Huston, Lila A., Osteogenesis Imperfecta: An analysis of the inner ear development in *Mus musculus* (house mouse) with comments on hearing quality. Master of Science in Medical Sciences – Anatomy, May 2022

Osteogenesis imperfecta (OI), a developmental disorder of type I collagen, is known to cause hearing loss in ~ 60% of the diseased population. Identified forms include conductive hearing loss (17.4% of OI patients), involving loss of function within the ossicular chain, and sensorineural hearing loss (25.8%), resulting from damage to the cochlea, with the most predominant form being mixed hearing loss (56.8%), involving damage to both the cochlea and ossicles. While OI-related pathologies have largely focused on the middle ear, the pathological appearance of the cochlea (the organ most often compromised in OI-related hearing loss) has gained little focus. In this study, we examine OI-related pathologies on the cochlea in a mouse model for the severe type III OI, to document 1) the morphological differences in the inner ear for adult wildtype mice compared to OI mice in order to determine the anatomy of the diseased state, and 2) intraindividual variation between cochlea of WT and OI mice to determine potential asymmetry in the etiology of the inner ear. We hypothesize that cochlea in mice with OI will have less consistent morphology overall than their WT counterparts due to abnormal growth of the bony capsule.

4 week and 16 week old OIM mice (B6C3Fe a/a-Col1a2^{oim}/J) (n=25) were compared to unaffected wildtype (WT) littermates (n=29) with no known hearing defects. High-resolution micro-CT scans were created for all specimens and 3D models and volumes of the cochlea were generated using 3D Slicer software. Two-tailed Mann-Whitney U-tests were used to investigate differences between 1) right and left ears of the same mouse to examine intraindividual symmetry and 2) differences in volumes between WT and OI cochlea.

No major morphologic differences between OI and WT were observed, except for minor areas of higher ossification at the base of the cochlea, mostly within the OI sample. Within WT specimens, we observed little intraindividual difference in the cochlear volume (0-3%). Within OI specimens, significant differences were observed in cochlear volume between right and left ears in the same animal, indicating potential unilateral effects (Mann-Whitney U, $p<0.05$). When average WT and OI volumes were compared, there was much overlap between the two samples although the OI volumes had a significantly larger range than the WT range (Mann-Whitney U, $p=0.704$ (w16), $p=0.703$ (w4)).

Overall, our results indicate that mice with OI are much more likely to have evidence of unilateral cochlear volume losses, despite very little difference in overall shape appearance, possibly due to bony capsule encroachment. This find indicates an extremely high potential for sensorineural and mixed hearing loss in OI-bred mice and elucidates at least one mechanism behind how this type of hearing loss might be occurring.

Little is known about the pathological appearance of the cochlea in OI, leading to difficulty in managing hearing loss. Further investigation of the etiology and progression of cochlear pathologies will allow for better outcomes in hearing for those patients afflicted with OI-related hearing loss.

OSTEOGENESIS IMPERFECTA: AN ANALYSIS OF INNER EAR DEVELOPMENT IN *MUS MUSCULUS* (HOUSE MOUSE) WITH COMMENTS ON HEARING QUALITY

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OSTEOGENESIS IMPERFECTA: AN ANALYSIS OF INNER EAR DEVELOPMENT IN *MUS MUSCULUS* (HOUSE MOUSE) WITH COMMENTS ON HEARING QUALITY

INTERNSHIP PRACTICUM REPORT

Presented to the Graduate Council of the University

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By Lila Athena Huston, MS, BA

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CHAPTER 1: SUMMARY

Osteogenesis Imperfecta (OI) is a congenital disease affecting the formation, integrity and amount of type I collagen in the body. It is often characterized by its effects on the bone, leading to increased fractures, and shortened height in humans. Additionally, OI has many effects on the soft tissue in the body, including blue sclera, loose joints, and, most importantly for the purposes of this study, hearing loss (Forin, 2007).

Hearing loss associated with OI was first reported in 1912 by Adair-Dighton, and since has been a commonly reported symptom associated with the disease (Carre *et al*., 2019). As discussed in Pillion *et al.'s* 2011 review, a range of 46% to 57.9% of adults with OI have self-reported hearing loss, while 62% of OI patients were further diagnosed with mild to profound hearing loss upon routine clinical examinations. In patients with type I OI a 95% rate of hearing loss has been reported after the age of 30 (Pillion *et al.*, 2011). This hearing loss has been observed as being conductive (affecting the middle ear), sensorineural (affecting the inner ear), or mixed (affecting both).

The primary interests of this study are in the bony otic capsule of the cochlea in the inner ear, as little attention has been directed toward OI pathologies in this region of the ear. In this study, the goal was to document the anatomical and developmental effects of OI in a sample of mice specifically bred to have the OI Type III variant. This study's aims are to 1) establish the morphological differences in the inner ear for adult wildtype mice compared to OI mice, 2)

examine and compare intraindividual variation between cochlea in WT and OI mice. These regions were examined using high resolution CT scans and visualized using 3D dissecting techniques.

CHAPTER 2: PROJECT GOALS

The goal of this project was to document the anatomical and developmental effects of OI in a sample of mice specifically bred to have the OI Type III variant. This project had two specific aims: 1) **establish the morphological differences in the inner ear for adult wildtype mice compared to OI mice** and 2) **examine and compare intraindividual variation between cochlea in WT and OI mice.** We hypothesized that cochlea in mice with OI will show less consistent morphology overall than their WT counterparts due to abnormal growth of the bony capsule. These regions were examined using high-resolution CT scans and visualized using 3D segmentation techniques. In addition, almost all studies have been performed on small experimental groups, many of which included only post-mortem samples. This has been remedied by using a larger sample size and gathering data from properly preserved specimens.

CHAPTER 3: SIGNIFICANCE

OI is often seen as a disease of fractures of the long bones. Lack of structural integrity and flexibility normally afforded by collagen leads to extremely brittle bones, leading in turn to increased fractures. This quality is what gives OI its colloquial name, “brittle bone disease”. However, as evidenced by the high rate of hearing loss in those with OI, it is not a disease exclusive to these large bones. This research aims to further what is already known about hearing loss in those with OI, specifically quantifying the morphological differences between those with hearing loss with OI and those without. Due to the limited research done in the inner ear, our work is among some of the primary efforts to quantify the detailed changes of anatomy in these areas during progression of OI.

The relative lack of in-depth study on the morphological differences of the cochlea in those with OI is a key force driving my study of these factors. By having a more concrete understanding of the morphological changes in the ear, specifically in the cochlea (the sensorineural hearing organ), steps can be taken in clinical practice to correctly diagnose and treat hearing loss in those suffering with OI. Further investigation of the etiology and progression of cochlear pathologies will allow for better outcomes in hearing for those patients afflicted with OI-related hearing loss.

CHAPTER 4: BACKGROUND

4.1 Osteogenesis Imperfecta

Osteogenesis Imperfecta (OI) is a congenital disease affecting the formation, integrity and amount of type I collagen in the body. It is often characterized by its effects on the bone, leading to increased incidence of fractures, and shortened height in humans. Additionally, OI has many effects on the soft tissue in the body, resulting in blue sclera, loose joints, and, most importantly for the purposes of this study, hearing loss (Forin, 2007).

There are several types of OI, with different symptoms and different population incidences. The most common variety, type I, is mild with a high incidence (60% of OI cases are type I), and results in increased fractures and little to no deformity, with a relatively high chance of hearing loss. Type II is the most severe kind of the disease, resulting in death at a very young age, so little is known about the differences in the phenotype as they age. Type II OI occurs in approximately 10% of the diseased population. Type III OI is an autosomal dominant form of the disease, with severe outcomes, and an incidence of approximately 10%. It has many of the same symptoms exhibited, but with more severe outcomes. These symptoms include severely decreased height, deformed limbs, and scoliosis. These individuals are also at high risk for hearing loss and exhibit more craniofacial symptoms overall.

Hearing loss associated with OI was first reported in 1912 by Adair-Dighton, and since has been a commonly reported symptom associated with the disease (Carre *et al.*, 2019). As discussed in Pillion *et al.*'s 2011 review, a range of 46% to 57.9% of adults with OI have self-

reported hearing loss, while 62% of OI patients were further diagnosed with mild to profound hearing loss upon routine clinical examinations. Of those with OI related hearing loss, 17.4% have hearing loss resulting from damage to the middle ear ossicles, 25.8% have damage to the cochlea, and 56.8% have mixed hearing loss, resulting from damage to both (Hartikka *et al.,* 2004).

Previous studies that endeavor to analyze hearing loss with OI are not discriminatory by phenotype of OI. Generally, as type I OI is the most common, and mildest form of the disease, most adults with OI that have hearing loss are type I. In studies including type III OI individuals, the population often consists of mixed OI phenotypes. Therefore, there is limited information on the rate of hearing loss in type III OI individuals alone.

Table 1: Targeted structures for hearing loss in OI, and biomechanics of diseased state.

Target Structure	**Pathology**	**Sources**
Cochlea	- Potential increased density of the bony labyrinth	- Swinnen *et al.*, 2012 - Shapiro *et al.*, 1982

Stapes	- Increased porosity - Compromised crus of the stapes - Otosclerotic fixation of the stapedial footplate	- Sando *et al*., 1981 - Swinnen *et al*., 2012 - Waissbluth *et al.*, 2020

4.2 Anatomy and biomechanics of hearing

To understand the effects of OI on hearing, it is important to understand the anatomy and biomechanics of sound transfer for hearing. The ear itself is divided into 3 parts: the outer, middle, and inner ear. Sound waves first enter the ear as soundwaves and are funneled through the ear canal to the tympanic membrane and the middle ear. As sound waves travel through the outer ear (consisting of the pinna or visible ear, and the auditory canal) sound is channeled towards the tympanic membrane. Medial to the tympanic membrane is the middle ear, which houses three small bones known as the ossicles. These are specialized bones that vibrate, and transmit sound through the oval window, which is a membranous connection to the inner ear. The inner ear contains fluid mediums that assist with transmission of vibrations to specialized nerve cells, which then transmit the vibrations of the soundwaves to the higher nervous system for processing. OI has been shown to specifically affect the conductive (middle) and neural (inner) parts of the ear, so the rest of this section will focus on those regions.

4.2.1. The middle ear

The middle ear houses three ear ossicles (malleus, incus, and stapes) that act as a conductive chain by transmitting sound vibrations from the vibrating tympanic membrane through the middle ear to the oval window of the cochlea (Keith *et al.*, 2006). Along this pathway, the ossicles act as a lever system, amplifying the sound waves as they move through the chain by vibrating the bones, so that the amplitude is increased by the time it gets to the cochlea, allowing for more accurate hearing (Keith *et al.*, 2006). Thus, this is referred to as conductive hearing.

The stapes is the most medial bone of the ossicular chain, and serves as the final conductor of sound, attaching directly to the oval window by the stapedial footplate (Fig. 1). A tendon attaches the stapes to the stapedius muscle at the neck of the stapes, superior to the anterior crus of the stapes (Svensson *et al.*, 2017). This tendon functions to dampen the vibrations of the ossicular chain, (reducing the amplitude of the waves traveling through the ossicular chain) to protect the oval window and cochlea from noise "blow-out" (Svensson *et al.*, 2017).

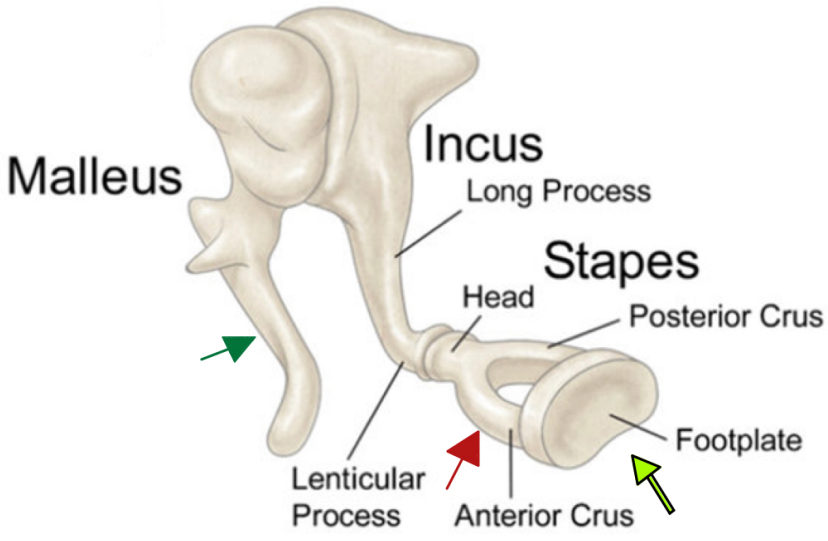

Figure 1: Human ossicles (Han *et al*., 2019). Above is an image of healthy human ossicles. Note the arrows indicating the footplate (neon green), the anterior crus (red) and the malleus (dark green).

4.2.2. The inner ear

The inner ear is comprised of a single membranous organ with dual function and its surrounding bony and neural tissue (Keith *et al.*, 2006). The vestibulocochlear organ can be divided into its vestibular portion, for balance, and the cochlea, for hearing (Keith *et al.*, 2006). In normal function, the cochlea is the primary sensory organ of hearing and transforms vibrations received from the ossicular chain of the middle ear into nerve impulses that are sent along cranial nerve VIII to be processed by the brain (Keith *et al.*, 2006). Within the cochlea there are three chambers, the scala tympani, scala media (cochlear duct), and scala vestibuli (Fig. 2) (Singh *et al.*, 2016). These chambers contain fluid mediums, perilymph (within scalas tympani and vestibuli) and endolymph (scala media) which transfer soundwaves through the organ. This fluid is responsible for the movement of the tectorial membrane, which lays over the hair cells (the specialized neuroreceptors within the ear) (Keith *et al.*, 2006). The movement of the tectorial membrane causes the cilia of the hair cells to bend, creating a neural impulse. This impulse is then sent to the brain along cranial nerve VIII and processed as sound (Keith *et al.*, 2006).

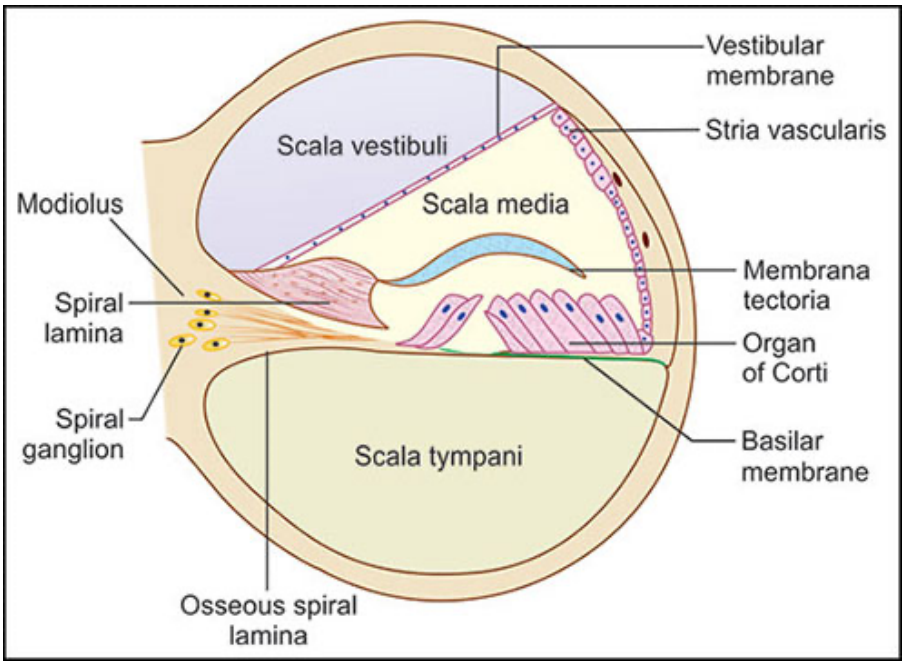

Figure 2: Cross section of the cochlea, as described above (Singh *et al*., 2016).

4.2.3 Development of the ear

In humans, cochlear and ossicular development and ossification are complete at birth. However, in mice, the model being used for this study, both the ossicles and the cochlea are cartilaginous at birth, allowing for observation of the ossification process. Ossicle ossification occurs at around day 5 in newborn mice, at which point the ossicular chain will be fully functional (Raft *et al*., 2014). However, cochlear bony capsule ossification is not complete until around day 15, preventing full ear function in newborn mice until this time point (Gao *et al*., 2011).

4.3 OI-observed anatomical pathologies

Currently, most research on OI-related hearing pathologies have focused on the middle ear. In the few studies that have examined anatomical changes associated with OI related hearing

loss, alterations have been identified in bone makeup and structure of the ossicular chain (Stewart and O'Reilly, 1989). In particular, the stapes is the key ossicle affected by OI as shown in observational studies in humans, with effects appearing as increased porosity of the crura (or bend) and increased otosclerotic fixation of the stapedial footplate (Waissbluth *et al.*, 2020). Stapedial footplate thickness leading to otosclerotic fixation has been observed in those with hearing loss due to OI (Waissbluth *et al*., 2020). If one of the ossicles cannot move, (as is present in otosclerotic fixation), that would likely either decrease the amplitude or completely halt the sound wave from traveling through that bone. If part of the chain is porous or fragile (as in the crus), disruption would occur, but an extensive literature search has not revealed a specific mechanism. A disruption of the sound waves through the ossicular chain would result in a condition of deafness known as conductive hearing loss.

While there is limited research on the effects of OI on the cochlea, studies performed in patients with OI-related hearing loss revealed increased bone density in the inner ear structures, particularly the bony otic capsule of the cochlea (Swinnen *et al.*, 2012). There are several hypotheses regarding the cause of loss of cochlear function in OI, which include encroachment of petrous bone on the cochlea, hemorrhage of blood into the labyrinth, atrophy of the stria vascularis, the blood supply of the cochlea, or atrophy of the cochlear hair cells (Shapiro *et al.*, 1982). However, research on this topic is limited and the exact cause of damage to the cochlea in patients with OI is mostly unknown. Deafness due to damage of the cochlea is referred to as sensorineural hearing loss, as it is considered damage to components of the peripheral nervous system, and not an issue related to the initial conduction of sound waves in the outer and middle ear.

It is also important to consider that, while there are many types of OI, the majority of individuals examined in human studies have type I OI. This is due to type I OI being the most common form of this pathology, and the form with the highest life span. As mentioned prior, studies of hearing loss often do not discriminate between types of OI, so this background will accordingly be a general overview, and not discriminatory between individual types of OI.

4.3.1 Stapes

In OI, given the compromised crura of the stapes and the otosclerotic fixation in the footplate, the transmission of soundwaves to the oval window is interrupted, possibly leading to conductive hearing loss. The porosity of the crura leads to decreased function in many individuals, given the increased fragility and continuous degradation throughout development (Sando *et al.*, 1981). In some cases, this fragility can even lead to complete disconnection from the footplate, rendering the stapes useless in the ossicular chain (Santos *et al*., 2012). Otosclerotic fixation of the stapedial footplate leads to the inability to move, therefore disallowing the stapes normal function in the ossicular chain, preventing the conduction of sound (Santos *et al.*, 2012). Both abnormalities would likely lead to an unstable and nonfunctional stapes, leading to conductive hearing loss, given the detriment to the ossicular chain (Raveh *et al.*, 2006).

Overall, the middle ear has been the subject of further research than the inner ear as it pertains to OI. Conductive deafness as caused by OI has been further defined and has a clearer etiology than sensorineural deafness resulting from OI. The intentions of this study are to expand on the biomechanics and etiology of the inner ear in those with OI.

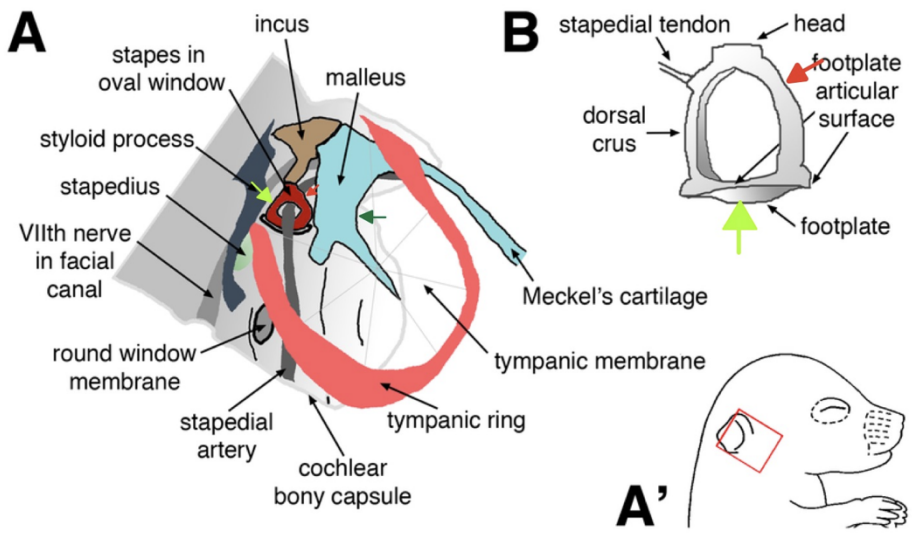

Figure 3: Mouse Ossicles at day 5 post birth (fully ossified) (Raft *et al.*, 2014). Note the similarities between the mouse and human ossicular chains (reference Figure 1), as well as the position and small size of the stapes.

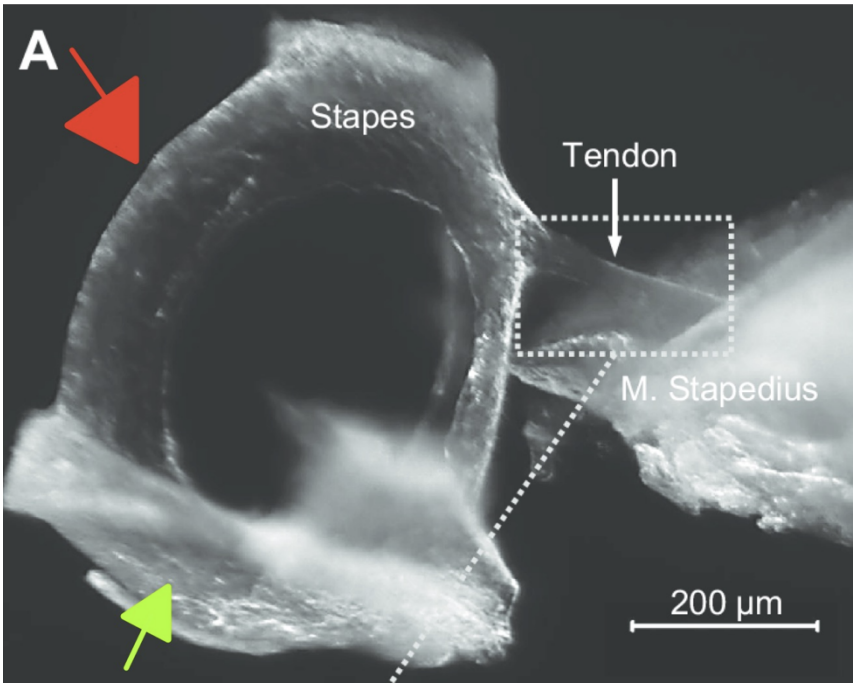

Figure 4: Mouse stapes and tendon, CT scan, post-mortem (Svensson *et al.*, 2017). Note again the positioning, with the oval window being below the stapedial footplate (neon green arrow).

4.3.2 Cochlea

Limited space due to potentially encroaching growth of the petrous bone has the potential to disrupt space for amplitude of sound waves traveling through perilymph and endolymph, which can affect cochlear function, as it relies on ample space for its fluid components and hair cells to sense sound waves. Furthermore, hemorrhage of endolymph or perilymph or atrophy of hair cells and blood supply could also lead to loss of function, all with the potential to cause sensorineural hearing loss. As OI is a disease primarily affecting collagen and bone, the cochlea's otic capsule has been observed in this study. Primarily, analysis to determine changes in the density of the otic capsule that have potential to damage soft tissue components has been completed, but little is understood about the morphology (De Paolis *et al.*, 2021). Changes to the bony capsule have the potential to affect the membranous cochlea within, which in turn could cause diminished or loss of sensorineural hearing. Therefore, in order to understand the etiology of this disease, the morphology must also be explored.

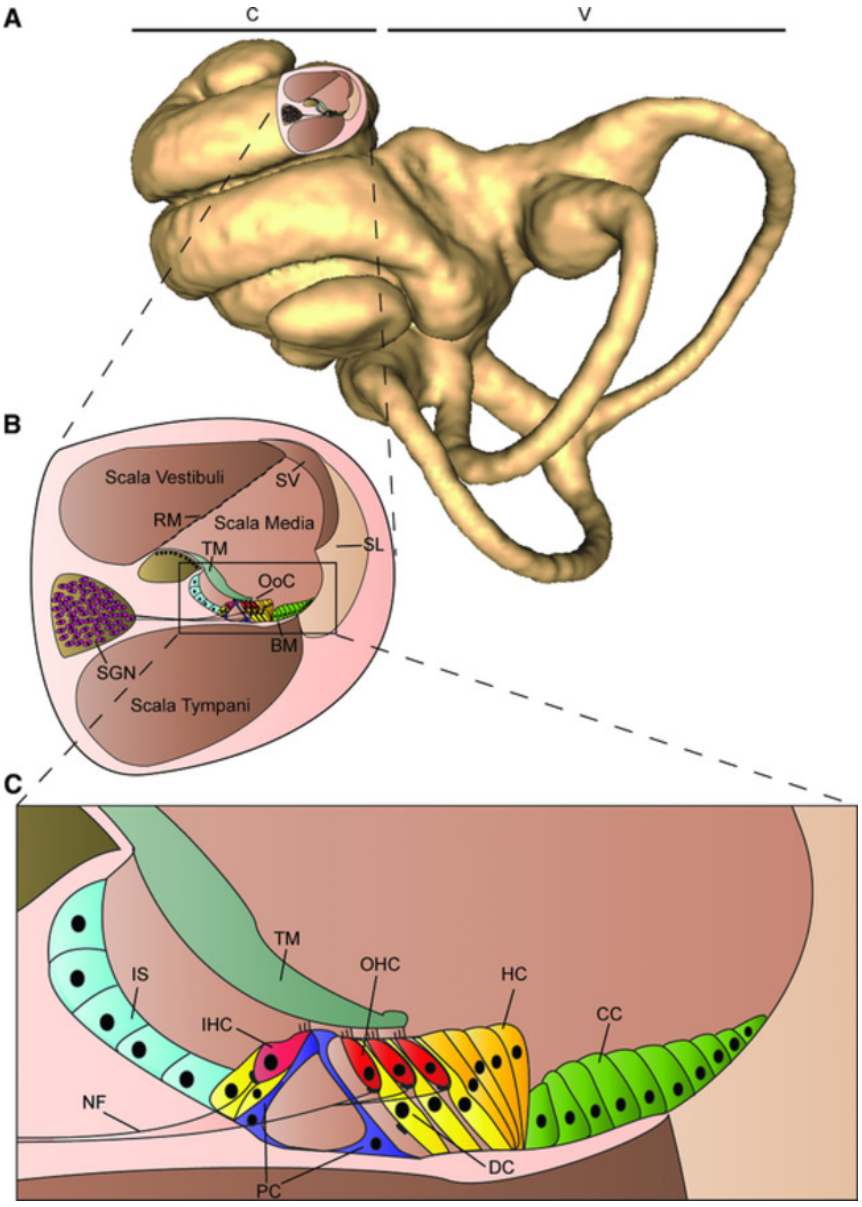

Figure 5: Mouse cochlea (Parker *et al.,* 2016). Illustration showing the general structure of a mouse cochlea, including hair cells (labeled HC in the figure).

4.3.3 Hearing loss with OI

Hearing loss affects approximately 60% of individuals with OI, and much of this hearing loss affects the cochlea (Swinnen *et al.*, 2012). It has been seen in past studies that OI related hearing loss that there is some asymmetry in those with OI related hearing loss (Hartikka *et al.*,

2004). This asymmetry was seen in individuals having different forms of hearing loss across right and left ears (such as sensorineural hearing loss in the right ear, and mixed hearing loss in the left ear). As OI is a disease resulting in pathological bone growth, this may contribute to these asymmetrical hearing loss patterns.

CHAPTER 5: RESEARCH DESIGN AND METHODS

5.1 Project Aims

Aim 1: Establish the morphological differences in the inner ear for adult WT mice compared to OI mice in order to determine the anatomy of the diseased state.

Analysis of the bony structures within the ear, and the differences in morphology between OI and WT mice is necessary for establishing the etiology of hearing loss related to OI.

Prediction:

H_1: We expect to observe bony intrusion of the otic capsule on the hollow portion of the cochlear spiral as evidenced by smaller cochlear volumes in specimens with OI.

Aim 2: Using established morphological differences from the first aim, examine intraindividual variation between cochlea of WT and OI mice, and use intraindividual differences to determine potential asymmetry in the etiology of the inner ear.

Analysis of bilateral cochlea in WT and OI mice might elucidate a potential mechanism (pattern) for sensorineural and/or mixed hearing loss in those with OI.

Prediction:

H_2: We expect that there will be evidence of asymmetrical volumes between intraindividual cochlea within the OI population.

5.2 Mouse colony and developmental time points

Our selected strain of mouse, B6C3Fe a/a-Col1a2^{oim}/J, referred to as the oim model or OI mouse, has similar symptoms and outcomes as individuals with either type I or type III OI, due to a mutation in the COL1A2 gene. A heterozygous mutation will result in a phenotype similar to OI type I, and a homozygous mutation will result in an OI type III phenotype. These distinct differentiations allow for specific study of the diseased state in the oim model. In this study, only homozygous specimens were used.

The first and youngest time point, week 4, will emphasize the focus on early development and examining how the ossicles are affected throughout growth and maturation. The second and oldest time point, 16 weeks post birth, is considered representative of the adult mouse, with a fully developed cranium and highest potential for capturing OI-induced abnormalities.

The sample group being studied includes roughly equal numbers of both wildtype and OI mutated mice that are homozygous for the COL1A2 oim gene. The adult group consists of 12 wildtype mice and 11 oim. The juvenile group consists of 17 wildtype mice and 14 oim.

Table 2: Population sizes at each selected time point.

Timepoint	**OIM**	**WT**
Week 4	14	17
Week 16	11	12

5.3 Digital data collection

All data has been collected from micro-CT scans, with scans of the adult population having been collected at Indiana University's Center for Musculoskeletal Health facility on

Skyscan 1176 micro-CT machine. The scanning resolution for the post-weaning populations is 0.008 or 0.017 mm^3 voxels, depending on cranial length.

Hounsfield units (hu) are used to determine density in CT scans, with higher hu representing dense matter, like bone, and lower hu representing soft tissue and cartilage. The otic capsule itself is composed of bone, but the hollow portion of the otic capsule is filled with fluid and membranous tissue, so analysis of the cochlea was completed by examining the low hu areas of the otic capsule.

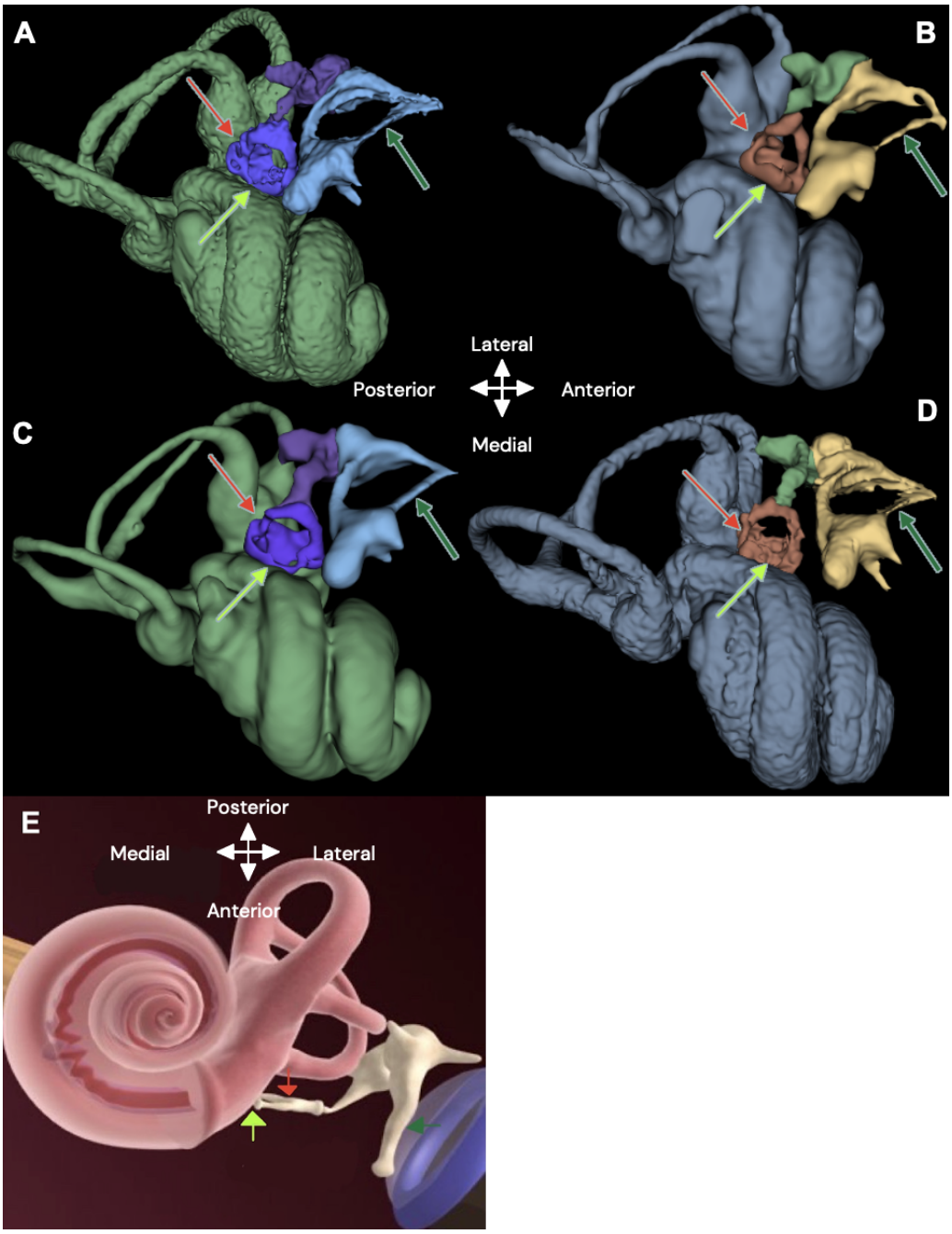

Figure 6: 3D renderings of mouse inner ear models compared to an artistic rendering of a human ossicular chain and cochlea (American Speech-Language-Hearing Association). A: Week 4 wildtype. B: Week 4 OI. C: Week 16 wild type. D: Week 16 OI. E: Human ossicles. Note the arrows indicating the footplate (neon green), the anterior crus (red) and the malleus (dark green), and the similarities in the structures to the human specimen, as indicated by the arrows.

5.3.1 Segmentation protocol

In order to assess changes to the bony anatomy, the inner ear was digitally segmented from CT scans using the imaging software 3D Slicer and Fiji ImageJ. The tools provided in 3D slicer were used to segment the otic capsule of the cochlea from CT scans, by adjusting the threshold observed of the CT scans. While normal thresholding techniques work for extracting most of the petrous bone and middle ear from CT scans, adjustments must be made when attempting to segment out the otic capsule, due to their lower threshold.

To create the 3D models, the respective CT scan was uploaded to the software ImageJ. Using the distinctive spiral of the cochlea in a coronal view, a crop of the image stack that included the left and right ears of the specimen was made to reduce the file size. After this cropping was saved, the CT scan was examined in the transverse view, leaving only the right and left ears and cranium in between in the scan. After cropping was completed, the CT was converted to a Nrrd file, which is compatible with the software 3D Slicer. This file was then uploaded to 3D Slicer in order to begin creation of the model, allowing for a view of both coronal, sagittal, and transverse views of the cropped scan.

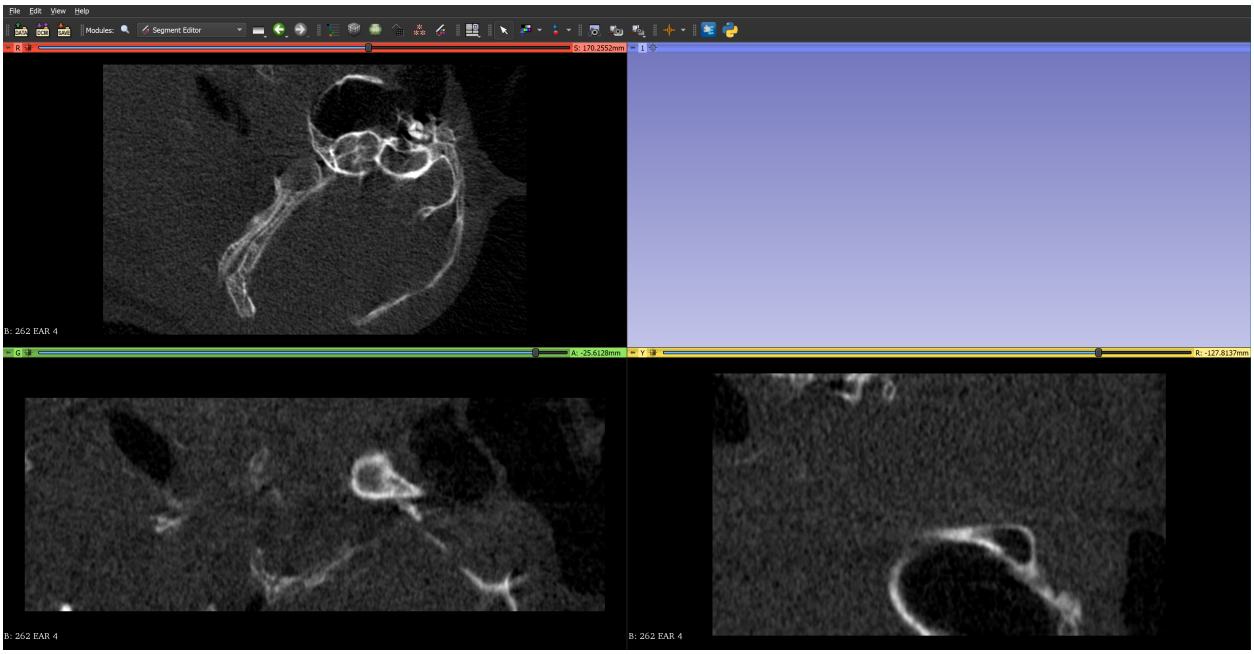

Figure 7: Example of a segmentation set up within 3D slicer with an uploaded CT scan.

The initial steps for segmentation were to digitally "fill" the majority of the bony cochlea, again using the spiral evident on the CT scan in order to make a 3D model of the hollow space within the bony cochlea (Fig. 7). The "paint" tool within 3D Slicer was used, with the threshold adjusted to paint in low hu units, as mentioned prior (Fig. 7). After the cochlea was filled in, the central aqueduct was removed so that a volumetric measure of only the cochlear duct was captured.

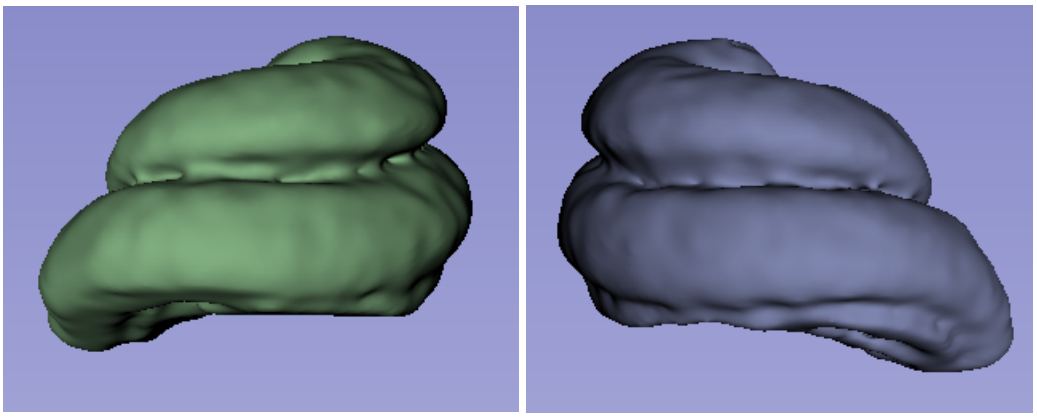

Figure 8: Bilateral WT cochlea 3D models as segmented in 3D slicer. Taken from specimen 79.

5.3.2 Data collection and analyses

The **two methods** are used to look for bone changes. In the **first method,** anatomical differences were observationally documented between the wild type and OI mice in both adult and juvenile populations. Observations were made of any potential bony intrusions within the cochlea, seen as high hu areas within the cochlear spiral.

In the **second method**, cochlear duct volumes were collected to assess quantitative changes in otic density relative to cochlear volume of the inner ear. To do this, the cochlea was cropped from the semicircular canals at the point of the vestibule, and volumetric data was collected using the measurement tool in 3D Slicer.

Raw cochlear duct volumes collected from 3D Slicer were scaled in order to get the true size of the cochlea within the cranium. Scaling was completed by use of centroids, a correcting factor that is calculated from data obtained from points on individual mouse crania and is used as an approximation of cranium. Centroid sizes used in this study were unique to each specimen use as they were calculated individually from landmarking data taken from each specimen's cranium. In order to calculate the scaled data, the raw cochlear volumes taken from the 3D models was divided by centroid size (Formula: Raw cochlear volume / centroid size = scaled cochlear volume). To compare nonparametric data within age groups, to test cochlear duct volume differences between OI and WT groups (**aim 1**), a Mann-Whitney U test was run. Additionally, to compare differences in intraindividual cochlea, an intraindividual variation variable was calculated (**aim 2**) and a Mann-Whitney U test was completed. This variable was

calculated by subtracting bilateral volumes from each other, and taking the absolute value of the result. (Formula: | Left cochlea – Right cochlea | = Intraindividual variation).

CHAPTER 6: RESULTS

Data generated from the 3D models is listed in tables 3 and 4. This includes the number of the specimen, their age, centroid size, raw data from right and left cochlea, phenotype, scaled data from right and left cochlea, and the differences of those scaled data. This data is further discussed throughout this section

Table 3: Week 4 cochlear volumes, including raw volumes, centroid sizes, volumes by centroid size, and calculated difference between intraindividual specimens.

Mouse Number	Week	Centroid	Volumes (L) (cm^3)	Volumes (R) (cm^3)	Phenotype	Scaled Volumes (L) (cm^3)	Scaled Volumes (R) (cm^3)	Differences
80	4	50.63	291.403	269.186	OI	5.756	5.317	0.439
81	4	50.828	241.059	247.078	OI	4.743	4.861	0.118
235	4	48.463	230.868	231.839	OI	4.764	4.784	0.02
194	4	49.411	264.794	263.11	OI	5.359	5.325	0.034
220	4	50.003	241.117	244.275	OI	4.822	4.885	0.063
262	4	48.714	247.355	252.987	OI	5.078	5.193	0.116
192	4	50.946	257.932	251.573	OI	5.063	4.938	0.125
115	4	48.869	296.335	303.5	OI	6.064	6.21	0.147
193	4	49.412	224.743	232.937	OI	4.548	4.714	0.166
82	4	49.412	228.556	239.807	OI	4.625	4.853	0.228
233	4	50.419	236.741	223.695	OI	4.695	4.437	0.259
221	4	51.084	229.488	245.157	OI	4.492	4.799	0.307
108	4	45.433	348.167	368.167	OI	7.663	8.104	0.44
234	4	48.15	271.985	247.45	OI	5.649	5.139	0.51
249	4	52.084	258.362	258.366	WT	4.96	4.961	0
257	4	54.01	260.314	260.433	WT	4.82	4.822	0.002
259	4	52.272	243.23	242.824	WT	4.653	4.645	0.008
256	4	53.46	262.422	261.449	WT	4.909	4.891	0.018
152	4	54.799	322.492	320.885	WT	5.885	5.856	0.029
254	4	53.415	254.389	252.685	WT	4.762	4.731	0.032
253	4	53.508	260.779	259.031	WT	4.874	4.841	0.033
153	4	54.493	312.95	315.012	WT	5.743	5.781	0.038
248	4	52.782	264.357	262.202	WT	5.008	4.968	0.041
156	4	54.116	299.982	302.98	WT	5.543	5.599	0.055
107	4	49.754	363.897	361.027	WT	7.314	7.256	0.058
246	4	53.255	262.616	259.148	WT	4.931	4.866	0.065
116	4	51.086	318.72	314.968	WT	6.239	6.165	0.073
86	4	50.115	260.081	254.726	WT	5.19	5.083	0.107
159	4	54.679	335.137	341.932	WT	6.129	6.253	0.124
154	4	52.922	301.915	308.873	WT	5.705	5.836	0.131

Table 4: Week 16 cochlear volumes, including raw volumes, centroid sizes, volumes by centroid size, and calculated difference between intraindividual specimens.

Mouse Number	Week	Centroid	Volumes (L) (cm^3)	Volumes (R) (cm^3)	Phenotype	Scaled Volumes (L) (cm^3)	Scaled Volumes (R) (cm^3)	Differences
80	16	54.674	328.915	318.89	OI	6.016	5.833	0.183
76	16	54.356	242.207	253.254	OI	4.456	4.659	0.203
194	16	53.815	224.42	213.396	OI	4.17	3.965	0.205
221	16	56.417	182.043	194.355	OI	3.227	3.445	0.218
81	16	59.216	297.443	314.153	OI	5.023	5.305	0.282
72	16	51.936	308.483	292.895	OI	5.94	5.64	0.3
220	16	54.996	176.456	192.972	OI	3.209	3.509	0.3
192	16	55.487	200.514	217.486	OI	3.614	3.92	0.306
193	16	53.067	170.733	201.742	OI	3.217	3.802	0.584
67	16	53.071	182.18	213.962	OI	3.433	4.032	0.599
115	16	54.379	273.071	236.615	OI	5.022	4.351	0.67
154	16	57.791	211.263	214.279	WT	3.656	3.708	0.052
152	16	58.76	239.598	239.516	WT	4.078	4.076	0.001
66	16	57.795	223.639	223.335	WT	3.87	3.864	0.005
86	16	57.582	256.667	257	WT	4.457	4.463	0.006
153	16	57.88	202.605	203.364	WT	3.5	3.514	0.013
156	16	58.487	237.233	234.281	WT	4.056	4.006	0.05
159	16	57.603	263.332	266.649	WT	4.572	4.629	0.058
73	16	53.176	303.23	300.082	WT	5.702	5.643	0.059
75	16	59.425	250.498	254.043	WT	4.215	4.275	0.06
79	16	59.019	247.33	251.508	WT	4.191	4.261	0.071
71	16	59.455	262.571	257.657	WT	4.416	4.334	0.083
116	16	56.519	214.347	220.297	WT	3.792	3.898	0.105

6.1 Gross cochlear shape changes

Gross morphological changes between WT and OI cochlea were observed visually. In OI mice, notable areas of higher ossification appear as protrusions or deformities throughout the cochlear spiral. Overwhelmingly these deformities were seen within OI specimens, in 29 out of 50 of OI cochlea across both age groups. These deformities occurred mostly unilaterally, with only 7 of the 25 OI mice having bilateral intrusions. These intrusions were not consistently seen in one side over the other, with 6 of the 15 unilateral intrusions occurring on the right side, and 9 occurring on the left side. In comparison, the cochlea of WT mice exhibited deformities in 9 of 58 specimens. Only 1 out of 29 WT mice had bilateral intrusions.

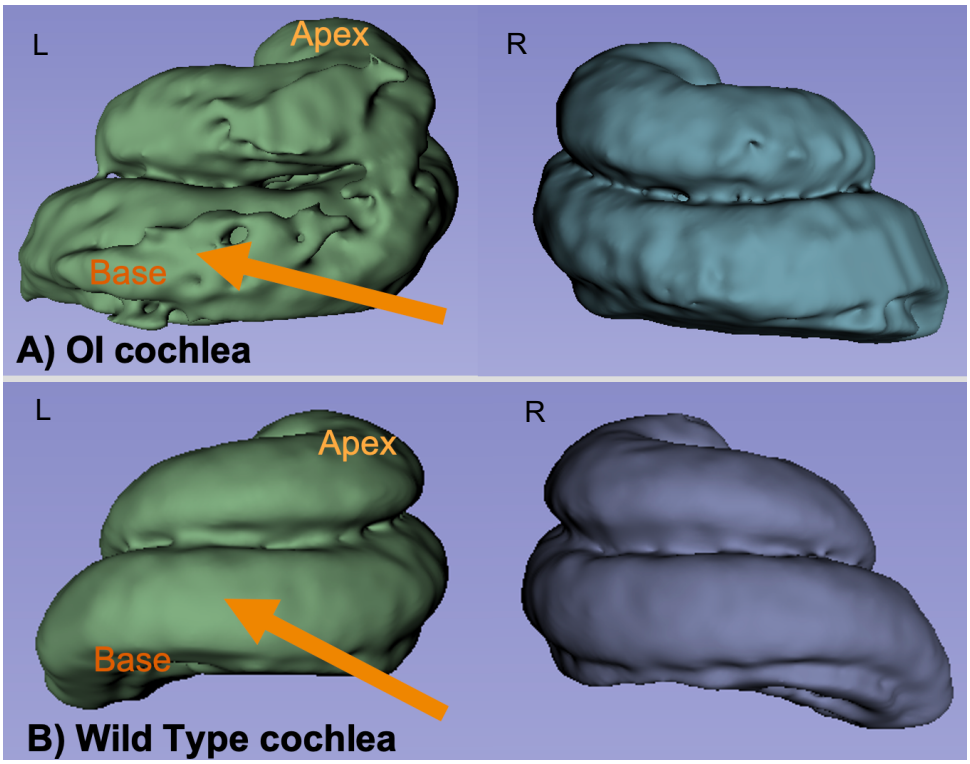

Figure 9: Areas of higher ossification (Orange arrow) were observed near the base of the cochlea within the A) OI cochlea (specimen 193, week 16) compared to the B) WT cochlea (specimen 79, week 16).

6.2 Volumetric cochlear analysis

6.2.1. WT and OI comparisons

From the 3D models, raw cochlear volumes were obtained for both week 16 and week 4 mice, which were scaled against centroid sizes to remove variation due to differences in body size. These volumes, presented in cm^3, were then run through a Mann-Whitney U-test to examine nonparametric differences between groups. When the averaged cochlear volume of OI mice was compared to WT mice, no statistical difference was observed in either week 16 (Fig. 10a) (Mann-Whitney U-test p=0.704) or week 4 (Fig. 10b) (Mann-Whitney U-test p=0.703). This indicates that the volumes of cochlea do not significantly change overall, regardless of the presence of disease. The volumetric ranges of the WT and OI samples overlap significantly and

are not statistically different from each other. However, for the week 16 specimens, a much wider range within the OI volumes was observed. An ANOVA was performed to analyze the variation between the groups, indicating that the volumes alone were not significantly different from each other (ANOVA p=0.542).

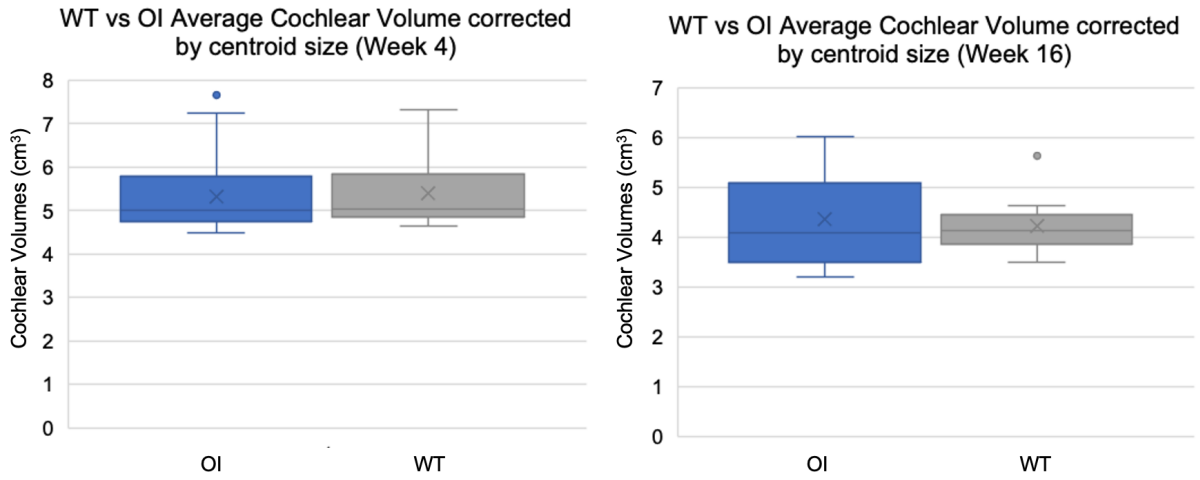

Figure 10: Box-&-whiskers plots showing the range of (averaged) cochlear volumes exhibited in WT and OI mice in both week 16 (left) and week 4 (right) samples.

6.2.2. Intra-individual comparisons

To test for quantitative differences in intraindividual cochlear duct volumes, the right and left sides were subtracted from each other to create a variation measurement of intraindividual volumetric cochlear differences (Tables 3 and 4). The OI sample's intraindividual variation compared to the WT intraindividual variation (Fig 10), shows notable significant differences in both age groups (Mann-Whitney U-test $p<0.05$), with the OI sample exhibiting much more variation between bilateral cochlea in the same mouse, compared to the WT sample. The range of differences between intraindividual cochlea in OI specimens evidences an erratic and potentially pathological pattern of bone growth. This is seen Figure 10, with cochlear differences

being far larger in OI specimens than in their WT counterparts. Within each sample, there was no consistency in which side appeared larger than the other (Fig. 11).

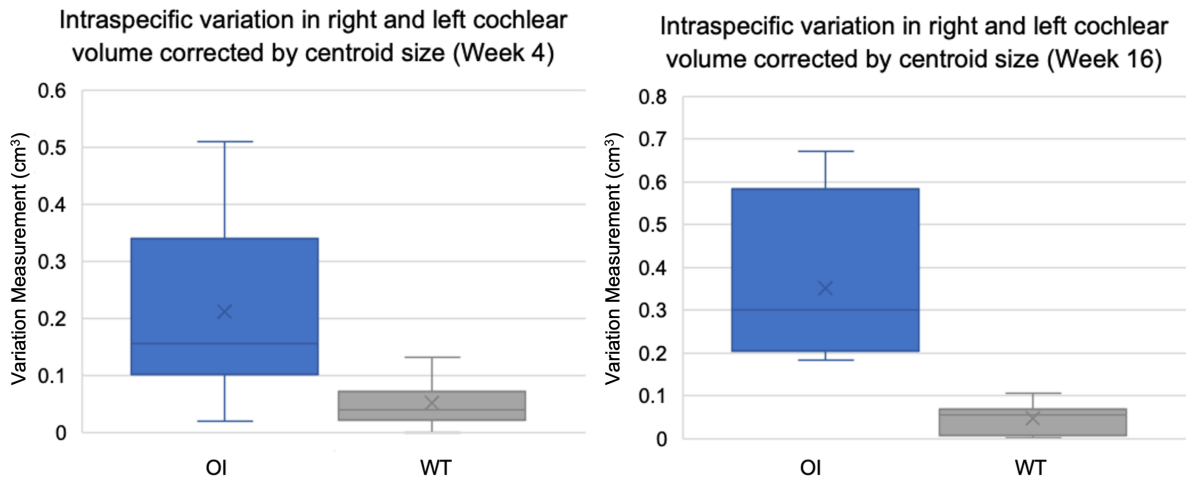

Figure 11: Box-&-whiskers plots showing volumetric intraindividual cochlear differences. Compared to the WT sample, notable significant differences were observed within the OI sample between right and left cochlear volumes within individuals.

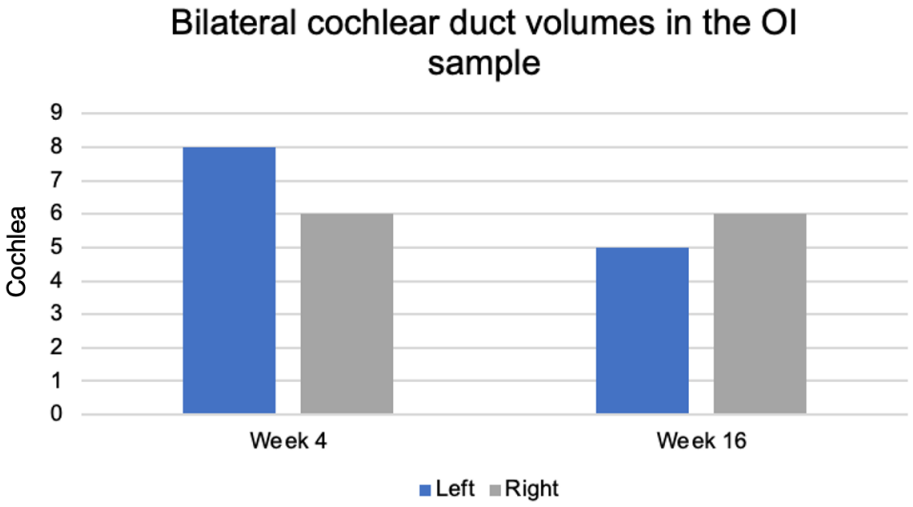

Figure 12: Bar graphs showing a comparison of right and left cochlea volumes with the OI mice sample. There was no statistical significance in which side was larger than the other.

CHAPTER 7: DISCUSSION

Osteogenesis imperfecta (OI) is a disease that has a significant impact on hearing, but the anatomical reasons for this remain relatively unknown. While most research has focused on the ossicular chain in the middle ear, the cochlea, which is the actual organ of hearing, has only been vaguely described and not quantified despite a large clinical indication that sensorineural (cochlear) damage affects 82.6% individuals with OI-related hearing loss (Hartikka *et al.*, 2004). As such, the primary goal of this practicum has been to document OI-related pathology on cochlear shape in order to better understand the factors that might contribute to hearing loss.

The project was divided into two aims. The first aim established the morphological differences in the inner ear for adult wildtype mice compared to OI mice in order to determine the anatomy of the diseased state. The second aim, using the established morphological differences accrued from the first aim, examined intraindividual variation between cochlea of WT and OI mice, and used intraindividual differences to determine potential asymmetry in the etiology of the inner ear. Overall, the results have shown that intraindividual differences between normal and pathological groups are very notable despite limited difference between overall volume, emphasizing OI's asymmetrical spread.

7.1 Gross cochlear shape changes

In order to understand the qualitative effects of OI on the cochlea, an observational analysis was completed. Macroscopic cochlear intrusions were observed within the majority of the OI specimens (58%), and they tended to only appear in one ear. The appearance of these intrusions indicates that the otic capsule of the surrounding petrous bone was growing into the hollow and membranous cochlea and decreasing total volume of the hollow cochlea. Observations yielded evidence that errant bony growth led to intrusions that could potentially affect the function of the membranous cochlea within the bony otic capsule.

7.2 Volumetric cochlear analysis

7.2.1 WT and OI Comparison

While gross cochlear shape showed some difference between the WT mice and the pathological mice, the overall cochlear volumes were not significantly different. However, while the volumes overlapped considerably, there was a notable, though not statistically significant, difference in the range of volumes exhibited by the OI mice compared to the WT. This would suggest that the pathological OI state introduces more variation to cochlear size.

7.2.2 Intraindividual differences

The OI pathological state was most obvious when looking within individuals. Consistently, the OI mice had a wider range of variation in cochlear volume, as well as more overall asymmetry in cochlear volumes than their wildtype counterparts. This excessive difference in volume between bilateral cochlea can potentially be contributed to OI's etiology, considering the potential for random and asymmetrical bone growth. These results indicate that

mice with OI are much more likely to have evidence of unilateral cochlear volume losses and asymmetry, despite very little difference in overall shape appearance and volume, possibly due to bony capsule encroachment. In post-mortem studies, those with asymmetrical OI related hearing loss due to damage to the cochlea exhibited increased porosity and fragility of the bony capsule (Santos *et al.*, 2012). This poor bony quality could be a contributor to asymmetrical improper growth, leading to damage to the soft tissue components of the cochlea. Additionally, as demonstrated in Hartikka *et al.*'s 2004 study, in OI there is a potential for different ears in the same individual to show different kinds of hearing loss. The asymmetry we have observed in these cochlear volumes may be a contributing factor to this asymmetrical hearing loss.

7.3. Comments on biomechanics and sensorineural hearing

The results of this project suggest that OI's effects on the cochlea are due to an intrusion of the otic capsule into the hollow bony coil of the cochlear duct.

These intrusions have the potential to disrupt the movement of soundwaves through endolymph and perilymph, the fluid medium that soundwaves affect in the cochlea to elicit neural response. This fluid is responsible for bending cilia on hair cells that line the membranous cochlea, which send nervous impulses to the brain to be interpreted as sound. Uninhibited movement of fluid within the membranous cochlea is essential for the accurate transmission of soundwaves. Therefore, interruptions to this movement, such as errant bony intrusions as seen in OI, will negatively affect the transmission of sound within the cochlea.

Another possible problem is that the intrusions could cause damage to soft tissue components of the cochlea. This damage could distress and rupture the membranous labyrinth, cause a hemorrhage of cochlear blood supply, a loss of either perilymph or endolymph, or cause

damage to hair cells lining the membranous cochlea. Loss of blood supply could lead to atrophy of the membranous cochlea, and damage to hair cells could lead to diminished neural impulses related to hearing. Any damage to either the bony or membranous cochlea could alter the interpretation and/or transfer of sound waves to the brain, ultimately resulting in loss of sensorineural hearing, and overall hearing loss.

Finally, the asymmetric nature of these intrusions would lead to diminished hearing in most the population, with less of the population having a total hearing loss due to the slight majority of intrusions being unilateral (51%) as opposed to bilateral (48%).

7.4. Comments on clinical treatments in humans

Understanding the etiology of OI as it pertains to hearing has been the primary goal of this study. With the strong evidence we have found supporting asymmetrical cochlear intrusions in the diseased state, it can be assumed that the etiology of OI-related sensorineural hearing loss in humans is also due to intrusions of the bony capsule. The primary treatment for sensorineural hearing loss, cochlear implants, requires electrodes to be placed directly on the cochlea. However, if the cochlea is damaged due to bony intrusions, this could potentially alter the possible treatment options available to those with mixed or sensorineural hearing loss caused by OI. Therefore, in those with OI caused sensorineural hearing loss, traditional treatments may be ineffective as their disease progression is different from those without OI.

CHAPTER 8: LIMITATIONS

The inability to test hearing, instead focusing on morphological changes, has been a limitation in this work. Comments can be made on potential hearing quality given the morphology presented, but unfortunately those claims are unverifiable with this sample population. This was amended by the focus being on volumetric quality of the data, and potential physiological effects of the diseased state's morphology.

CHAPTER 9: CONCLUSIONS

As evidenced by the relatively high rate of intrusions observed in our OI sample, there is a high potential for bony intrusions within the OI diseased state. While these bony intrusions were not visibly apparent on all diseased cochlear specimens analyzed, asymmetrical volume patterns were seen throughout most of our OI population. Fluctuating asymmetrical intraindividual volumes can also be attributed to errant bony growth. These bony intrusions can have many potential effects on soft tissue and fluid components of the cochlea, ultimately leading to anatomical damage to the sensory components of hearing.

The results of this practicum indicate that there is a high potential for sensorineural (damage to the cochlea resulting in diminished or lost hearing) and mixed hearing loss (damage to the cochlea or ossicles resulting in diminished or lost hearing) in OI-bred mice and elucidates at least one mechanism behind how this type of hearing loss might be occurring.

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