2Yah – Ts65Dn – Double, the Landmark 15 showed a more serious deformation than Landmark 4.
6 Discussion

Here we show how the Euclidean distance matrix-based approach for comparison of shapes suggested by Lele and also the comparison of two groups statistically. Then trying to identify those areas where the differences are prominent. For this method, if we can have more Landmarks, the more accurate result we will get. So here comes the problem to get more Landmarks’ information. I also did much research about .ply file so that the Landmark could be put automatically and we could put as many landmarks as we want. Unfortunately it is just can be done manually, because the data stock in .ply file is not matched, for instance, the vertex 1 in file 1 is not the same in file 2.

As mentioned before, the \( t \)-test could always get a signification result because the size of sample was large enough so we used Effect Size to study the deformation.

For Simple Linear Regression, there existed aberrant values (sometimes they appear in pairs, because the distance from Landmark \( i \) to \( j \) is equal to Landmark \( j \) to \( i \)), in this way, the relationship would be effected. So when we want to study the symmetry, we should combine the simple linear regression with the number of values which are out of confidence interval and how they distribute(Figure 13).
7 Conclusion

During my internship, I learned a new statistical method EDM A (Euclidean Distance Matrix Analysis) which is widely used in the field of biology, medicine for morphometrics. With this method, I could compare two groups’ shape. I determined the confidence interval for all control groups and for every control group in order to observe if the deformation caused by genotype is more serious than the difference caused by mouse model. Then we could find which genotype will lead to the deformation.

I strength my ability of programming on software $R$, all my project realized on it, in my laboratory, before we do the analysis in different software separately, and now with my new program, getting the new dataset and graphs become easier. Besides, when I tried to collect data from the image I learned a lot about how to read and write a file especially the format .ply with software $matlab$. 
References


Appendix A

First appendix

Figure 17: The 22 Landmarks of mouse mandibular

Using these 22 Landmarks we can explore the morphometric.

Figure 18: The form difference matrix of the mouse model (mandibular)

Ts2Yah – Ts65Dn – double

The form difference matrix for mandibular (Figure 18 similar to Figure 9) is a symmetry matrix of dimension 22 * 22. Then we plot this matrix by column.

Figure 19 (similar to Figure 12) is the graph corresponding the matrix in Figure 18, the all control groups’ confidence interval of mandibular is [0.93, 1.057]. There are still many values are out of the interval, that means this
A. FIRST APPENDIX

Figure 19: The graph form difference matrix of the mouse model (mandibular) $Ts2Yah - Ts65Dn - double$

genotype will cause serious deformations.

We can also define the confidence interval for every genotype, then we have the result showing in Table 5 and Figure 20.

If we plot the scatter diagram between two symmetry Landmark, we observe a linear relationship. Figure 21 is the Linear Regression corresponding the Table 4, we can see the relationship between the symmetry landmarks.

Figure 22 is an example showing that there are not always as many values out of the confidence interval as Figure 12. Here we take the confidence interval of all control group [0.95, 1.06] as before. So we can consider that the genotype $Ts2Yah$ has less effect than genotype $Ts5Yah-Ts65Dn-Double$ in phenotype (skull’s deformation) of Down Syndrome, the corresponding genetic region of human chromosome 21 effect not as much as $Ts5Yah-Ts65Dn-Double$. 

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A. FIRST APPENDIX

<table>
<thead>
<tr>
<th>mouse model</th>
<th>Ts5Yah</th>
<th>Ts3Yah</th>
<th>Ts2Yah</th>
<th>Ts2Yah-Ts65Dn</th>
<th>Ts2Yah-Ts1Cje</th>
<th>Ts1Yah-Ts65Dn</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI(2.5%)</td>
<td>0.8690</td>
<td>0.8372</td>
<td>0.9303</td>
<td>0.9610</td>
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<td>0.9384</td>
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<td>CI(97.5%)</td>
<td>1.1173</td>
<td>1.0604</td>
<td>1.1339</td>
<td>1.1599</td>
<td>1.1178</td>
<td>1.1395</td>
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</table>

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<thead>
<tr>
<th>mouse model</th>
<th>Ts1Yah-Ts65Dn-F</th>
<th>Ts1Yah-Ts65Dn-M</th>
<th>Ts1Yah-Ts1Cje</th>
<th>Ts1Rhr</th>
<th>Tc1-Ms4Yah</th>
<th>Ms5Yah</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI(2.5%)</td>
<td>0.9455</td>
<td>0.9230</td>
<td>0.8831</td>
<td>0.9225</td>
<td>0.7530</td>
<td>0.8797</td>
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<tr>
<td>CI(97.5%)</td>
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<td>1.1341</td>
<td>1.1125</td>
<td>1.1327</td>
<td>1.1270</td>
<td>1.0689</td>
</tr>
</tbody>
</table>

Table 5: The confidence intervals of every control group (mandibular)

Figure 20: The bar chart of every control group (mandibular) correspondent Table 5
A. FIRST APPENDIX

Figure 21: The symmetric Landmarks’ Simple Linear Regression of $Ts2Yah - Ts65Dn - Double$
Appendix B

Second appendix

Bootstrap Procedure

$X = (X_1, X_2, \ldots, X_n)$ is the sample from a population with distribution function $F(x)$, $\theta$ is the parameter we are interested in, $\hat{\theta} = \hat{\theta}(X_1, X_2, \ldots, X_n)$ is the estimator of $\theta$.

Supposing the distribution $F$ is unknown, $x = (x_1, x_2, \ldots, x_n)$ is the observations from the sample $X = (X_1, X_2, \ldots, X_n)$ of $F$, $F_n$ is the empirical distribution. When $n$ is large enough, according to Glivenko - Cantelli theorem [6], $F_n$ has an approximating distribution to $F_n$.

We pick $x^*_i$, $i=1,2,\ldots,n$ from $x$ randomly and with replacement. We have a new sample $x^*=(x^*_1, x^*_2,\ldots,x^*_n)$, then we can determine $\hat{\theta}^* = \hat{\theta}(x_1, x_2, \ldots, x_n)$.
We repeat this procedure B times and get $\hat{\theta}_i^*$, for $i = 1, 2, \ldots, B$, we can consider $\bar{\theta}^* = \frac{1}{B} \sum_{i=1}^{B} \hat{\theta}_i^*$ is a new estimator of $\hat{\theta}$

**The Simple Linear Regression**

Suppose there are $n$ data points $(X_i, Y_i), i = 1, 2, \ldots, n$. The model is:

$$Y_i = a + bX_i + e_i$$

we use linear least squares method to estimate the unknown parameters. This method minimizes the sum of squared vertical distances between the observed responses in the dataset and the responses predicted by the linear approximation. we are trying to find:

$$\min Q(a, b) \text{ for } Q(a, b) = \sum_{i=1}^{n} (Y_i - a - bX_i)^2$$

By using calculus, we can obtain the estimators of $a$ and $b$.

$$\hat{b} = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{\sum_{i=1}^{n}(X_i - X)^2}$$

$$\hat{a} = \bar{Y} - \hat{b}\bar{X}$$