

Ladd, Summer H., The Effect of Diet on Craniofacial Growth in Osteogenesis Imperfecta Mouse Model. Master of Science (Medical Sciences-Anatomy), May 2018

Osteogenesis imperfecta (OI, or “brittle bone disease”) is a rare disorder that is caused by genetic point mutations (COL1A1/COL1A2) that affect type 1 collagen. In OI type III (severe) patients, limb bones are more susceptible to skeletal fractures and the bones of the craniofacial region are underdeveloped. Some OI type III patients also suffer from dental malocclusions or fractures (dentinogenesis imperfecta, DI). The goals of this project are 1) to describe the facial phenotype in an OI mouse model, to see if this model can be used to test potential behavioral and pharmaceutical interventions; and 2) to determine if diet and masticatory loading affect the development of the craniofacial region in the OI model. The homozygous OI murine (OIM<sup>-/-</sup>), a mouse strain with a nonlethal recessively inherited mutation of the COL1A2 gene, is a potential model for the human OI type III. OIM<sup>-/-</sup> and wild type (WT) littermates were raised from weaning (21 days) to adulthood (16 weeks). Digital 3D craniofacial landmarks were taken from in-vivo micro CT scans, and Kuskal-Wallis ANOVAs, along with Mann-Whitney tests, were used to compare centroid size and interlandmark distances among treatment groups. This practicum focuses on the Week 10 mice, with 3 treatment groups: OIMxM, WTxM, and WTxP. We acknowledge that the sample is incomplete due to factors beyond our immediate control, such as OIM<sup>-/-</sup> survivability.

Adolescent OIM<sup>-/-</sup> mice (week 10) were found to have on average smaller cranial and mandibular centroid sizes compared to WT mice regardless of diet. Week 10 OIM<sup>-/-</sup> mice also show several morphological similarities to the OI type III human phenotype, such as shortened

cranial vault height, shortened jaw length, and altered dental spacing secondary to a shortened tooth row.

We conclude that the OIM mouse model shows potential for future investigations of the growth mechanisms underlying the craniofacial presentation of OI. Furthermore, preliminary results suggest that masticatory loading during the early growth period can be used to stimulate craniofacial bone growth and improve bone quality in the OIM mouse model. Future studies will continue to improve sample size by treatment and age groups. The significance of this project is that it will give a better understanding of the role of type 1 collagen and the biomechanical mechanics of craniofacial development, which are important in the search for a new treatment method in OI.

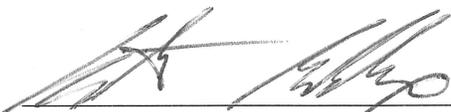
THE EFFECT OF DIET ON CRANIOFACIAL GROWTH  
IN OSTEOGENESIS IMPERFECTA MOUSE MODEL

Summer H. Ladd, B.M.A.

APPROVED:



Rachel Menegaz, Ph.D, Major Professor



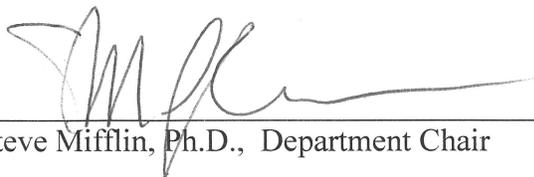
Scott Maddux, Ph.D., Committee Member



Rustin Reeves, Ph.D., Committee Member



Julian Borejdo, Ph.D., University Member



Steve Mifflin, Ph.D., Department Chair

Johnny He, Ph.D., Interim Dean  
Graduate School of Biomedical Sciences

THE EFFECTS OF DIET ON CRANIOFACIAL GROWTH  
IN OSTEOGENESIS IMPERFECTA MOUSE MODEL

INTERNSHIP PRACTICUM REPORT

Presented to the Graduate Council of the  
Graduate School of Biomedical Sciences  
University of North Texas  
Health Science Center at Fort Worth  
in Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Summer H. Ladd, B.M.A.

Fort Worth, Texas

May 2018

## ACKNOWLEDGEMENTS

This work was supported by an Indiana University Collaborative Research Grant and the Ralph W. and Grace M. Showalter Research Trust.

I would like to first thank my principal investigator and major professor, Dr. Rachel Menegaz, for her relentless effort and understanding while working with and teaching me throughout this practicum. Her technological skills and especially her insane enthusiasm for Microsoft Excel never ceased to amaze me.

I would next like to thank the Graduate School of Biomedical Sciences at the University of North Texas Health Science Center for allowing me the opportunity to study in this program to achieve a graduate degree. I specifically would like to thank my committee and the professors that make up the Center for Anatomical Sciences, Dr. Rustin Reeves, Dr. Rehana Lovely, Dr. Scott Maddux, Dr. Magda Muchlinski, and Dr. Armando Rosales for their encouragement and support throughout the program and this project.

To my exceptional friends who were always willing to offer advice and reassurance throughout this chapter of my life, I offer my deepest gratitude. Lastly, I would like to thank my family, who have always offered unwavering support throughout my education, and especially throughout this program.

## TABLE OF CONTENTS

	Page
LIST OF TABLES.....	iv
LIST OF ILLUSTRATIONS .....	v
Chapter	
I. INTRODUCTION .....	1
II. INTERNSHIP SUBJECT .....	4
BACKGROUND AND LITERATURE.....	4
Section 1: OI.....	4
Section 2: Biomechanical Properties of Bone.....	9
SPECIFIC AIMS.....	11
SIGNIFICANCE.....	12
MATERIALS AND METHODS.....	12
RESULTS.....	14
DISCUSSION.....	19
SUMMARY AND CONCLUSIONS.....	23
III. INTERNSHIP EXPERIENCE.....	25
DESCRIPTION OF INTERNSHIP SITE AND EXPERIENCE.....	25
JOURNAL SUMMARY.....	27
BIBLIOGRAPHY.....	38
APPENDIX.....	42

## LIST OF TABLES

Table 1: Week 10 Mandible and Cranium Centroid Sizes .....	15
Table 2: Pair-wise Comparisons (Mann-Whitney).....	19
Table 3: Preliminary Results from Week 16 Hemi-Cranium and Hemi-Mandible Centroid Sizes .....	21

## LIST OF FIGURES

Figure 1: Week 10 Centroid Size (M=meal; P= Pellet).....	15
Figure 2: Week 10 Interlandmark Distance vs Treatment Group.....	16
Figure 3: Preliminary Results of Week 16 Hemi-Cranium and Hemi-Mandible Centroid Sizes. Note that these centroid sizes are calculated from only right-side landmarks and are not directly comparable to the week 10 centroid values.....	20
Figure 4: Week 16 Preliminary Results. Sample size: OIMxM: n=5; OIMxP: n=3; WTxM: n=1; WTxP: n=9.....	21

## CHAPTER I

### INTRODUCTION TO THE STUDY

The following practicum report was performed as a requirement for the Master of Science-Anatomy Track program, from May 2017-May 2018, at the University of North Texas Health Science Center. The study was conducted under the direct supervision of Rachel Menegaz, PhD, in the Center for Anatomical Sciences and Department of Physiology and Anatomy at the University of North Texas Health Science Center.

Osteogenesis imperfecta (OI) is a rare genetic bone disorder that is caused by a mutation in type I collagen, resulting in life changing symptoms. The primary symptom is frequent bone fractures, many seemingly with no cause. Other symptoms include blue sclera, brittle teeth, and cardiac and pulmonary issues. The symptoms are sometimes fatal (Alharbi, 2016). OI can range from mild (type I) to perinatal lethal (type II) to severe (type III). In the severe type III OI, craniofacial dysmorphologies are often seen. These include a triangular facial form, relatively large head size, and soft calavaria (Renaud, 2013). Many individuals with type III OI have related dental issues, which ultimately lead to a lower quality of life (Lanyon, 1985). Among the most common orthodontic problems found in OI patients are cross bite and class III occlusal relationship.

With an incidence rate between 1/10,000 and 1/25,000 worldwide, OI is often misunderstood, leading to frequent misdiagnoses and less than ideal treatment. Current treatments include physical therapy, surgical treatment, pharmacological therapy such as bisphosphonates, and stem cell transplantation. Increasing the strength and flexibility of an OI patient can improve the ability to bear weight and help to prevent fractures in the limbs (Alharbi,

2016). However, with a nickname of “brittle bone disease,” it can easily be understood how parents, with the purpose of protecting their child, often times keep their child from participating in tasks or activities that may seem dangerous, but could actually help to strengthen the bones in an OI patient. With regards to the craniofacial region, current dental interventions are limited by the fragility of the alveolar bone supporting the teeth. Dental extractions are commonly used in juveniles (O’Connell, 1999), but this approach treats only the symptoms and not the cause. Just as exercise improves bone outcomes in the limbs, we propose that targeting bone growth and bone quality in the craniofacial skeleton of young OI patients will improve dental outcomes.

Previous studies have shown that bone growth in the facial skeleton, jaws, and cranial vault can be modulated through altering the mechanical properties of diet. Harder, more fibrous foods, compared to softer foods, require increased masticatory activity in the form of increased cyclical and peak bite forces, stimulating bone growth and remodeling (Paschetta, 2010). In this study, we propose that diets of different material properties will influence muscle behavior and structure, and ultimately the size and quality of the craniofacial bones. We chose the OI murine (OIM) model and wild-type (WT) littermates for this study. Mice of both genotypes were fed a soft meal diet and a hard pellet diet from weaning (3 weeks) to adulthood (16 weeks). Individuals were imaged using *in-vivo* micro-CT, and 3D landmarks collected from the scans. Distances between landmarks were analyzed and compared among the treatment groups (genotype x diet) for a purpose of developing a model for future investigations of the growth mechanisms underlying the craniofacial presentation of OI.

There are two aims of this project. The first aim is to determine if the OIM mouse model replicates the human craniofacial phenotype, thus showing potential for future investigations of the growth mechanisms underlying the craniofacial presentation of OI. We hypothesize that the

OIM<sup>-/-</sup> phenotype will have decreased cranial and mandibular centroid sizes compared to the WT model, along with a smaller maxilla, shorter cranial vault height, smaller mandible, and a shorter tooth row leading to crowded dentition. The second aim is to test if increased biomechanical loading through altered diet can recover the craniofacial phenotype in the OIM model with a hypothesis that OIM<sup>-/-</sup> pellet mice will have increased cranial dimensions, with more similarity to that of the WT genotype than the OIM<sup>-/-</sup> meal mice, secondary to increased masticatory loading.

## CHAPTER II

### INTERNSHIP SUBJECT

#### BACKGROUND AND LITERATURE

##### Section 1: OI

Osteogenesis Imperfecta (OI) is a rare genetic mutation, with the majority of mutations caused by a dominant mutation in a gene coding for Type 1 collagen (Alharbi, 2016). Type 1 collagen is a trimer, consisting of three polypeptide chains that intertwine to form a triple helix structure with a glycine residue at every third position. A point mutation affecting the glycine residues in either the COL1A1 or COL1A2 polypeptide chains produces a mixture of normal and abnormal collagen in a cell, causing the four major types of OI. The chain that is affected, the position of the substitution in the triple helix or the amino acid that is substituted for glycine, determines the resulting OI phenotype (Rauch, 2004).

There are four major types of OI, with severity ranging from mild to lethal. Type 1 is considered the mild type, often clinically diagnosed by characteristics such as multiple fractures usually occurring in the neonatal period, blue sclera, brittle teeth and hearing loss (Alharbi, 2016). A mutation that creates a premature stop codon within the COL1A1 gene is typically associated with this phenotype. Genes that hold this mutation have unstable transcription products that are deleted by nonsense-mediated decay. This allows only normal collagen type 1 chains to be produced, however, overall production is decreased (Rauch, 2004). Type II is considered the most severe, with death occurring either before birth or shortly after secondary to respiratory issues. Type III is the most severe in children, with characteristics such as scoliosis, severe deformity of bones, and brittle teeth (Alharbi, 2016). Type IV has characteristics that are

consistent with Type I, however, are moderately severe rather than mildly (Alharbi, 2016; Rauch, 2004).

Type I collagen, produced by fibroblasts, is an important component of the body and is found in bones, cartilage, tendons, ligaments, eye sclera, skin, and the walls of arteries (Phillips, 2000). Particularly in bones, type I collagen makes up a majority of the bone matrix, contributing to the stability of the extracellular matrix and working as a template for initial mineral deposition (Boskey, 2013). A mutation in type I collagen therefore causes a disorganized assembly of collagen along with lower bone tissue quantity and decreased trabecular and cortical thickness (Boyde, 1999). The quantity of OI type I and both the quantity and quality of the bone of OI type II-IV patients are reduced causing low bone material strength and an increase in fractures and skeletal deformities, with symptoms possibly starting during development in the uterus (Alharbi, 2016; Renaud, 2013). While still in utero, the fetus may already have fractures or deformities to the skull, ribs, spine, and limbs, especially in Type II. The use of an ultrasound during the second trimester of pregnancy, or a low-dose CT scan after 26 weeks gestation, can help to diagnose OI (Renaud, 2013).

Postnatally, depending on the severity, OI patients often present with multiple or repeating non-traumatic skeletal fractures. Other characteristics that might be found are shorter stature, osteopenia, scoliosis, hyperplastic callus formation, and rib fractures. For OI patients, a fracture in the diaphysis of the long bone is very common. The thinning and bending in this region of the lower extremities, along with multiple healed fractures causing angulation, is what is thought to cause the discrepancy in lower leg length in OI patients. However, while this thinning is common, in young children with more severe forms of OI, there is often a lack of

bone modelling, leading to thicker, more broad bones, secondary to multiple healed fractures and bone deformities (Renaud, 2013).

Clinically, OI patients are also often found to have a triangular face, macrocephaly, and soft calvaria, along with several orthodontic issues; the most common being a cross bite<sup>1</sup> and a class III occlusion. A class III occlusion (“underbite”) is when the lower dental arch is pushed out more anteriorly than the upper dental arch (Waltimo-Siren, 2005). Waltimo-Sirén et al. postulates that this occlusion is secondary to a closing mandibular growth rotation. An underdeveloped condyle and alveolar bone cause the occlusion by pushing the mandible forward (Waltimo-Siren, 2005). O’Connell et al. studied children affected by OI types III and IV, finding a triangular shaped face and broad bossed forehead in all patients along with low bone mass and a prevalence of 80% for dentogenesis imperfecta in primary teeth. Class III malocclusions were found in greater than 70% of the patients, with a posterior cross bite in 36% and 47% percent of type III and IV patients, respectively. Mandibular protrusion, mandibular hyperplasia, maxillary hypoplasia, and inhibition of maxillary growth have all been found to contribute to the class III malocclusion (O’Connell, 1999).

Dentinogenesis imperfecta (DI) is a disorder affecting the dentin of the teeth, resulting in increased risk of dental fracture and a color change of the teeth to yellow, brown, or gray (Hartsfield, 2006, O’Connell, 1999). DI affects approximately 50% of OI patients, with incidence varying based on primary vs permanent teeth and age of the patient. While primary teeth tend to be affected more than permanent, it has also been found in permanent teeth that typically the mandibular incisors are affected at a higher rate than maxillary teeth (Hartsfield, 2006).

---

<sup>1</sup> Misalignment of the dental arches.

Cranial base abnormalities, such as basilar invagination and platybasia, are found to be significantly higher in patients with OI. Cheung et al. (2001) provided a longitudinal study on skull base abnormalities in patients with OI that showed that about 20% of OI patients have skull abnormalities, most commonly platybasia (Cheung, 2011). One theory is that the bones of the skull base are too weak to hold up the weight of the brain, and over time, the cranial base becomes shaped abnormally (Kamoun-Goldrat, 2013). Waltimo-Siren et al. proposes that the normal mass of the brain causes depression of the sella<sup>2</sup> and eventual depression of the skull base along with shortening of the anterior cranial base. The rest of the skull then must compensate to fit the brain, resulting in macrocephaly (Waltimo-Siren, 2005). However, Cheung et al. argues that bone found in OI patients is in fact harder than normal bone, with increased brittleness, causing microcracks that can lead to basilar abnormalities. As can be assumed from this theory, as clinical severity increases, so does the probability of a cranial skull base abnormality (Cheung, 2011).

While there is no current cure for OI, continuous efforts are being made to help prevent worsening symptoms and increase functionality. Several options are available, which include physical therapy, surgical treatment, pharmacologic therapy, and stem cell transplantation (Alharbi, 2016). Currently one of the most popular pharmacological treatments in children with moderate to severe OI is bisphosphonate therapy. Bisphosphonates (BPs) inhibit osteoclasts that mediate bone resorption, slowing the loss of existing bone (Alharbi, 2016; Renaud, 2013). BPs bind to hydroxyapatite crystals in the bone mineral and are released during bone turnover, helping to suppress the process of bone resorption and offering beneficial effects in the spine and long bones (Monti, 2010, Renaud, 2013). The reduction of bone resorption helps to increase

---

<sup>2</sup> A depression at the base of the skull where the pituitary gland is located.

bone mineralization density, increase mobility, and decrease the incidence of fractures.

Intravenous administration of bisphosphonates has been reported to reduce arthralgias and fractures while improving vertebral compressions and overall shape (Alharbi, 2016). Long term BP use is not well studied, but has been found to reach a maximal beneficial affect and after discontinuation leaves localized areas of bone fragility secondary to low density metaphyseal bone tissue (Monti, 2010).

Surgical treatment is often used to help correct long bone deformities, by inserting rods in the long bones, but each case must be given careful consideration as to which treatment will be use (Alharbi, 2016, Monti, 2010). Long bones are often very brittle, with bowing, and “rodding” is a treatment that is performed to reduce this and to strengthen the bone (Alharbi, 2016). This method can sometimes help to achieve increased mobility and even allow handicapped patients to stand and walk (Rauch, 2004). OI patients that are older than 2 years old are often treated using intramedullary telescopic rods, which require less frequent changing than non-elongating rods, until linear growth stops (Monti, 2010). The complexity of this surgical procedure can cause osteoporosis of the limb bones, but developments have been made to decrease the number of surgical corrections needed over a patient’s lifetime. If an OI patient is too young or sick to have this procedure completed, there are percutaneous non-extensible intramedullary rods. If the patient is a young adult with good cortical bone, they can use flexible intramedullary rods. Soft bracing can be used to help with spinal deformities, and for more severe cases surgical intervention followed up by spinal stabilization can be performed (Monti, 2010). Dental implants, dental restorative bonding, dentures, crowns, brackets, and orthodontic bands are a few of the more common methods of treatment for patients with DI and other dental implications.

When planning these treatments, the fragility of each tooth must be carefully considered to avoid injury during treatment (Hartsfeild, 2006).

Physical therapy is another common method of treating OI patients. Although OI patients have fragile bones, inactivity can decrease bone mass, and cause contractures, making the bones even more susceptible to fracture (Monti, 2010, Rauch, 2004). Physical therapy in OI patients focuses on increasing muscle strength through light activity to improve the ability to bear weight and ultimately prevent fractures (Alharbi, 2016). Light exercise in water is a very common method to strengthen muscle without too much risk of injury, due to the non-weight bearing nature of aquatic exercises, as well as the use of wheelchairs or walking aids (Monti, 2010, Rauch, 2004). With the initial program beginning in a specialized rehabilitation center or hospital, physiotherapy is continued after discharge with a plan to develop increased range of motion, mobility and reach other short and long term goals (Monti, 2010). The plan is usually developed with a general curriculum starting with handling and positioning and developing into a focus on strengthening exercises.

## Section 2: Biomechanical Properties of Bone

Hydroxyapatite crystals that associate with type I collagen, along with several other ions, are the basic make up of bone and teeth. Phillips et al. performed a longitudinal study on  $OIM^{-/-}$  mice compared to  $OIM^{-/+}$  and WT mice that showed a reduced BMC, and BMD in the  $OIM^{-/-}$  mice (Phillips, 2000). In their study, they found that the composition of hydroxyapatite crystals and associated ions in the femurs and incisors of the  $OIM^{-/-}$  mice was altered compared to the WT. The crystals from tibial diaphyseal bone that had been ground up and scanned via small-angle X-ray scattering, were thinner and more disorganized in  $OIM^{-/-}$  mice. One associated ion,

magnesium, was found to be increased in OI patients. Magnesium is thought to affect the quality of the mineral matrix by inhibiting hydroxyapatite formation, resulting in smaller and more disorganized crystals. OIM<sup>-/-</sup> and OIM<sup>-/+</sup> femurs have also showed decreased biomechanics, such as bending and torsional rigidity, with OIM<sup>-/-</sup> severity higher than OIM<sup>-/+</sup>. These findings suggest that bone and teeth may not just be affected by the quantity of collagen but also by the ability for the collagen and mineral to interact and form bone (Phillips, 2000).

While the composition of bone is crucial in development of the skeleton, in order to attain functional shape and size for load bearing, bones must undergo functional adaptation. Functional adaptation is the adaptive response to load bearing to achieve the appropriate structure for function (Lanyon, 1985). Bones have a basic genetic template, but the architecture of the tissue must be modified by functional adaptation to satisfy the demands of load bearing. A prime example of this process is the differential training of athletes. There may be some genetic factor that leads an athlete to a particular sport, but they must undergo training to maximize their physical potential. This is done through functional adaptation via training. An athlete training for tennis will perform different exercises than an athlete training for football because they have different load bearing responsibilities and train different muscles so that they can fulfill those responsibilities on game day. This can also attribute to why the bone mass of an athlete may be higher than the bone mass of a sedentary individual. However, continued training, or adaptation, is required to prevent loss of the attained architecture (Lanyon, 1985).

Studies show that some functional adaptation can also be seen in the craniofacial region through functions such as masticatory loading, such as mastication (chewing). Paschetta et al. (2010) performed a study using an archaeological sample from the Ohio Valley to determine if a difference in hardness of diet affected craniofacial shape, which showed results that determined

that a dietary shift to softer foods affected the craniofacial regions associated with mastication (Paschetta, 2010). Menéndez et al. (2014) performed studies on Southern South American human populations to determine if morphometric variations in the skull are correlated with bite force and diet composition. They did not find a correlation between bite force and morphometric variation, but did find a correlation between diet composition and cranial variation (Menéndez, 2014). While bite force was studied here, a question lies in the impact of cyclical chewing against peak bite force, such as chewing beef jerky vs trying to crack a nut, on masticatory performance.

This project will discuss the craniofacial differences between the OIM mouse model and wild type littermates, as well as the effect of hard vs soft diet on the craniofacial growth and development of the OIM mouse model.

## SPECIFIC AIMS

**Aim 1:** Determine if the OIM mouse model replicates the human craniofacial phenotype, and thus shows potential for future investigations of the growth mechanisms underlying the craniofacial presentation of OI.

Hypothesis 1: The craniofacial region of the osteogenesis imperfecta murine mouse model will have decreased cranial and mandibular centroid sizes than the wild-type model. The OIM<sup>-/-</sup> phenotype will display a smaller maxilla, shorter cranial vault height, smaller mandible, and a shorter tooth row leading to crowded dentition.

**Aim 2:** Test if increased biomechanical loading through altered diet can recover the craniofacial phenotype in the OIM model.

Hypothesis 2: OIM<sup>-/-</sup> pellet mice will have increased cranial dimensions, with more similarity to that of the WT genotype than the OIM<sup>-/-</sup> meal mice, secondary to increased masticatory loading.

## SIGNIFICANCE

Describing the facial phenotype of an OI mouse model provides the opportunity to find a mouse model to work with for potential behavioral and pharmaceutical interventions for human patients. In the case of OI, it is important to understand the cellular mechanics behind the type I collagen and how it is involved with skeletal growth and quality. We know that type III OI patients have dental malocclusions (O'Connell, 1999; Waltimo-Siren, 2005), but it is currently unknown exactly which jaw dimensions underlie these dental issues or whether the maxilla and mandible are equally effected. It is equally important to understand the biomechanics of this region, and how load bearing throughout life effects bone growth and bone quality in the craniofacial region. The timing of growth in the craniofacial skeleton varies among different modular regions (Menegaz & Ravosa, 2017), so it is also necessary to look longitudinally at skeletal growth to understand *when* these changes occur and when interventions might be most effective. A better understanding of the role of type I collagen and biomechanical loading in craniofacial development are both significant in the search for a new treatment method for OI.

## MATERIALS AND METHODS

All procedures and animal care were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee (IACUC protocol 11133). The homozygous OI murine (OIM<sup>-/-</sup>) is a mouse strain with a nonlethal recessively inherited mutation

of the COL1A2 gene. OIM<sup>-/-</sup> and wild type (WT) littermates were raised from weaning (21 days) to adulthood (16 weeks). OIM<sup>-/-</sup> and WT mice were each separated into hard pellet and soft meal diet. Specimens were anesthetized with inhalation isoflurane at 3-5%, then maintained at 1.5%, in preparation for in-vivo micro-CT scans at 4 weeks (post-weaning juveniles), 10 weeks (adolescents), and 16 weeks (adults). Scans were gathered using a Skyscan 1176 micro-CT machine at a resolution of 8 or 16  $\mu\text{m}^3$  voxels. eTDIPS software was used to generate 3D models and collect digital 3D craniofacial landmarks (Mullick, 1999). A repeatability study (n = 3, trials = 3) was conducted to ensure precision in landmark placement with resulting standard errors (0.00–0.19 mm) below 1% of mean skull length during week 16 (mean = 21.83 mm, 1% of mean = 0.22 mm). Centroid sizes and interlandmark distances were generated using PAST 2.0 software (Hammer, 2001). Measurements of interlandmark distances (ILDs) were completed on the right side of the skull with the assumption of symmetry. These measurements were then analyzed using non-parametric ANOVAS (Kruskal-Wallis) ( $\alpha=0.10$ ) to compare genotypes (Aim 1) and treatment groups (Aim 2). While  $p < 0.05$  is typically considered statistically significant, we used  $p < 0.10$  as our cut off for statistical significance bearing in mind the considerably small sample size we were working with. Pair-wise comparisons (Mann-Whitney) ( $\alpha=0.10$ ) were made when indicated.

The group of littermates used for this study is the beginning of a new sample that is being bred for further research in OI. Sample size is small (n = 8) due to the recent restart of this breeding colony and also due to the difficulty of OIM<sup>-/-</sup> survival. In the week 10 and week 16 results, there is no data for any OIM pellet mice as all but one expired before the week 10 time period, and the CT scans for Week 10 on the surviving specimen had too much movement blur in the micro-CT scan to obtain any reliable data.

A previous undergraduate student, Adam Guth (IUPUI), worked with Dr. Menegaz to construct models of the Week 16 scans. He left the project and I took over his position and worked with Dr. Menegaz to analyze the week 16 data. I continued to construct and analyze data for Week 10 mice and began working on construction of models for Week 4 mice. The data for the Week 4 mice are not yet complete and are not discussed in this practicum report.

## RESULTS

### Week 10

The use of a Kruskal-Wallis test was used to analyze the data found in this project. Our sample size was a total of  $n = 8$ , with OIM<sup>-/-</sup> meal (OIMxM;  $n = 2$ ), WT meal (WTxM;  $n = 3$ ) and WT pellets (WTxP;  $n = 3$ ) (Table 1). There was an OIM<sup>-/-</sup> pellet (OIMxP) group, but results are not shown for the reasons discussed above.

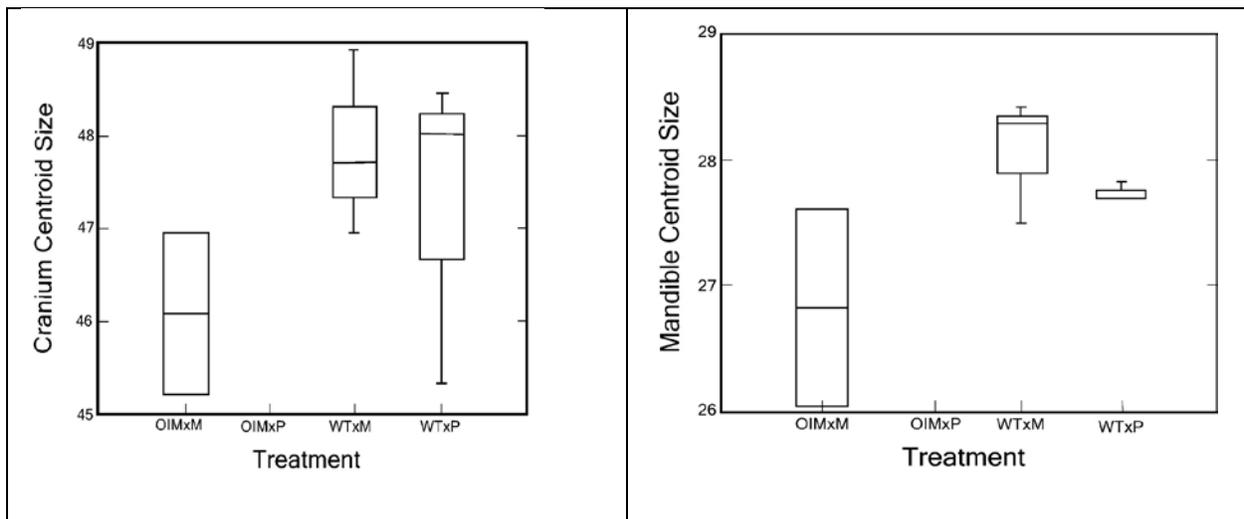
### Centroid Size Results

Results showed a p-value of 0.405 for cranium centroid size and a p-value of 0.24 for mandible centroid size. While these are not statistically significant, we do feel that these are biologically significant due to the trend for separation between the OIM<sup>-/-</sup> and WT genotypes (Figure 1). We expect that the non-significant p-value is due to the small sample size, and that results will be statistically significant once the sample size is increased to a valid size of approximately  $n=20$  per treatment group.

Table 1: Week 10 Mandible and Cranium Centroid Sizes

Week 10 Mandible Centroid Size				Week 10 Cranium Centroid Size			
Treatment	Mean	SD	Kruskal-Wallis p-value (OI-WT)	Treatment	Mean	SD	Kruskal-Wallis p-value (OI-WT)
<b>OIM x M</b> (n = 2)	26.82	1.12	0.24	<b>OIM x M</b> (n = 2)	45.21	1.24	0.405
<b>WT x M</b> (n = 3)	28.07	0.51		<b>WT x M</b> (n = 3)	47.87	0.99	
<b>WT x P</b> (n = 3)	27.73	0.08		<b>WT x P</b> (n = 3)	47.28	1.69	

Figure 1: Week 10 Centroid Size (M=meal; P= Pellet)

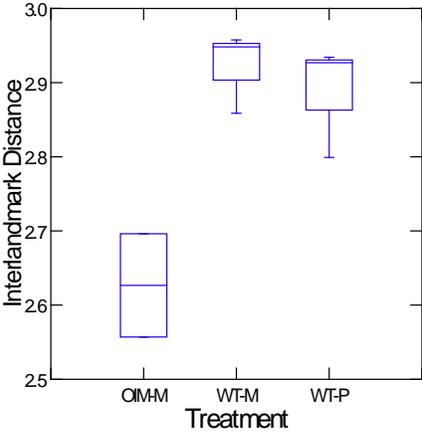


### Interlandmark Distance Results

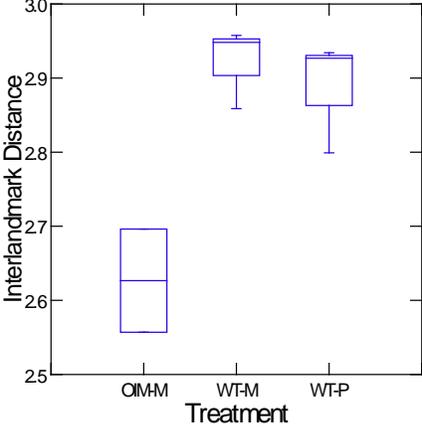
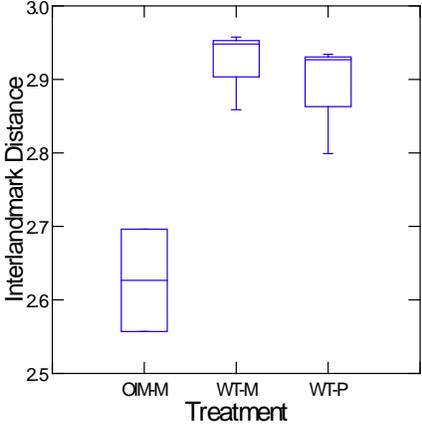
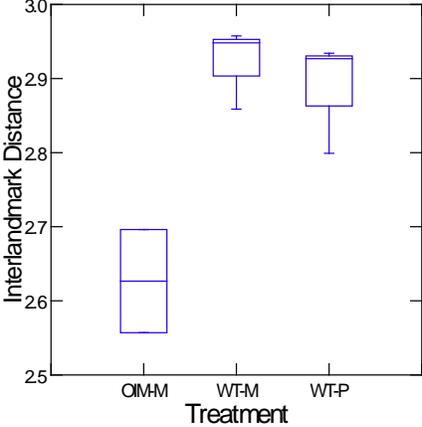
In week 10 mice (adolescent), we found that the posterior portion of the ramus of the mandible is smaller in the OIM<sup>-/-</sup> genotype than in the WT. The ramus height (coronoid-angular process) (Figure 2A) and the distance between the condyle and the coronoid process were both statistically significant with  $p < 0.096$  (Figure 2B). The jaw length, distance from the condyloid process to the incisor alveolus, was also statistically significant with  $p < 0.096$  (Figure 2C).

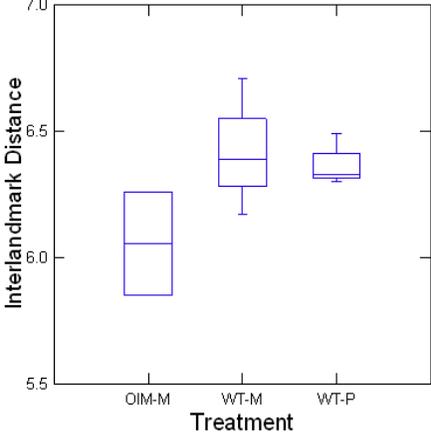
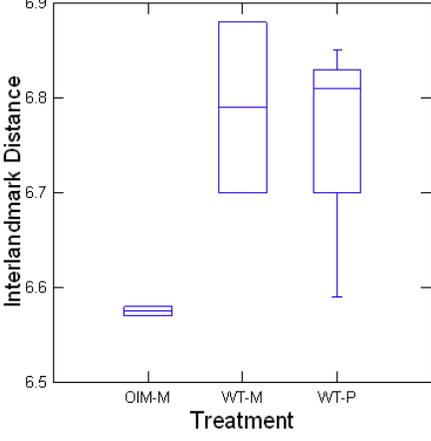
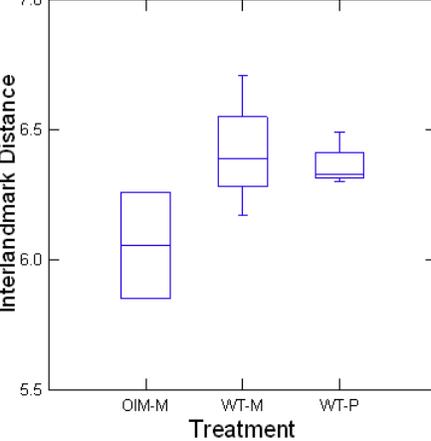
In the cranial region, the cranial vault height was significantly shorter with a  $p < 0.077$  and the maxillary tooth row was smaller with  $p < 0.096$  (Figure 2D). Basicranial length was not found to be significantly decreased in the week 10 OIMxM mice, with  $p < 0.757$ .<sup>3</sup> Pair-wise comparisons found that the differences of size in the posterior ramus, the jaw length, and the maxillary tooth row were all between the OIMxM vs WTxM as well as the OIMxM vs WTxP. However, in the cranial vault, the difference was found in the OIMxM vs WTxM and WTxM vs WTxP rather than in OIMxM vs WTxP (Table 2).

Figure 2: Week 10 Interlandmark Distance vs Treatment Group

Interlandmark Distances		Kruskal-Wallis p-value (OI-WT)
93-94 (Ramus height)	 <p>The box plot displays the distribution of interlandmark distances for three treatment groups: OIMM, WFM, and WTP. The y-axis represents the interlandmark distance, ranging from 2.5 to 3.0. The OIMM group shows a median distance of approximately 2.63, with a box from 2.56 to 2.70 and whiskers extending from 2.56 to 2.99. The WFM group has a median distance of approximately 2.93, with a box from 2.91 to 2.95 and whiskers from 2.86 to 2.99. The WTP group has a median distance of approximately 2.91, with a box from 2.87 to 2.93 and whiskers from 2.80 to 2.99.</p>	0.096

<sup>3</sup> Figure of mouse skulls with landmarks and descriptions provided in appendix.

<p>89-93 (Condyle- coronoid process)</p>	 <p>Box plot showing Interlandmark Distance (Y-axis, 25 to 30) for three treatments (OIMM, WFM, WTP) for landmark 89-93 (Condyle-coronoid process). The OIMM group has a median distance of approximately 26.3, while the WFM and WTP groups have medians around 29.3 and 29.1, respectively. All groups show a similar range of values, with whiskers extending to the minimum and maximum values.</p>	<p>0.096</p>
<p>89-96 (Jaw length)</p>	 <p>Box plot showing Interlandmark Distance (Y-axis, 25 to 30) for three treatments (OIMM, WFM, WTP) for landmark 89-96 (Jaw length). The OIMM group has a median distance of approximately 26.3, while the WFM and WTP groups have medians around 29.3 and 29.1, respectively. All groups show a similar range of values, with whiskers extending to the minimum and maximum values.</p>	<p>0.096</p>
<p>39-42 (Maxillary tooth row)</p>	 <p>Box plot showing Interlandmark Distance (Y-axis, 25 to 30) for three treatments (OIMM, WFM, WTP) for landmark 39-42 (Maxillary tooth row). The OIMM group has a median distance of approximately 26.3, while the WFM and WTP groups have medians around 29.3 and 29.1, respectively. All groups show a similar range of values, with whiskers extending to the minimum and maximum values.</p>	<p>0.096</p>

<p>16-52 (Cranial width)</p>		<p>0.119</p>
<p>54-55 (Basicranial length)</p>		<p>0.140</p>
<p>17-67 (Zygomatic arch-internal pterygoid)</p>		<p>0.249</p>

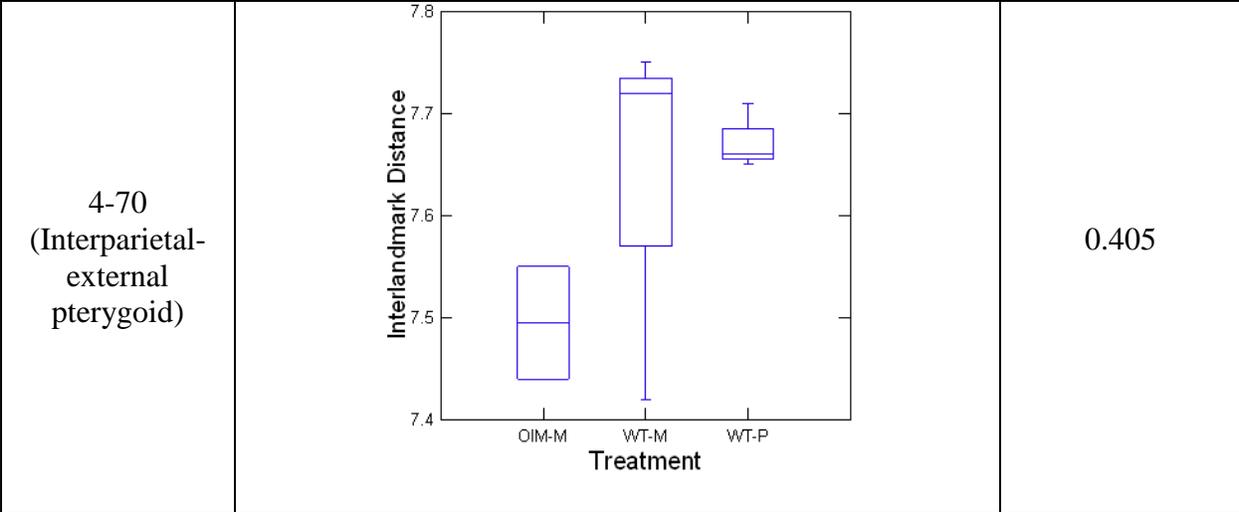


Table 2: Pair-wise Comparisons (Mann-Whitney)

	<b>OIMxM vs WTxM</b>	<b>OIMxM vs WTxP</b>	<b>WTxM vs WTxP</b>
Ramus Height	0.083	0.083	0.275
Condyle-Coronoid Process	0.083	0.083	0.275
Jaw Length	0.083	0.083	0.275
Maxillary Tooth Row	0.083	0.083	0.275
Cranial Vault Height	0.083	0.564	0.050

**DISCUSSION**

The goal of this study was (Aim 1) to describe the craniofacial phenotype in an OI mouse model, to see if this model can be used to test potential behavioral and pharmaceutical interventions, and (Aim 2) to determine if diet and masticatory loading affect the development of the craniofacial region. The hypothesis for Aim 1 was supported, as the data show that the OIM model has decreased cranial and mandibular centroid sizes compared to the WT model as well as

more localized differences including a smaller mandible, shorter cranial vault height, and a shorter tooth row leading to crowded dentition.

The week 10 centroid sizes for the cranium and the mandible were analyzed using the Kruskal-Wallis test and were not found to be statistically significant as hypothesized. However, while the statistics of the centroid sizes of the mandible ( $p < 0.24$ ) and the cranium ( $p < 0.405$ ) were not statistically significant, we do believe that these results are biologically significant and will become statistically significant with a larger sample size. Results from studies done in humans and another mouse model with larger sample sizes have shown statistically significant reduced maxillary length, anterior cranial base length, and mandible length when compared to controls (Cheung, 2011; Kamoun-Goldrat, 2013; Waltimo-Siren, 2005). Preliminary results from the week 16 show a general trend that supports the idea that on average,  $OIM^{-/-}$  mice have smaller centroid sizes than WT mice (Menegaz & Organ, 2018) (Figure 3, Table 3).

Figure 3: Preliminary Results of Week 16 Hemi-Cranium and Hemi-Mandible Centroid Sizes. Note that these centroid sizes are calculated from only right-side landmarks and are not directly comparable to the week 10 centroid values.

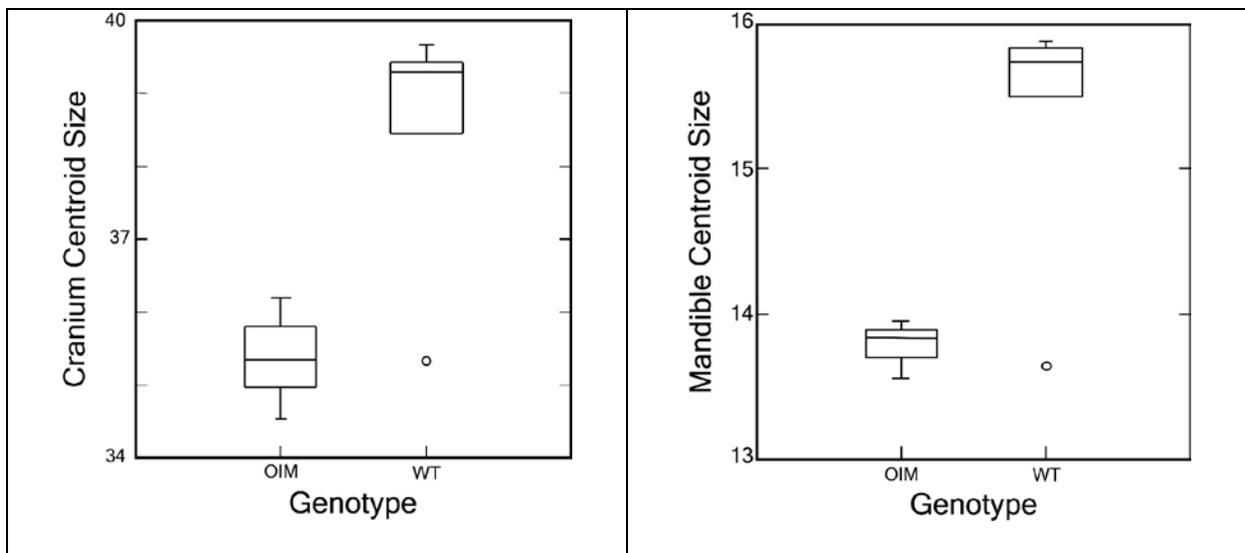
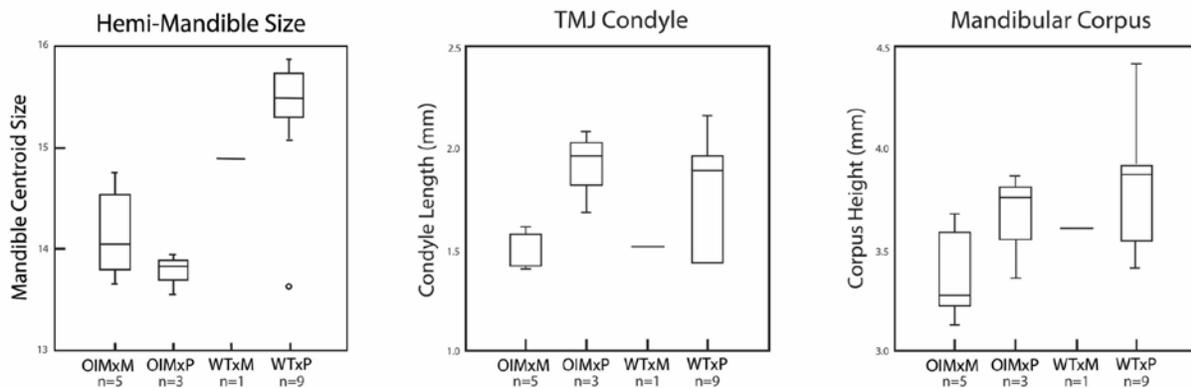


Table 3: Preliminary Results from Week 16 Hemi-Cranium and Hemi-Mandible Centroid Sizes

Week 16 Hemi-Mandible Centroid Size				Week 16 Hemi-Cranium Centroid Size			
Treatment	Mean	SD	Kruskal-Wallis p-value (OI-WT)	Treatment	Mean	SD	Kruskal Wallis p-value (OI-WT)
WT	15.313	0.951	0.101	WT	38.429	1.795	0.101
OIM	13.781	0.200		OIM	35.377	0.832	

The hypothesis for Aim 2 remains more ambiguous due to our small week 10 sample size. However, preliminary results from week 16 (unpublished data, R. Menegaz) suggest that increased dietary biomechanical loading can partially recover some localized morphologies, such as TMJ length, in the OIM genotype compared to the WT genotype (Figure 4).

Figure 4: Week 16 Preliminary Results. Sample size: OIMxM: n=5; OIMxP: n=3; WTxM: n=1; WTxP: n=9.



In the week 10 mice, the posterior portion of the ramus of the mandible for the OIM x M was found to be smaller than both the WT x M and WT x P mice. The difference in ramus height

(coronoid-gonial process) was statistically significant among the treatment groups, with the OIM x M having the shortest ramus (Figure 3A). The distances between the condyle and the coronoid process were also found to be smaller in the OIM x M, which leads to an assumption that these muscle attachment sites are better developed in the WT mice. Avis performed a study to determine if mandibular angles decreased in the event of removal of the masticatory muscles. It was found that when the superficial masseter muscle and the internal pterygoid muscles were removed, the mandibular angles all decreased (Avis, 1961). From this study and our results from week 10, we can assume that the angle between the condyle and the coronoid process was decreased in our OIMxM mice due to the consumption of a meal diet rather than a pellet diet since a meal diet requires less masticatory loading than a pellet diet would.

As previously discussed, the week 10 results showed a smaller posterior ramus in the OIM mice compared to the WT. The week 16 results also show a decreased ramus height in the OIM mice compared to the WT mice, but did not show a significant difference in the distance between the condyle and the coronoid process. The lack of a decreased condyle angle in the week 16 OIM mice could be attributed to the continued growth of the mice. As the masticatory muscles further develop, the coronoid and condyloid processes may be adapting to the muscular attachment needs, thus increasing the condyle angle. Further validation of this idea lies in the fact that in week 10 OIM<sup>-/-</sup> the jaw length (ILD 89-96) is significantly decreased. However, in the week 16 OIM<sup>-/-</sup> mice, there is no significant decrease in length of the same ILD. If the condyloid process is lengthening, this could cause there to be no significant difference in the jaw length, as well as no significant difference in the condyle angle.

As expected, the maxillary tooth row is shortened in week 10 OIM x M mice (Figure 3D), causing altered dental spacing and overcrowding of the dentition. The week 16 results

showed no significant difference in the tooth row of OIM<sup>-/-</sup> mice compared to the WT, however, they did show significant shortening in the maxillary length of the OIM<sup>-/-</sup> mice vs the WT mice. Basicranial length in the week 10 OIMxM mice was not found to be significant or even near significance. However, in the week 16 sample, basicranial length was found to be significantly shorter in OIM<sup>-/-</sup> mice compared to WT. The week 16 sample also showed significantly decreased maxillary length in the OIM<sup>-/-</sup> mice compared to WT, whereas the week 10 OIMxM mice did not. However, considering the near significance of the shorter length in the posterior portion of the cranial base in week 10 OIM<sup>-/-</sup> mice, but not the anterior portion, I would consider that the basicranial length in the week 10 OIM<sup>-/-</sup> mice is influenced by the non-significance of the maxillary length in the week 10 mice. Therefore, it appears that the region of importance in the basicranial length lies in the maxillary length relative to age. The cranial vault height was significantly decreased in the week 10 OIMxM mice compared to the WT mice. This height was also found to be significantly decreased in the OIM<sup>-/-</sup> mice of the week 16 sample. Other cranial distances were not found to be statistically significant, but results did show that overall the OIM<sup>-/-</sup> mice tend to have smaller interlandmark distances (ILDs) than the WT.

## SUMMARY AND CONCLUSIONS

Osteogenesis imperfecta is a genetic mutation in the COL1A1/COL1A2 gene that affects type I collagen. This study aimed to (1) determine if the OIM mouse model replicates the human craniofacial phenotype, thus showing potential for future investigations of the growth mechanisms underlying the craniofacial presentation of OI and (2) test if increased

biomechanical loading through altered diet can recover the craniofacial phenotype in the OIM model.

For Aim 1, we hypothesized that the craniofacial region of the OIM mouse model, compared to their diet-matched WT littermates, would have decreased cranial and mandibular centroid sizes and would display a smaller maxilla, shorter cranial vault height, smaller mandible, and a shorter tooth row leading to crowded dentition. While the trend for smaller centroid sizes in the OIM<sup>-/-</sup> mice were not statistically significant in our results, the posterior portion of the ramus, the mandible, the maxilla, and the tooth row length all had significantly smaller lengths ( $p < 0.1$ ) in the OIM<sup>-/-</sup> mice than in the WT mice.

For Aim 2, we hypothesized that increased masticatory loading in the OIM<sup>-/-</sup> model would affect the development of the craniofacial region and help to recover the phenotype. Due to several technical issues (re-establishment of the breeding colony, poor survivability of the OIM<sup>-/-</sup> genotype, and micro-CT imaging errors), we were unable to include a OIMxP treatment group in the week 10 study described here. Preliminary results from week 16 show no change in mandibular centroid size but do demonstrate recovery of localized measurements, such as TMJ and corpus size, in the adult OIMxP treatment group.

In conclusion, our findings of smaller dimensions of the craniofacial region in the OIM mice compared to the WT mice are consistent with previous results (Menegaz & Organ, 2017) that show that type I collagen mutations impede craniofacial growth. In the future, a continuation of this study should be completed with a larger sample size in order to evaluate the role of biomechanical loading and dietary properties on craniofacial growth in the OIM mouse.

## CHAPTER III

### INTERNSHIP EXPERIENCE

#### DESCRIPTION OF INTERNSHIP SITE AND EXPERIENCE

This internship practicum was performed at the University of North Texas Health Science Center in Fort Worth, TX under the direct supervision of Rachel Menegaz, PhD over the course of a year as a partial requirement for the degree of Master of Science. I was introduced to the project and previous studies performed by Dr. Menegaz when I began the program. A general schedule was created for the year, which was maintained and adjusted throughout the project by both my PI and myself.

The mice in our study sample were housed at Indiana University School of Medicine, where micro-CT scans were performed and sent to us for further evaluation. Once we received these scans, Dr. Menegaz trained me on the software programs that I would need to use to perform the modeling, measuring, and analyzing of the scans. In the beginning, I spent the majority of my time working with the micro-CT scans, reconstructing them into 3D models to attain geometric landmarks. Once the landmarks were placed, I worked with Dr. Menegaz to measure and analyze interlandmark distances for the results of this project. These results have been reported in this practicum report.

Through my experience, I also learned a lot about the administrative side of research. Thanks to the incredible professors in the anatomy department, organizing a committee was easily managed. However, the completion of the paperwork required throughout this process always proved much more difficult, typically due to unforeseeable and uncontrollable

circumstances. Through these experiences, I was given the opportunity to work closely with my PI and committee members, allowing me to better understand the networking and “behind-the-scenes” work that takes place throughout a research project.

## JOURNAL SUMMARY

**9/26/2017**

-Week 4 reconstruction of CT scans into 3D models.

**9/29/2017**

-Landmarking of 3D model anatomical landmarks, precision trials.

-Week 16 OIM 80 Trial 1. 1-23 landmarks completed.

**10/23/2017**

-Landmarking, precision trials.

-Week 16 OIM 80 Trial 1. 1-49 completed with 1-23 completed again.

**10/24/2017**

-Landmarking, precision trials.

-Week 16 OIM 80 Trial 1. 50-72 completed.

-Issues: 69 and 70-could not visualize.

**10/25/2017**

-Landmarking, precision trials.

-Week 16 OI 80 Trial 1. 72-104 completed. Unable to visualize 69 and 70 on inferior view.

**11/6/2017**

-Landmarking, precision trials.

-Week 16 oIM 81 Trial 1. Completed all landmarks.

-Issues: Difficulty visualized 54 on inferior.

Extraction threshold: 71-255.

### **11/17/2017**

-Landmarking, precision trials.

-Week 16 OIM 86 Trial 1. Completed.

-No issues.

-Extraction threshold: 55/75-255.

### **11/30/2017**

-Landmarking, precision trials.

-Week 16 OIM 80 Trial 2. Completed.

-Issues: 1,6,7,24,37 not marked due to CT cutting off nasal area.

-Extraction threshold: 71-255.

### **12/1/2017**

-Landmarking, precision trials.

-Week 16 OIM 81 Trail 2. Completed

-Issues: 24, 37 cut off and not marked. 54 suture not found.

-Extraction threshold: 81-255.

### **12/6/2017**

-Landmarking, precision trials.

-Week 16 OIM 86 Trial 2. Completed superior view, & 31, 44.

-No issues.

-Extraction threshold: 80-255.

**12/11/2017**

-Landmarking, precision trials.

-Week 16 OIM 86 Trial 2. Completed.

-No issues.

-Extraction threshold: 71-255

**12/15/2017**

-Landmarking, precision trials.

-Week 16 OIM 80 Trial 3.

-Issues: 1,6,7,24,37 not marked due to CT cut off.

-Extraction threshold: 81-255.

**12/22/2017**

-Landmarking, precision trials.

-Week 16 OIM 81 Trial 3. Completed all but inferior view. Application quit responding.

-Issues: 24,37 not marked. CT cut off.

**12/26/2017**

-Landmarking, precision trials.

-Week 16 OIM 81 Trial 3. Completed.

-Issues: 54, cannot visualize.

-Extraction threshold: 65-255.

-Week 16 OIM 86 Trial 3. Completed.

-No issues.

-Extraction threshold: 81-255.

### **1/12/2018**

-Meeting with PI to determine semester timeline and to discuss any issues with data.

### **1/13/2018**

-Landmarking.

-Week 10 OIM 194. Completed superior view.

-Issues: 1,6,7,13,22 not marked. Nasal region cut off. Scan very difficult to visualize landmarks.

-Extraction threshold: 30-255.

### **1/14/2018**

-Landmarking.

-Week 10 OIM 194. Completed mandibles.

-Issues: Will need to discuss points with PI. Having to use multiple extraction to determine individual landmarks.

### **1/18/2018**

-Landmarking.

-Week 10 OIM 194. Completed to best of ability.

-Issues: Very difficult to visualize landmarks. Multiple landmarks unable to be visualized. Will discuss with PI at next meeting.

-Week 10 OIM 193. Completed to best of ability.

-Issues: Also very difficulty to visualize landmarks. No paracondylars were seen.

Will also discuss with PI at next meeting.

### **1/19/2018**

-Meeting with PI. Determined that Week 10 OIM 193 and 194 have too much movement blur to use. Checked other week 10's for similar issues. OIM 81, 86, 152, and 192 were also found to have movement blur and will not be landmarked.

### **1/20/2018**

-Reconstruction.

-Week 10 OIM 81 and 107. File too large for eTDIPS.

-Landmarking.

-Week 10 OIM 80. Completed

-No issues.

-Extraction threshold: 25/30-255.

-Week 10 OIM 153. Completed.

-Issues: Nasal region cut off. No 1,6,7. 43, 56 not visualized.

-Extraction threshold: 60-255.

### **1/21/2018**

-Landmarking.

-Week 10 OIM 153. Checked and completed.

-Week 10 OIM 156. Reconstructed and then completed.

-Issues: 30, 55, 56 cut off, not visualized.

-Extraction threshold: 55/90-255.

-Week 10 OIM 159. Reconstructed and Completed.

-Reconstructed TIF stack, 50% scale.

-Issues: 32 and 45: difficult to visualize sutures.

-Week 10 OIM 154.

-Issues: Nasal bones skewed. Discuss with PI. Consider movement blur.

### **1/24/2018**

-Reconstruction.

-Week 10 OIM 81, 107, 115, and 116.

-TIF stack, 25% scale.

### **1/25/2018**

-Meeting with PI to discuss issues or concerns with data. Discuss current literature and outline ideas for practicum report.

-Landmarking.

-Week 10 OIM 116. Did not complete.

-No current issues.

-Extraction threshold: 65-255.

-Week 10 OIM 152.

-Issues: Movement blur. Unable to use.

### **1/26/2018**

-Landmarking.

-Week 10 OIM 116. Completed.

-No issues.

-Extraction threshold: 65-255.

-Week 10 OIM 154.

- Issues: Nasal bones skewed, possible genetic abnormality. Unable to mark 1, 6, 7.

-Extraction threshold: 60-255.

### **1/30/2018**

-Landmarking.

-Week 10 OIM 107. Completed.

-No issues.

-Extraction threshold: 55/75-255.

-Reconstruction.

-Week 10 OIM 192.

-Issues: movement blur. Unable to use for landmarking.

### **1/31/2018**

-Landmarking.

-Week 10 OIM 115. Completed.

-Issues: CT scanner circle in scan over the nasal region. Cutting off 1,6,7,38. Right zygomatic arch is broken, may affect 17 and 19.

-Extraction threshold: 50/60-255.

**2/1/2018**

-Landmarking.

-Week 10 OIM 81.

-Issues: movement blur. Unable to use.

**2/2/2018**

-Meet with PI to discuss any issues and concerns. Discuss literature.

-Landmarking.

-Week 4 OIM 259.

-Issues: 6,7 marks are unable to be visualized. Appears as if the extraction is too high. However, the extraction is at 38 and cannot go lower. 43 is cut off.

-Extraction threshold: 38/60-255.

**2/5/2018**

-Landmarking.

-Week 4 OIM 262.

-Issues: Right lower teeth difficult to differentiate. 38 and 45 difficult to visualize.

-Extraction threshold: 50-255.

**2/9/2018**

-Meeting with PI to discuss any issues or concerns with data. Discussed further ideas for outline of practicum and background of sample litter.

-Landmarking.

-Week 4 OIM 257. Completed.

-No issues.

-Extraction threshold: 50/60-255.

-Week 10 landmark data imported into Excel.

## **2/10/2018**

-Landmarking.

-Week 4 OIM 256. Completed.

-Issues: 1,6,7-nasal region cut off. 54 not present.

-Extraction threshold: 50/55-255.

-Week 4 OIM 254. Completed.

-No issues.

-Extraction threshold: 50/60-255.

## **2/13/2018**

-Landmarking.

-Week 4 OIM 253. Completed.

-No issues.

-Extraction threshold: 45/55-255.

## **2/15/2018**

-Landmarking.

-Week 4 OIM 249. Completed.

-34 may be affected by abnormally running suture.

-Extraction threshold: 40/55-255.

**2/16/2018**

-Meeting with PI to discuss any issues or concerns with data.

**2/21/2018**

-Landmarking.

-Week 4 OIM 248. Completed.

-No issues.

-Extraction: 40/60-255.

**2/22/2018**

-Landmarking.

-Week 4 OIM 246. Completed.

-No issues.

-Extraction threshold: 40/55-255.

**2/23/2018**

Meet with PI to discuss timeline, data issues and concerns and practicum outline.

**2/26/2018**

-Landmarking.

-Week 4 OIM 234.

-Issues: Reconstruction performed prior to landmarking. 32 and 45 not visualized.

-Extraction threshold: 40/50-255.

-Week 4 OIM 235.

-Reconstruction performed prior to landmarking. 32 and 45 not visualized.

### **3/2/2018**

-Finished outline and sent to PI for review. Organized and set practicum date and time with committee.

### **3/5/2018**

-Filed intent to defend.

-Met with Dr. Borejdo, university member to discuss project.

### **3/9/2018**

-Met with PI to analyze and organize current data.

### **3/16/2017**

-Met with PI to discuss and organize results.

### **3/21/2018**

-Sent practicum report draft to committee for review.

## BIBLIOGRAPHY

1. Alharbi, S. A Systematic Overview of Osteogenesis Imperfecta. *Molecular Biology* 5(1):150. 2016.
2. Avis, V. The significance of the angle of the mandible: An experimental and comparative study. *American Journal Physical Anthropology*. 19:55–61. 1961.
3. Bachrach, L., Acquisition of optimal bone mass in childhood and adolescence. *Trends in Endocrinology & Metabolism*. 12 (1):22-28. 2001.
4. Boskey, A. Bone composition: relationship to bone fragility and antiosteoporotic drug effects. *BoneKEy Reports*. 447. 2013.
5. Boyde, A., Travers, R., Glorieux, F., Jones, S.J. The mineralization density of iliac crest bone from children with osteogenesis imperfecta. *Calcified Tissue International* 64: 185. 1999.
6. Cheung, M., Arponen, H., Roughley, P., Azouz, M., Glorieux, F., Waltimo-Siren, J., Rauch, F. Cranial base abnormalities in osteogenesis imperfecta: phenotypic and genotypic determinants. *Journal of Bone and Mineral Research*. 26(2):405-413. 2011.

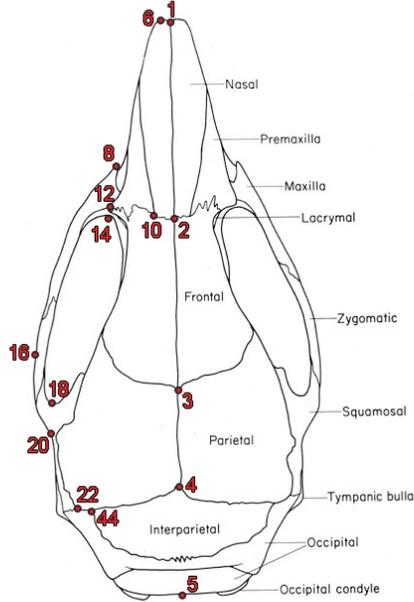
7. Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 9pp.
8. Hartsfield Jr., J. K., Hohlt, W. F., Roberts, W. E. Orthodontic treatment and orthognathic surgery for patients with osteogenesis imperfecta. *Seminars in Orthodontics*. 12 (4): 254-271. 2006.
9. Kamoun-Goldrat, A., Marchadier, A., Le Merrer, M., Goldberg, M. Animal models of osteogenesis imperfecta and craniofacial development. *Hard Tissue*. 2(2):11. 2013.
10. Lanyon, L., Rubin, C. T. Functional adaptation in skeletal structures. *Functional Vertebrate Morphology*. pp. 1-25. 1985.
11. Menegaz, R. A., Organ, J. M. Craniofacial growth in a mouse model of osteogenesis imperfecta. *Proceedings of the Regional Meeting of the American Association of Anatomists (Craniofacial Research Themes)*. Pittsburgh, Pennsylvania. 2017.
12. Menegaz, R. A., Ravosa, M. J. Ontogenetic and functional modularity in the rodent mandible. *Zoology*. 124: 61-72. 2017.

13. Menéndez, L., Bernal, V., Novellino, P., Perez, S. I. Effect of bite force and diet composition on craniofacial diversification of southern south american human populations. *American Journal of Physical Anthropology*. 155:114-127; 2014.
14. Monti, E., Mottes, M., Frascini, P., Brunelli, P., Forlino, A., Venturi, G., Doro, F., Perlini, S., Cavarzere, P., Antoniazzi, F. Current and emerging treatments for the management of osteogenesis imperfecta. *Therapeutics and Clinical Risk Management*. 6:367-381; 2010.
15. Mullick, R., Warusavithana, S.V., Shalini, V., Pang, P., 1999. Plug-ins: a software model for biomedical imaging and visualization research. <http://www.cc.nih.gov/cip/software/etdips>.
16. O'Connell, A.C., Marini, J.C. Evaluation of oral problems in an osteogenesis imperfecta population. *Oral Surgery Oral Medicine Oral Pathology & Oral Radiology*. 87(2):189-196. 1999.
17. Paschetta, C., de Azevedo, S., Castillo, L., Martinez-Abadias, N., Hernández, M., Lieberman, D., González-José, R. The influence of masticatory loading on craniofacial morphology: A

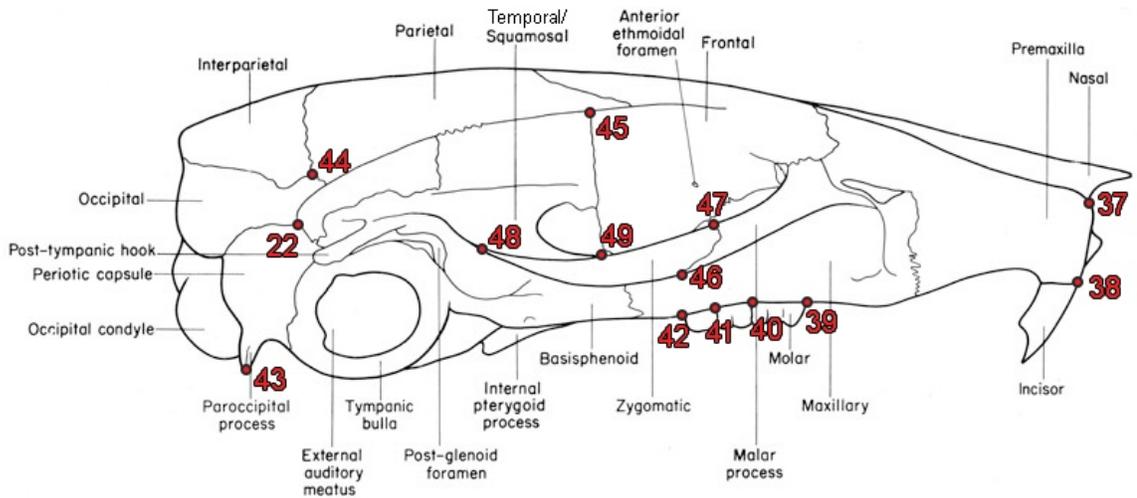
- test case across technological transitions in the ohio valley. *American Journal of Physical Anthropology*. 141: 297-314. 2010.
18. Phillips, C., Bradley, D., Schlotzhauer, C., Bergfeld, M., Libreros-Minotta, C., Gawenis, L. R., Morris, J. S., Clarke, L. L., Hillman, L. S. Oim mice exhibit altered femur and incisor mineral composition and decreased bone mineral density. *Bone*. 27(2): 219-226. 2000.
19. Rauch, F., Glorieux, F. Osteogenesis imperfecta. *Seminars*. 363:1377-85. 2004
20. Renaud, A., Aucourt, J., Weill, J., Bigot, J., Dieux, A., Devisme, L., Moraux, A., Boutry, N. Radiographic features of osteogenesis imperfecta. *Insights into Imaging*. 4(4):417-429. doi:10.1007/s13244-013-0258-4. 2013.
21. Waltimo-Siren, J., Kolkka, M., Pynnönen, S., Kuurila, K., Kaitila, I., Kovero, O. Craniofacial features in osteogenesis imperfecta: A cephalometric study. *American Journal of Medical Genetics* 133A:142-150. 2005.

# APPENDIX

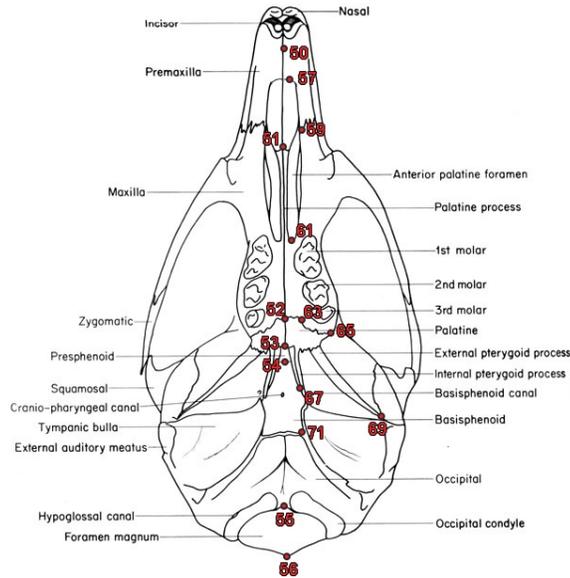
## LABORATORY MOUSE - DORSAL



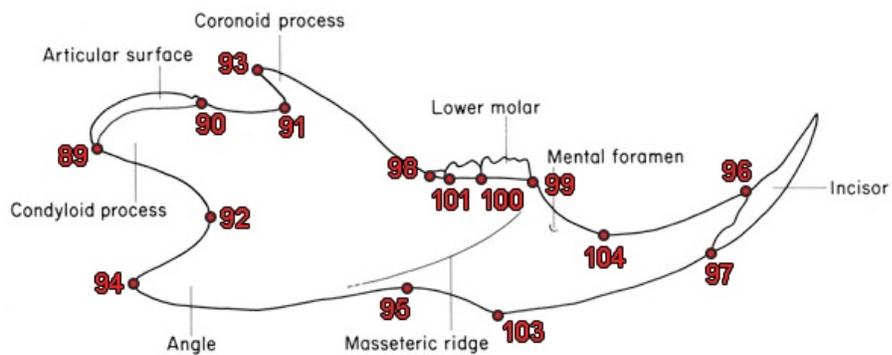
## LABORATORY MOUSE - LEFT LATERAL



## LABORATORY MOUSE - INFERIOR



## LABORATORY MOUSE - LATERAL MANDIBLE



*\*102 hidden by bone of coronoid process*

Number Name Description

Superior View

- 1 Nasale Most anterior-medial point of the nasal bones
- 2 Nasion Intersection of frontal and nasal bones on midline
- 3 Bregma Intersection of the frontal and parietal bones on midline
- 4 pip Intersection of the parietals and interparietal on the midline
- 5 operan Most caudal point on the midline
- 6 Lnas Most anterior-lateral point of the left nasal bones
- 7 Rnas Most anterior-lateral point of the right nasal bones
- 8 Lzyg Most anterior point on the left zygomatic bone
- 9 Rzyg Most anterior point on the right zygomatic bone
- 10 Lfrnaspr Intersection of the left nasal and premaxilla and frontal bones
- 11 Rfrnaspr Intersection of the right nasal and premaxilla and frontal bones
- 12 Lfrzygpr Intersection of the left zygomatic and premaxilla and frontal bones
- 13 Rfrzygpr Intersection of the right zygomatic and premaxilla and frontal bones
- 14 Lantfoss Most anterior point of the left zygomatic fossa on the maxillary bone
- 15 Rantfoss Most anterior point of the right zygomatic fossa on the maxillary bone
- 16 Lzygarc Most lateral point on the left zygomatic arch
- 17 Rzygarc Most lateral point on the right zygomatic arch
- 18 LPzygfs Most posterior point of the zygomatic fossa on the left temporal bone
- 19 RPzygfs Most posterior point of the zygomatic fossa on the right temporal bone
- 20 LPzygrt Most posterior point of the left zygomatic root
- 21 RPzygrt Most posterior point of the right zygomatic root
- 22 Lpateoc Intersection of the left parietal and temporal and occipital bones
- 23 Rpateoc Intersection of the right parietal and temporal and occipital bones

Right Lateral View

- 24 Rprenas Intersection of premaxilla and nasal bones on the right nasal notch
- 25 Rmaxinc Most anterior point on the right premaxilla the incisal alveolus
- 26 Rmol1 Rostral point on the right first molar on the alveolus
- 27 Rmol2 Rostral point on the right second molar on the alveolus
- 28 Rmol3 Rostral point on the right third molar on the alveolus
- 29 Rretro Caudal point on the right third molar on the alveolus
- 30 Rparacn Right paracondylar process on the caudoinferior point
- 31 Rintpar Lateralmost intersection of the right parietal and interparietal and and occipital bones
- 32 Rfrpatm Intersection of the right frontal and parietal and temporal bones
- 33 Rlnfmz Inferior aspect of the intersection between right maxillary and zygomatic bones
- 34 RSupmz Superior aspect of the intersection between right maxillary and zygomatic bones
- 35 Rlnftz Inferior aspect of the intersection between right temporal and zygomatic bones
- 36 RSuptz Superior aspect of the intersection between right temporal and zygomatic bones

Left Lateral View

- 37 Lprenas Intersection of premaxilla and nasal bones on the left nasal notch
- 38 Lmaxinc Most anterior point on the left premaxilla the incisal alveolus
- 39 Lmol1 Rostral point on the left first molar on the alveolus
- 40 Lmol2 Rostral point on the left second molar on the alveolus
- 41 Lmol3 Rostral point on the left third molar on the alveolus
- 42 Lretro Caudal point on the left third molar on the alveolus
- 43 Lparacn Left paracondylar process on the caudoinferior point
- 44 Lintpar Lateral most intersection of the left parietal and interparietal and occipital bones
- 45 Lfrprtm Intersection of the left frontal and parietal and temporal bones
- 46 Llnfmz Inferior aspect of the intersection between left maxillary and zygomatic bones
- 47 LSupmz Superior aspect of the intersection between left maxillary and zygomatic bones
- 48 Llnftz Inferior aspect of the intersection between left temporal and zygomatic bones
- 49 LSuptz Superior aspect of the intersection between left temporal and zygomatic bones

Inferior View

- 50 incisiv Incisive foramen anterior border at midline
- 51 premax Midline intersection of the premaxilla and maxilla bones
- 52 mxpalat Midline intersection of the maxilla and palate bones
- 53 cns Caudal nasal spine
- 54 presphn Midline intersection of the presphenoid and sphenoid bones
- 55 basion Midline on the rostral border of the foramen magnum
- 56 opisth Midline on the caudal border of the foramen magnum
- 57 Lantpal Most anterior point on the left mid-palate foramen
- 58 Rantpal Most anterior point on the right mid-palate foramen
- 59 Llnfmax Intersection of the left maxilla and premaxilla bones lateral to mid-palate foramen
- 60 Rlnfmax Intersection of the rightmaxilla and premaxilla bones lateral to mid-palate foramen
- 61 Lpospal Most posterior point on the left mid-palate foramen
- 62 Rpospal Most posterior point on the right mid-palate foramen

63 Lmaxpal Most lateral aspect of the intersection between the left palate and maxilla bones  
64 Rmaxpal Most lateral aspect of the intersection between the right palate and maxilla bones  
65 Lpalsph Most lateral aspect of the intersection between the left palate and sphenoid bones  
66 Rpalsph Most lateral aspect of the intersection between the right palate and sphenoid bones  
67 Linpter Most inferior point on the hamulus of the left internal pterygoid process  
68 Rinpter Most inferior point on the hamulus of the right internal pterygoid process  
69 Lexter Most inferior point on the left external pterygoid process  
70 Rexter Most inferior point on the right external pterygoid process  
71 Lbasocc Left lateral aspect of the intersection between occipital and sphenoid bones  
72 Rbasocc Right lateral aspect of the intersection between occipital and sphenoid bones

Mandible: Left Lateral View

73 mLcond Caudal point on the left mandibular condyle  
74 mLrcond Rostral point on the left mandibular condyle  
75 mLnotch Left mandibular notch  
76 mLsubcn Rostral point on the notch above the left gonial process (subcondylar notch)  
77 mLcor Superior point on the left coronoid process  
78 mLgon Caudal point on the left gonial process  
79 mLprean Superior point on the bottom margin of the left corpus (preangular notch)  
80 MLSupin Superior point on the left incisal alveolus  
81 mLInfin Inferior point on the left incisal alveolus  
82 mLalvc Intersection of alveolar rim and coronoid process on the left side  
83 mLmol1 Rostral point on the left first molar on the alveolus  
84 mLmol2 Rostral point on the left second molar on the alveolus  
85 mLmol3 Rostral point on the left third molar on the alveolus  
86 mLretro Caudal point on the left third molar on the alveolus  
87 mLinram Inferior point on the left incisal ramus  
88 mLinnch Left incisal notch

Mandible: Right Lateral View

89 mRcond Caudal point on the right mandibular condyle  
90 mRrcond Rostral point on the right mandibular condyle  
91 mRnotch Right mandibular notch  
92 mRsubcn Rostral point on the notch above the right gonial process  
93 mRcor Superior point on the right coronoid process  
94 mRgon Caudal point on the right gonial process  
95 mRprean Superior point on the bottom margin of the right corpus  
96 mRSupin Superior point on the right incisal alveolus  
97 mRInfin Inferior point on the right incisal alveolus  
98 mRalvc Intersection of alveolar rim and coronoid process on the right side  
99 mRmol1 Rostral point on the right first molar on the alveolus  
100 mRmol2 Rostral point on the right second molar on the alveolus  
101 mRmol3 Rostral point on the right third molar on the alveolus  
102 mRretro Caudal point on the right third molar on the alveolus  
103 mRinram Inferior point on the right incisal ramus  
104 mRinnch Right incisal notch