The Role of *Prickle 1* in determining the Craniofacial Morphology of Beetlejuice mice

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Submitted to the Graduate Faculty of

The University of Pittsburgh School Of Dental Medicine in partial fulfillment

Of the requirements for the degree of

Master of Dental Science

University of Pittsburgh

2017

UNIVERISTY OF PITTSBURGH

SCHOOL OF DENTAL MEDICINE

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As one of the most common congenital cranio-facial defects, cleft lip/palate (CL/P) occurs in approximately one per 750 live births in the United States. Cleft lip and palate may not be life threatening but affects functions like feeding, speech, hearing, respiration, facial and dentoalverloar development, just to name a few. These problems can cause emotional, psychosocial, and educational difficulties. Cleft lip and palate require extensive treatment that require a team approach of many specialists, which costs patients, insurances and the county billions of dollars each year.

The etiology of cleft lip/palate (CL/P) is complex and is believed to be the result of both genetics and environmental inputs. Studies have been done that implicate certain craniofacial phenotypes and variation in the craniofacial morphology as an etiological factor for cleft lip in embryonic mice and in humans. Wide faces are hypothesized to increase susceptibility to CL in both mice and humans. There are many studies done supporting the link between genetic variation and specific craniofacial phenotypes. We know now that genetically similar individuals vary in the specific trait, which means genetics alone cannot be the source of dysmorphology like CL and CP. Both mutations and environmental effects can change the phenotype of an individual. Mutations can

affect the size, position, and maturation of different developmental processes and prominences that are important for proper development and function of the face.

The *Beetlejuice (Bj)* mutants have compressed faces, compared to their wild type littermates. We observed a ~ 50% rate of cleft palate in *Bj* mutants. The purpose of this study was to investigate if *Prickle1* differentially affects the craniofacial morphology of Beetlejuice mice.

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1.0 BACKGROUND

1.1 Mice Cranial Base

Mammalian skull can be divided into three components: Calvarium which houses the brain, basicranium (base of the skull) and the face. These three components have different tissue origin and are formed differently. Bones of the face are neural crest in origin (Jiang et al.,2002), bones of the vault are derived from both neuro-crest cell and mesoderm (Jiang et al.,2002), the bones of the basicranium are also derived from both neuro-crest cells and mesoderm. Bones of the Calvarium and face are formed by intramembranous bone formation while cranial base is formed by endochondral bone formation. Cranial base is first formed as a solid cartilage(chondrocranium) (McBratney-Owen et al., 2008) which later ossifies to form the bones of the base of the skull. This solid cartilage is formed by fusion of different cartilages and that is why different bones of the basicranium formed from these cartilages have different tissue origin (McBratney-Owen et al., 2008)

Basicranium grow by endochondral ossification of the synchondrosis. Mice basicranium has two synchondrosis: spheno-occipital synchondrosis and pre-sphenoidal synchondrosis. As stated earlier, the basicranium has both neural crest and mesoderm origin. The neural crest and mesoderm boundary lies between the cartilages that form the basioccipital and basisphenoid bones

(Parsons et al.,2015). In general, the anterior cranial base is derived from neural crest whereas the posterior cranial base is from mesoderm (Noden DM, Trainor PA, 2005).

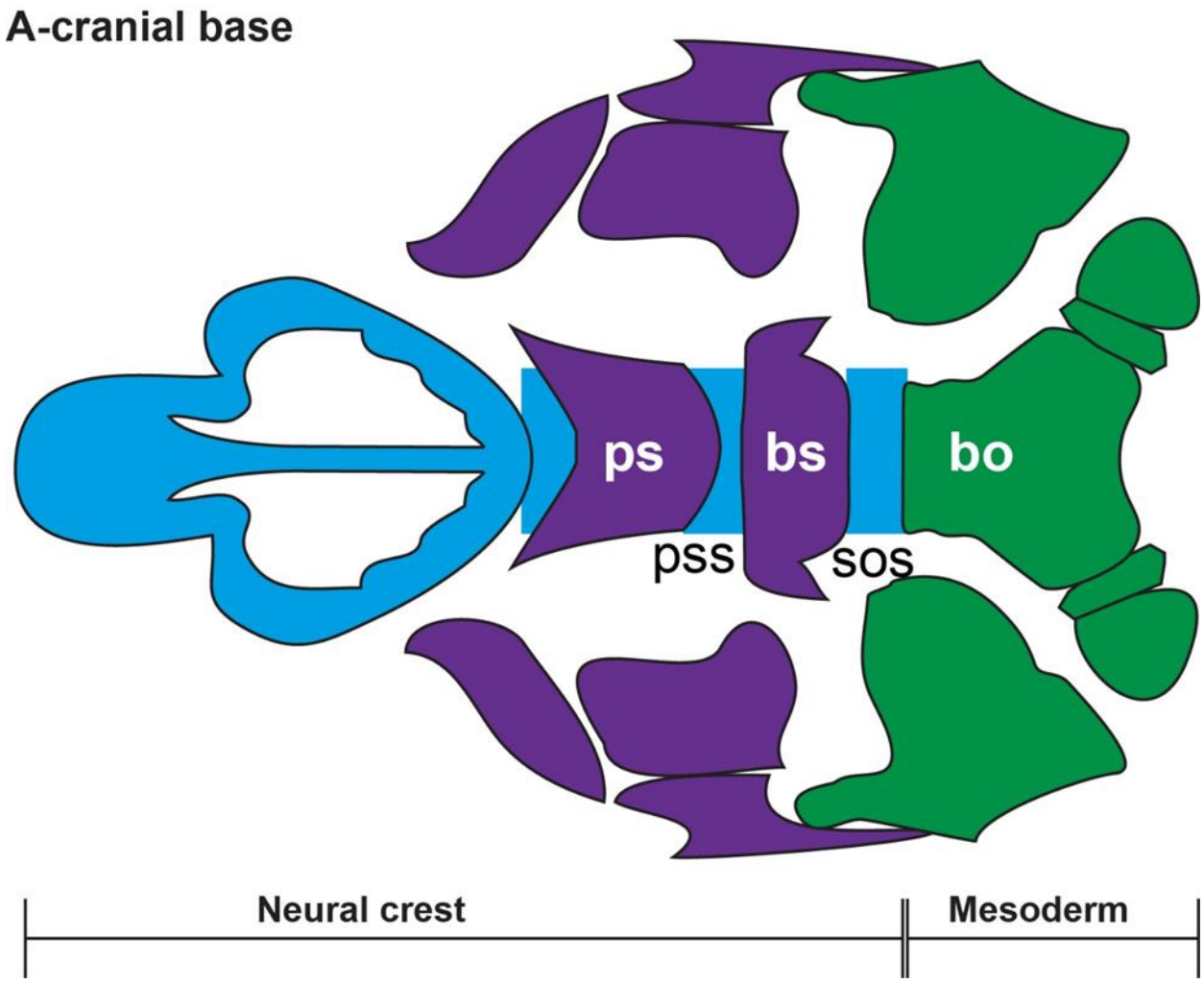


Figure 1. Schematic of the ventral view of mouse cranial base.
Anterior cranial base is derived from neural crest whereas the posterior cranial base is from mesoderm.bo, bassioccipital; bs, basisphenoid; ps, presphenoid; pss, pre-sphenoidal synchondrosis; sos, spheno-occipital synchondrosis.

Besides the differences describes above between the three parts of the skull, one other difference is in the control of growth of these three parts. The growth and shape of the basicranium has been suggested to be under intrinsic control (since it is formed by endochondral bone formation) (Scott, 1958), growth and shape of the calvarium is regulated by brain expansion (Richtsmeier et al., 2006) while growth and shape of the face is believed to be controlled by growth hormones (M.J Waters; P.L Kaye,2002).

1.2 CRANIOFACIAL MORPHOLOGY AND CLEFT LIP AND PALATE (CL/CP)

Cleft lip and palate is of the most common congenital cranio-facial anomaly that occurs in approximately 1 per 750 live births in the United States (Bornstein et al., 1970). Clefts occur more frequently among Asians (about 1:400) and certain American Indians than Europeans or European descendants. Clefts are relatively less common among Africans and African Americans (about 1:1500) (Slavkin HC, 1992). Cleft lip and palate may not be life threatening but affects functions like feeding, speech, hearing, respiration, facial and dentoalveolar development, just to name few. These problems can cause emotional, psychosocial, and educational difficulties. Cleft lip and palate require extensive treatment that require a team approach of many specialists, which costs patients, insurances and the county billions of dollars each year.

A cleft lip if formed earlier in the embryonic life when maxillary prominence and medial and lateral nasal prominences fail to fuse during embryonic development (Kaufman & Bard, 1999).

Cleft palate is formed when the palatal shelves fail to fuse in the midline. We know that secondary palate formation starts as outgrowth from the maxillary prominence in the form of palatal shelves. In the beginning the palatal shelves grow vertically down along the side of the tongue. These palatal shelves then ascend above the tongue at the same time when the tongue lowers down at the floor of the mouth. Further growth of the palatal shelves brings them in close proximity to each other which leads to their final fusion in the middle. This whole process from beginning to the end is a well-coordinated process (Wenli et al., 2009). Any disturbances along this process, e.g., defects in palatal shelf growth, uncoordinated timing, and blocked fusion, can cause cleft palate (CP) (Ferguson MW, 1988; Christensen K, Juel K, Herskind AM, Murray JC., 2004). Cases of CL/P that occur without other craniofacial abnormalities are called non-syndromic CL/P. These cases make up about 70% of the CL/P cases (Dixon et al.,2011). The remaining cases are associated with different syndromes.

The etiology of cleft lip with or without palate (CL/P) is complex and is believed to be the result of both genetics and environmental inputs (Murray JC, 2002; Gritli-Linde A, 2008). It is understood that the cleft affliction is produced early in the embryonic stage when nasal, maxillary, and mandible facial prominences develop. Developments in gene targeting technology using animal models have led to the identification of some genes associated with etiological factors (Dixon et al., 2011).

Besides genes there are factors that have been strongly associated with CL/CP, like maternal smoking and alcohol use or use of anti-convulsant medications (Wyszynski DF, Duffy DL, Beatty TH, 1997; Shaw GM, Lammer EJ, Zhu H, Baker MW, Neri E, Finnell RH,2002). On the other hand, studies have been done that shows protective role of folic acid on CL/P (Boot et al., 2003; Briggs, 1976; Finnell et al., 2004).

Studies have been done that associate certain craniofacial phenotypes and variation in the craniofacial morphology as an etiological factor for cleft lip in embryonic mice (Trasler, 1968; Juriloff & Trasler, 1976) and in humans(Fraser & Pashayan, 1970; Hermann et al. 1999). In the study done by Fraser and Pashayan, they looked at the parents of children with CL, with or without CP, and compared them to a control group. They looked at 11 different dimensions of the head of these parents. They observed higher frequency of rectangular and trapezoid heads, less prominent maxilla and upper lip, higher interzygomatic and interocular chin measurements in the experiments group than the control. The study done on A/J strain (with a spontaneous frequency of about 12% cleft lip) and the C57BL/6J strain (almost never has cleft lip), the medial nasal processes of A/J embryos were more prominent, more medially placed, and were less divergent than those of C57BL/6J (Trasler, 1968)

In a study done by Dr. Seth Weinberg (Weinberg et al. 2009), 3 D surface imaging technology along with morphometric were used to evaluate the facial shape of unaffected parent from Cleft lip/palate families and compared them to a control group. In this study, they identified certain features associated with CL/P. Some of the feature that they noticed in the unaffected parents of cleft lip and palate were: loss of convexity of the face (because of retrusion of the nasolabial structures and protruded mandibles and forward projection of the orbital-nasal bridge) and reduction in height of middle and upper portions of the face.

Study done by Parsons et al., 2015, found out that abnormalities of the cartilage of the basicranium will produce changes in adult mice face and Calvarium. In this study, they took one type of transgenic mice strains whose endochondral bone development was intentionally under activated (they designated this group as UG) and took another type of transgenic mice whose endochondral bone development was intentionally over activated (they designated this group as

OG). They have the unaffected littler mates as the control group. They examined the neonatal and adult skulls of these mice. They observed mean differences in the basicranium, Calvarium and faces of these three group of mice. The group that had the cartilage growth inhibited(UG) had shortened but widened basicranium, shortened faces and taller more dome shaped Calvarium than the control group. Such changes were not seen in the OG mice. They found significant correlation between the shape of the basicranium and shape of the face and calvarium. Since only the endochondral bone formation was affected in the two group, in theory there should have been no changes in the faces and Calvarium in these mice, as face and calvarium are not formed by endochondral bone formation. But they suggested that changes in the basicranium are correlated to the changes in face and calvarium. They called the changes in the length of the basicranium as "direct or genetic" effect and the changes in the width of the basicranium and face as "in direct or epigenetic effect". According to them the width of the basicranium is affected by the shape of the basicranium and not by the endochondral bone formation.

Studies done by Young et al., 2007 and Parsons et al., 2008 suggested the possible involvement of morphological variation in the etiology of CL in mice. In these studies, they compared the 2D and 3D craniofacial morphology of mice that have high frequency of CL and CP to those that rarely get CL and CP. They found out the mice that have high frequency of CL and CP have higher phenotypic variability than the ones with no CL or CP.

1.3 MOUSE MODELS AND CRANIOFACIAL DYSMORPHOLOGIES

Animal models, more specifically mouse models, that has craniofacial dysmorphologies like CL and CP have shown to be useful in the research of human genetic mutations that cause the same craniofacial dysmorphologies (Bornstein et al., 1970). Since earlier facial development and morphology of mice is very similar to that of human, this makes mice the perfect animal model for human palate formation and malformation. In addition, some mic models have who have dysmorphology like one CL and CP have the same genetic and clinical presentation (like incomplete penetrance, variable expression and frequent unilateral expression. A good example is the A/WySnJ mice. They are inbred strain that has a high frequency of cleft lip with or without palate concept (Halgrimmson et al., 2005). Like humans, they have incomplete penetrance and have variable and frequent unilateral expression of CL/P. This make these mice ideal model for studying human dysmorphology like CL and CP.

Most mouse models currently available for genetic research are inbred strains and genetically engineered mutants. During early embryonic stages, growth factors stimulate migration, patterning, and differentiation of the face. Global changes to these growth factors can influence the shape and width of the face. This is observed in the *Beetlejuice (Bj)* mouse line where the mice have compressed faces when compared to the wild type (Gibbs et al., 2016), serving as a model for cleft anomalies.

1.4 Beetlejuice mice and *Prickle-1* gene

For this study, we used the *Beetlejuice* mice. *Beetlejuice* mice have a missense mutation in *Prickle1(Pk1)*. The *Pk1* gene is part of noncanonical Wnt signaling (β-catenin not involved in the Noncanonical pathway) pathway, also known as the planar cell polarity (PCP) signaling pathway. In addition to the apical basolateral polarity, epithelial cells have as additional axis of polarity called the epithelial planer cell polarity(PCP). So, PCP play a role in polarization of epithelial cells, tissue formation by regulation of convergent-extension movements, and migration of neuro-crest cells (Gibbs et al., 2016). Mutations of *Prickle1* in humans have been associated with familial epilepsy and orofacial clefting (Tao et al., 2011). *Prickle* proteins were first discovered in *Drosophila*. A single prickle protein has One PET and three LIM domains (Gubb et al., 1999). *Prickle-1*^{Beetlejuice} (*Prickle1* $^{Bj/Bj}$) is a missense mutation (p:C161F) in the first LIM domain of the Prickle 1 protein.

All the *Prickle1* $^{Bj/Bj}$ mutants develop a median cleft lip while only 46% develop a cleft palate associated with a cleft lip. For our study, we include *Beetlejuice* mice that had over CP (FIGURE: 2)

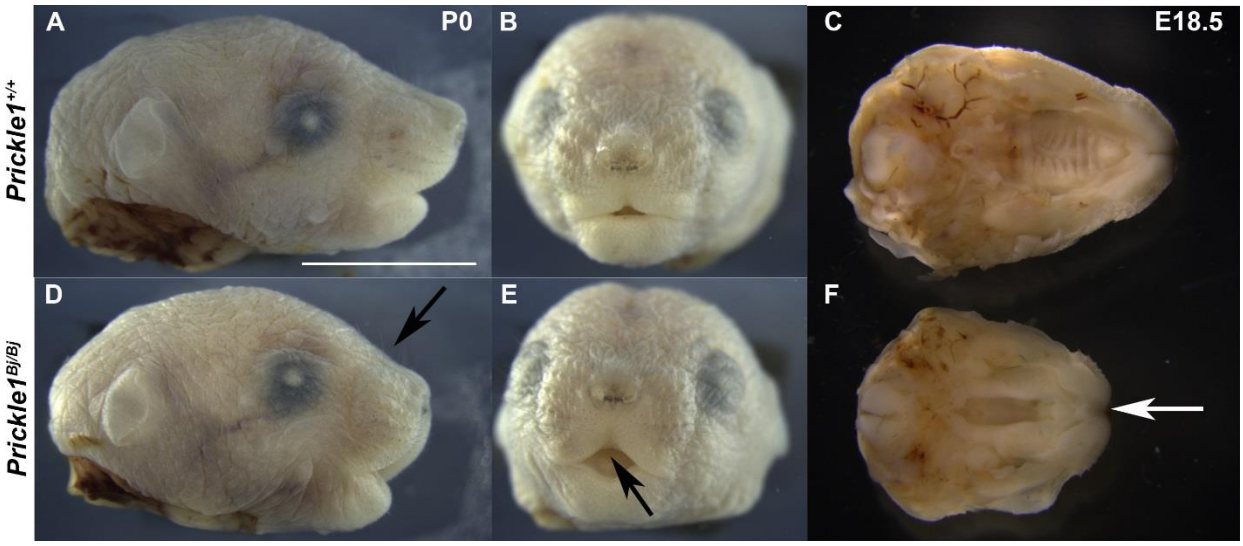


Figure 2. Craniofacial dysmorphology in the Bj mic.
A-C (top) control, D-F (bottom) Beetlejuice mutant mice. Arrow in D pointing to the short and domed shaped head, Arrow in E pointing to the midfacial cleft while arrow in F pointing to midfacial and palatal clefts in of the Beetlejuice mice (Wan et al., submitted 2017)

Unlike other *Pk1* mutants, the *Bj* mutant survives to term. *Bj* mutants exhibit a wide spectrum of developmental anomalies that include congenital heart defects, skeletal and craniofacial anomalies, cochlea defects, and biliary ductal hypoplasia (Gibbs et al., 2016).

2.0 PURPOSE OF THIS STUDY

The purpose of this study is to investigate the role *of Prickle1* in determining the craniofacial morphology of *Beetlejuice* mice. We further hypothesized that these morphology changes make the *Beetlejuice* mice susceptible to CL and CP.

Preliminary data has shown that *Prickle1 Beetlejuice* mutants have craniofacial morphology that is different from the wild types. Their head are shorter in the anterior-posterior axis and expanded in the medial-lateral axis (Gibbs et al., 2016). Basioccipital has mesodermal origin while the rest of the bones (premaxilla, presphenoid and basisphenoid) and synchondrosis have neural crest cell origin. Since PCP has been suggested to regulate directional migration of neural crest cells, we hypothesized that the anterior most region of the skull is affected in these mutant mice.

3.0 MATERIALS AND METHODS

We examined the heads of *Prickle1*$^{Bj/Bj}$ and their wild type littermates after in situ hybridization at E 12.5, in both vertex (top of the head) and ventral view (inferior). We took micrographs of the prepared samples (both vertex and ventral), saved them and printed them on paper. We measured the length and width of head of both wild and mutant types.

We also collected several neonates of the *Prickle1* $^{Bj/Bj}$ and their wild type littermates at embryonic age (E17.5) and at post-natal day 0(P0). They were than stained with Alizarin red (pink) and Alcian blue for bone and cartilage respectively using standard protocols. Mandibles were cut out to look at the bones of the bones of basicranium(Fig 3). We included only *Prickle1* $^{Bj/Bj}$ mutants that developed cleft palate for our study.

We took micrographs of these prepared samples. Images were printed on paper and cranial base components were measured using a mm ruler.

All data was recorded on excel spreadsheet. We plotted bar graphs to compare the length and width and ratios of width to length measurements between wild and mutant types.

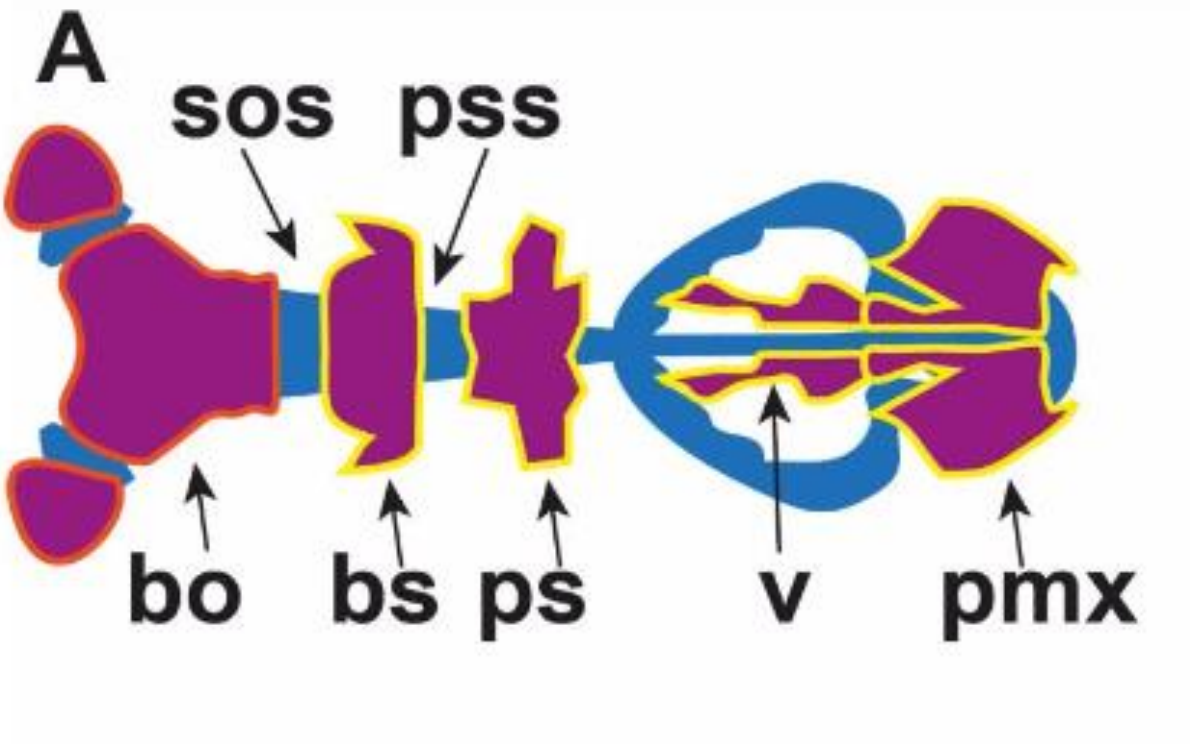


Figure 3. Schematic representation of Bones of the cranial bones.
*bo, basioccipital; bs, basisphenoid; ps, presphenoid; sos, spheno-occipital synchondrosis; pss, presphenoid synchondros*is; v, vomer; pmx, premaxillary bones

To minimize any distortion of the images, all images were saved as "TIF" files. We used a mm ruler for all our measurements. Images were taken at different resolution of the microscope and all measurements were fist calibrated before the start of the data analysis.

Figure 4.Ruler used in this study for measurements.

I measured the width of 3 bones in the cranial base and the length of four segments of the cranial base(Fig.5). Figure below represents the Length and width measurements of the cranial base that we measured.

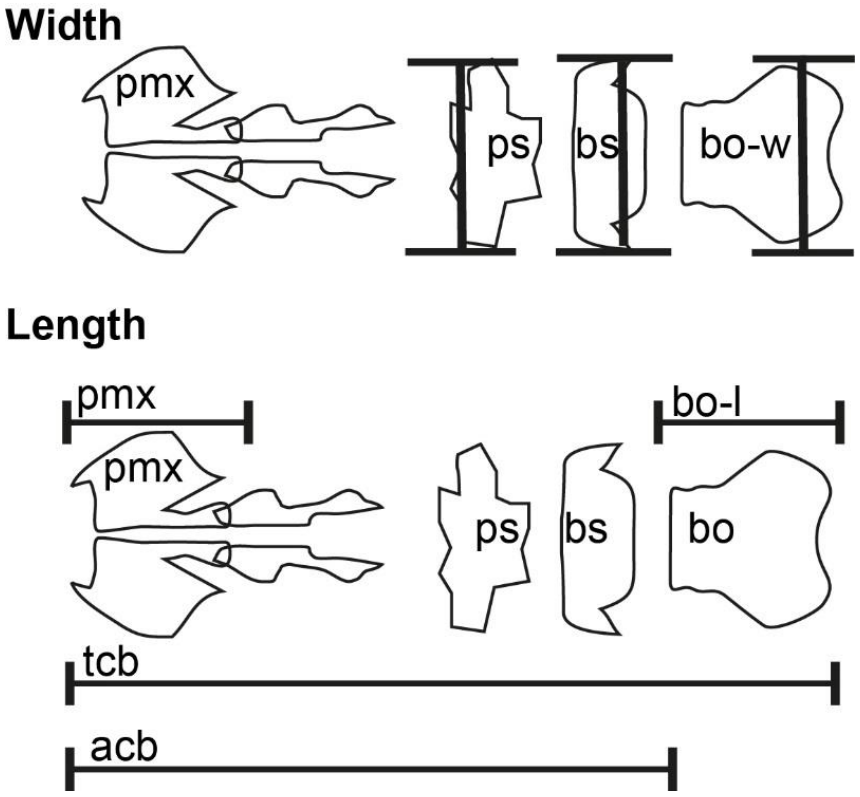


Figure 5.Measurements made to compare the cranial base.
Eight cranial base linear measurements: Distal premaxillary bones (pmx) Maximum width of the preshenoid (ps), Maximum distance of the basisphenoid(bs), Maximum distance of the basioccipital (bo-w), total cranial base length(tcb), Length of the basioccipital (bo-l), anterior cranial base length(acb)

4.0 RESULTS

4.1 Vertex view of the head at E12.5

We examined the heads of two mutant and three wild type mice at E 12.5. We compared the morphology of the head of the wildtype to mutant type. We clearly observed differences in the morphology of the wild and mutant types. The proximal-distal dimension of the head of the mutant mice, compared to the wildtype, were shorter while expanded in the medio-lateral axis in the vertex view (figure 6).

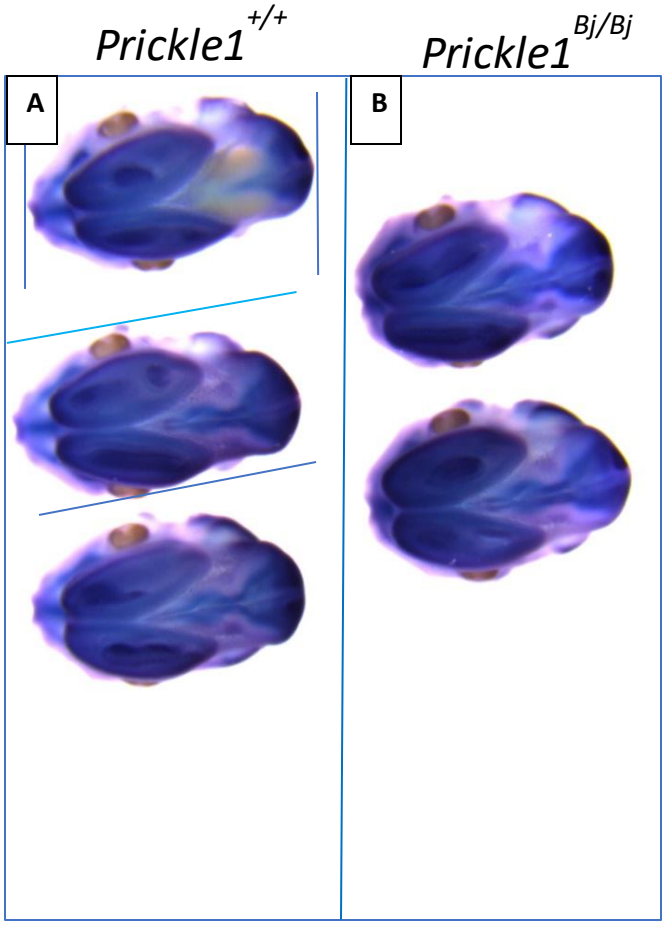


Figure 6. Vertex (top) view at E 12.5. A, wt at 12.5; B, mut at 12.5. The proximal-distal dimension of the head of the mutant mice, compared to the wild type, were shorter while expanded in the medio-lateral l axis

In order to quantify the changes that we observed, we took length and width measurements of the head of both wild type and mutant mice on the printed images of the vertex view. We noticed the lengths of the head of mutant mice were reduced while widths were increased in the mutant mice(Fig.7). We than took width to length ratio of our measurements and observed the ratios increased in the mutant mice(Fig.8)

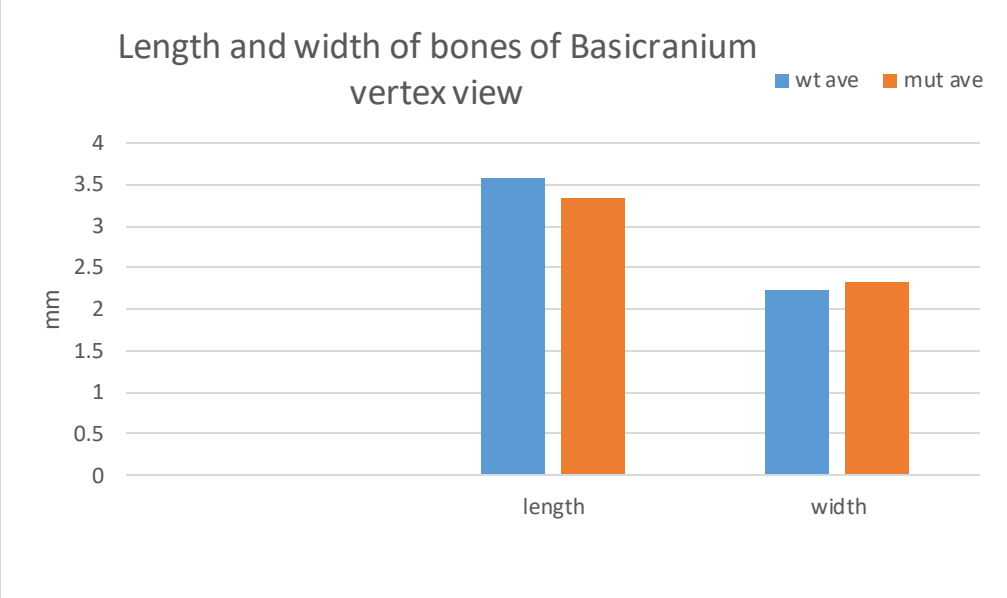


Figure 7. This graph shows length and width of the skull in the vertex (from the top) view Early in development at E12.5, the BJ morphology is shortened in length.

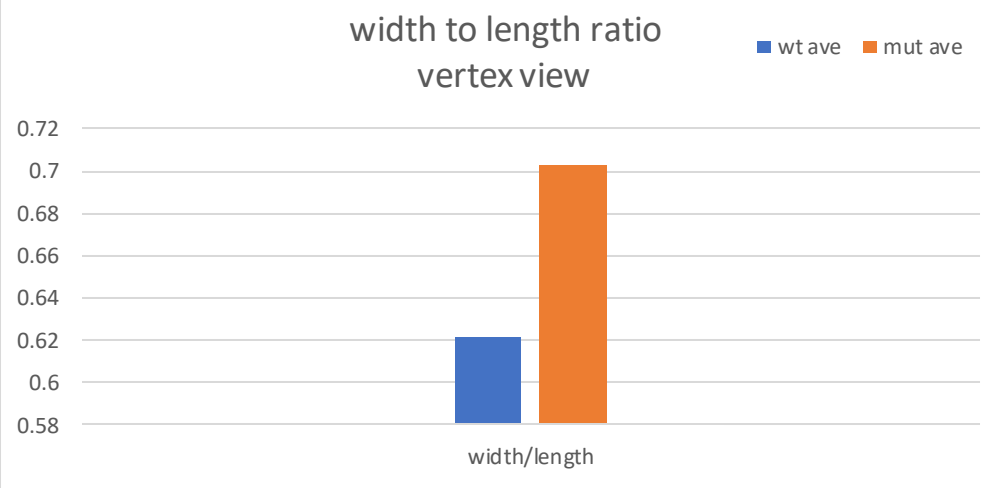


Figure 8. Graph showing width to length ratio at E 12.5(vertex view). Ratio is increased for mutants

4.2 Ventral view of the head at E 12.5

The change in length and width in the vertex view of the E12.5 was confirmed with the ventral view of the head of an additional four individuals at E12.5. The head of the *Beetlejuice* mice were shortened in the proximal-distal and expanded in the medio-lateral axis in the ventral view (figure 9).

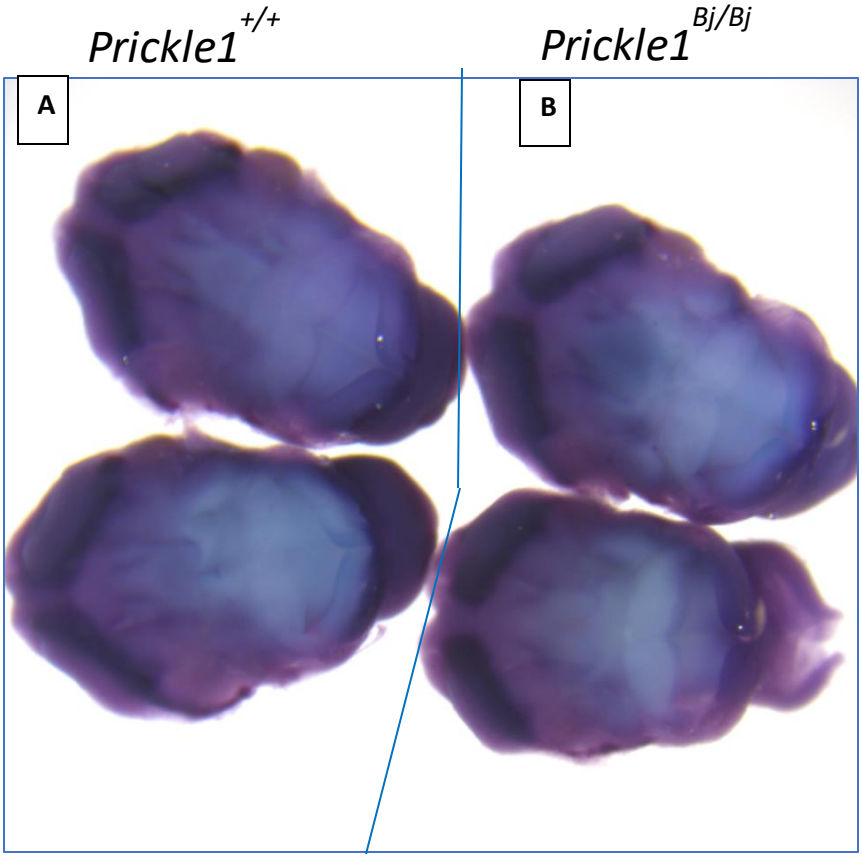


Figure 9. Inferior (below) view at E 12.5. A, wt at E12.5; B, mut at E 12.5. The proximal-distal dimension of the head of the mutant mice, compared to the wild type, were shorter while expanded in the medio-lateral axis

We took length and width measurements of the head of both wild type and mutant mice on the printed images of the ventral view also. We noticed the lengths of the head of mutant mice were reduced. The widths of the head were not much different at this view (Fig. 10) but when we took width to length ratio of our measurements, we observed the ratios increased in the mutant mice (figure 11)

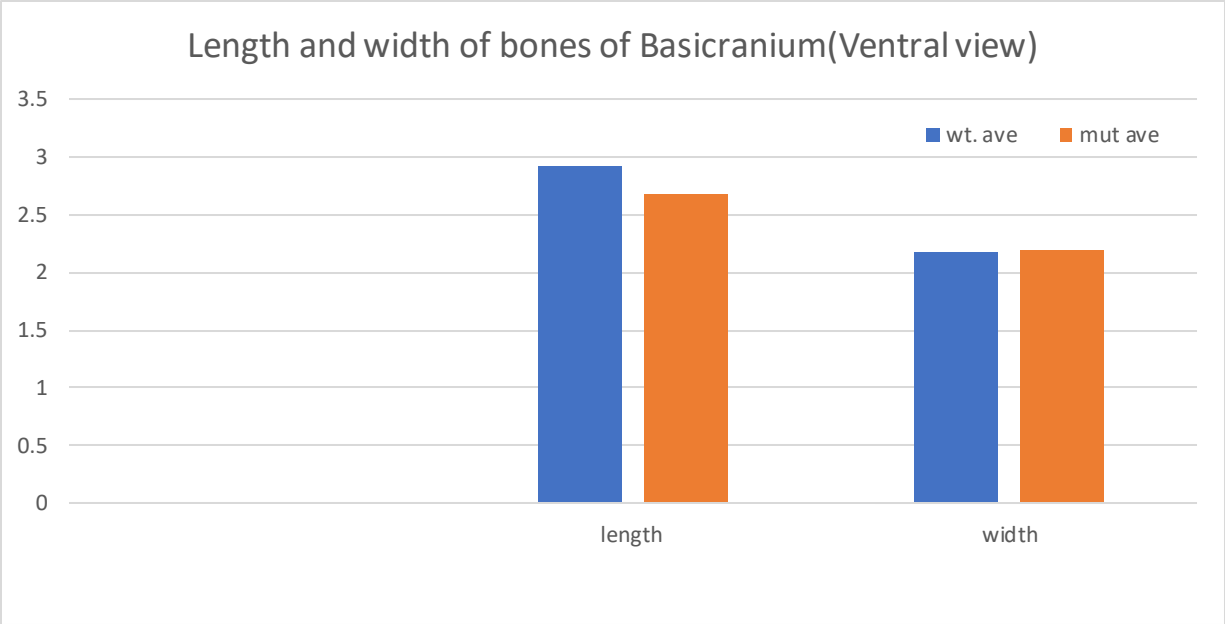


Figure 10. Graph comparing length and width of mutant and wild type in the ventral view. Early in development at E12.5, the BJ morphology is shortened.

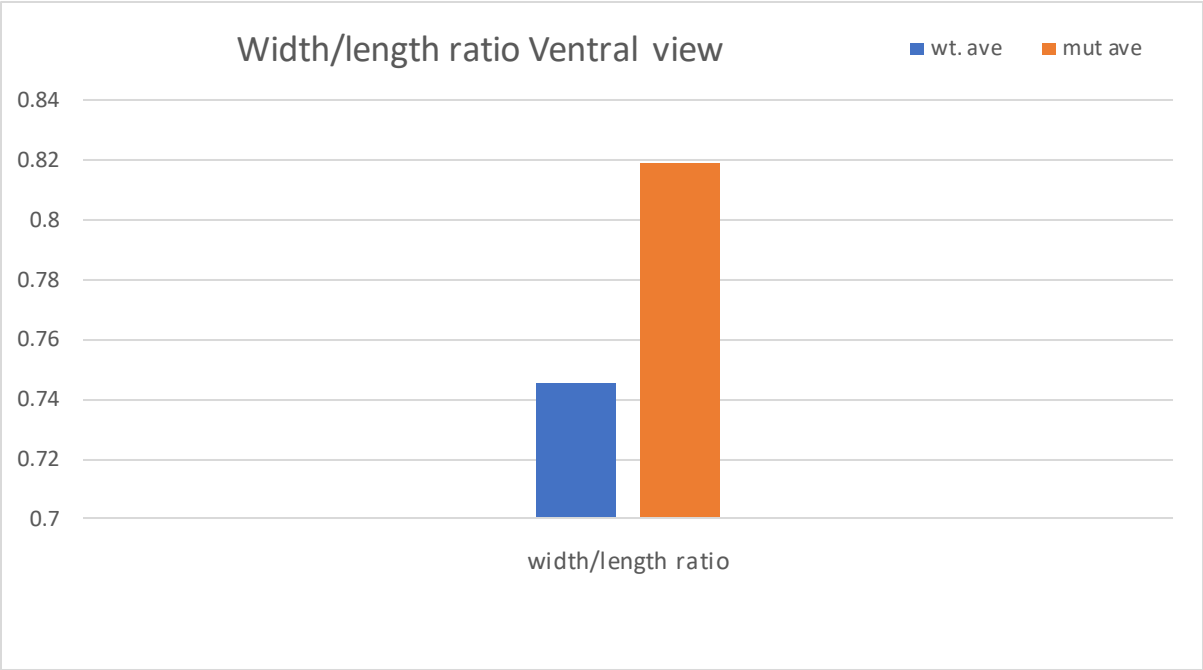


Figure 11. Width to length ratio at E 12.5(inferior view). Ratio is increased for mutants

4.3 Skeletal Preparation

To determine if these changes were just an intermediate stage, or if they had an affect on later development, we performed an experiment to test the width and length of the cranial base from skeletal preparation of the heads in both wild-type and *Beetlejuice* mutants littermates just prior and after birth at 17.5, and P0.

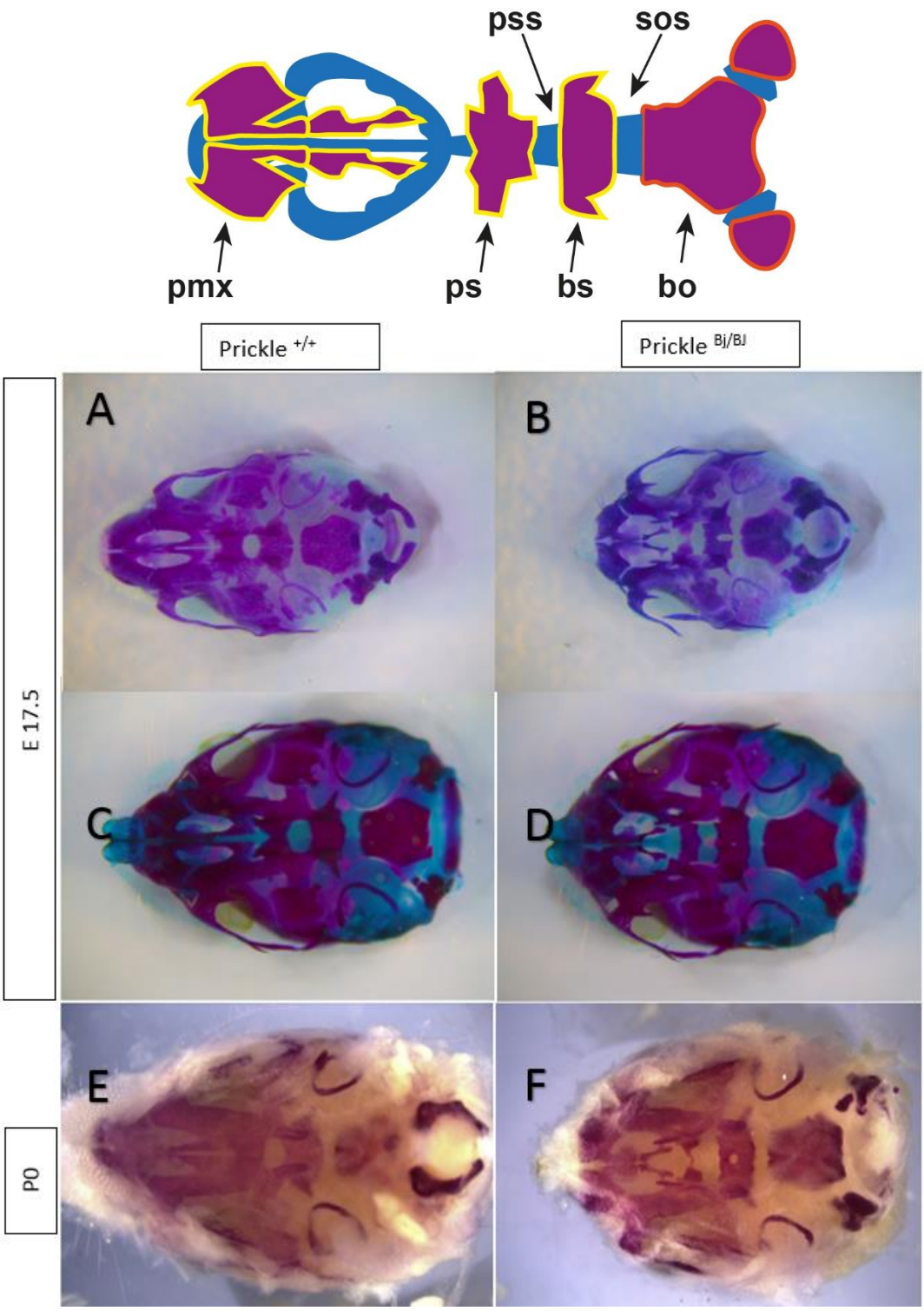


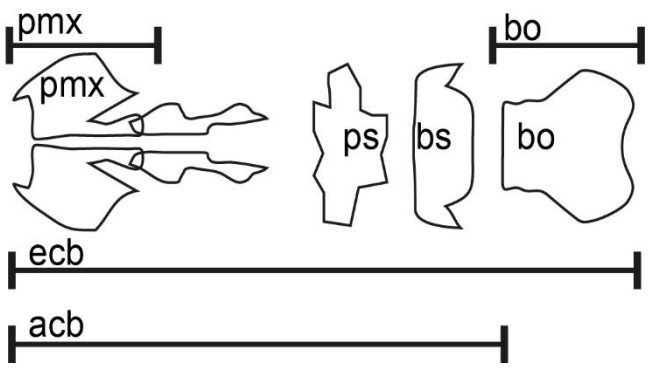
Figure 12. Inferior view of Skeletal Preparation at P0 and E 17.5.
A, wt at 17.5; B, mut at 17.5; C, wt at 17.5; D, mut at 17.5; E, wt at P0 and F, mut at P0.

We took length and width measurments of the bones of the basicranim and find significant differences between the wild and mutant types. These differences are described in the following sections.

4.4 Length measurements

We took four length measurements at the basicranium at E17.5 and P0. We observed that premaxillary length, total cranial base length and anterior cranial base length to be shorter in the *Prickle*$^{Bj/Bj}$ mutants compared to the wild type littermates. We noticed that the length of basioccipital was not statistically significant between the genotypes.

A



B

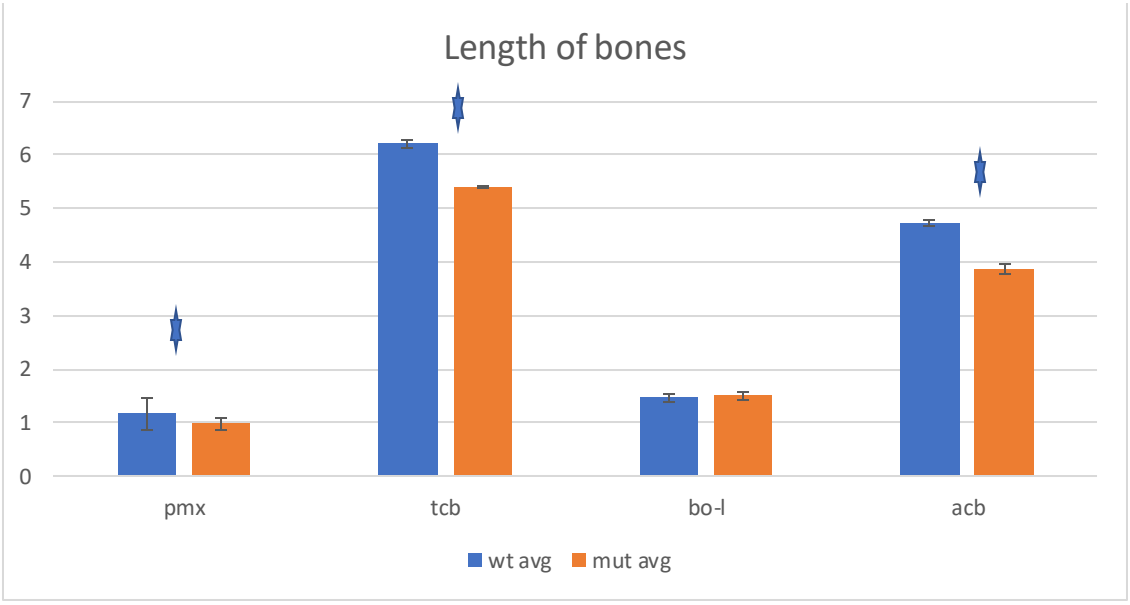
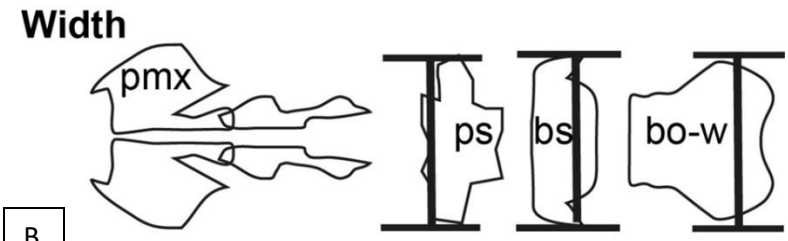


Figure 13. The length of the neural crest derived cranial base is shorter in the mutants.
A) Schematic of length measurement. B) Graph showing four length measurements of the basicranium at E12.5 and P0. pmx, tcb and acb are greater in wild type than the mutant type, while bo-l is the same between the two. pmx, distal premaxillary bones; tcb; total cranial base length; bo-l, length of basioccipital bone; acb, anterior cranial base length

4.5 Width measurements

We measured width of three bones at the basicranium at E17.5 and at P0. There was a statistically significant difference in the width of Basisphenoid bs) between the two groups. There was no statistically significance in Basioccipital width(bo-w) of wild and mutant types. There was a difference in width of the presphenoid but was not statistically significant.

A



B

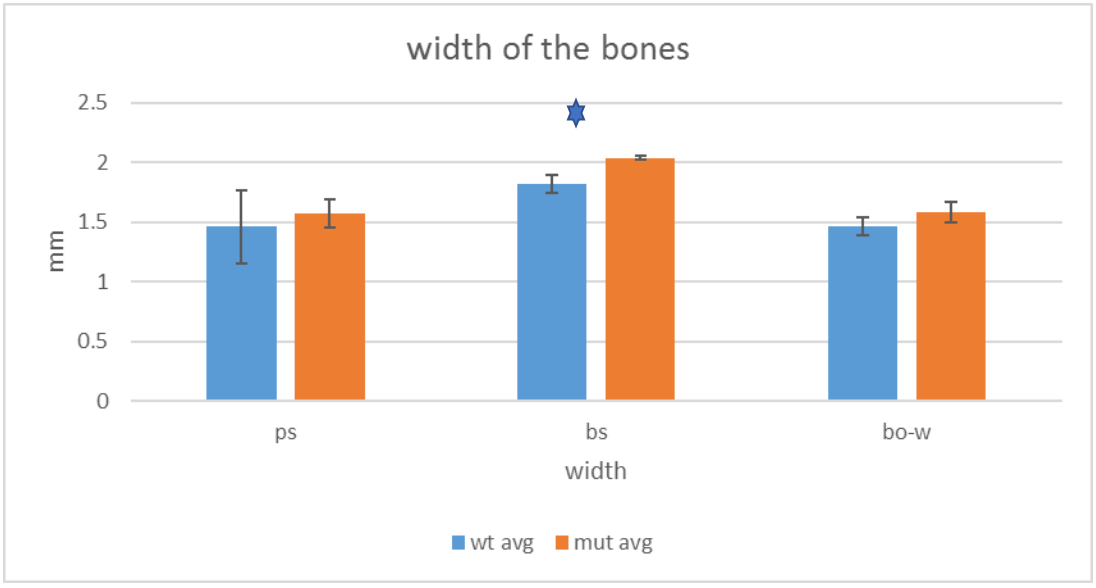


Figure 14.Graph showing width of three bones of the basicranium at E12.5 and P0.
A) Schematic of the cranial base and width measurements. B) Width of the three bones is greater in the mutant than the wild type but only bs is statistically significant ps, Presphenoid; bs, basisphenoid; bo-w, length of basioccipital.

We took percent ratio of mut bo-l/wt bo-l that was 102%, which mean these measurements were almost the same for the two groups. We took percent ratio of mut acb/wt acb, which was 81 %, indicating a decrease in the length of G for the mutant type. We took percent ratio of mut tcb/wt tcb, we got a ratio higher (87%) than that for the mut acb/wt acb (81%). The percent ratio of mut bs/wt bs was 112%, indicating a much wider basisphenoid in the mutant type than the wild type. All of these measurements indicate that the changes are more pronounced in the anterior than the posterior region of the basicranium.

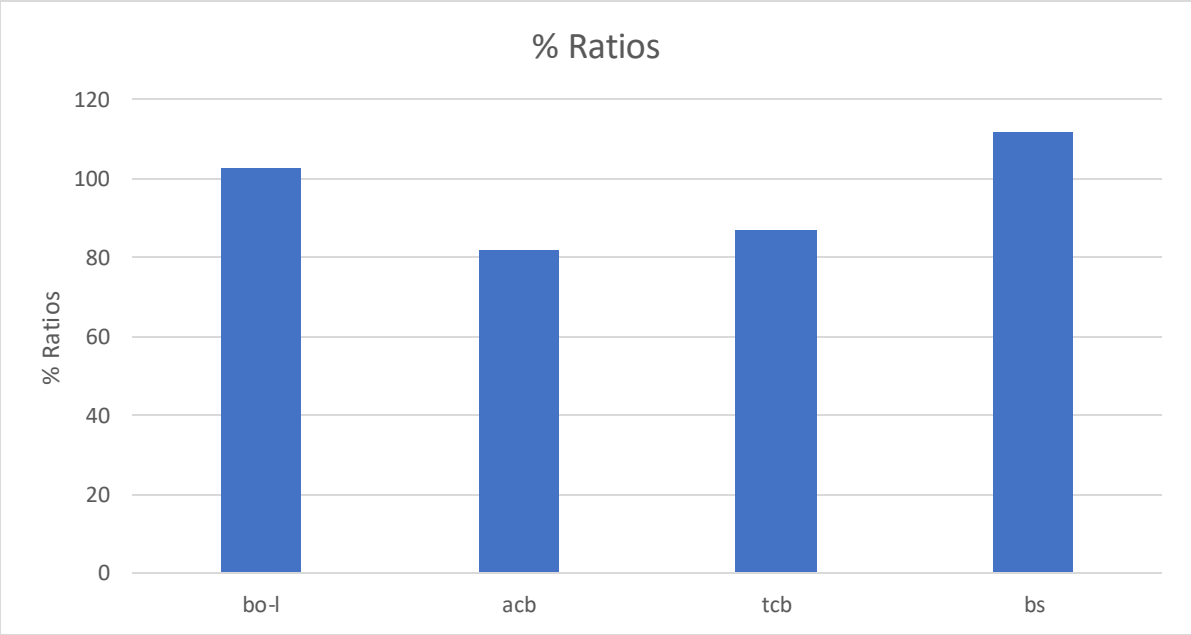


Figure 15. Prickle1 $^{Bj/Bj}$ mutants have shorter cranial base length but the basisphenoid is wider, and the length of the basioccipital is not affected. The ratio of mutants to wildtype measurements is almost on at basioccipital, decreases to the lowest at anterior cranial base, increases at total cranial base and increases even more at basisphenoid

We used Stata software program to perform two sample t-tests to compare the mean lengths, width the basicranium of the wild and mutant types.

Table 1: Lengths and widths of the bones of the basicranium

Table 1: Lengths and widths of the bones of the basicranium							
Genotype	pmx	ps	bs	bo-w	tcb	bo-l	acb
Prickle1$^{+/+}$	1.17125 +/- 0.05268	1.46466 +/-0.30608	1.82 +/-0.07621	1.46733 +/-0.07408	6.203 +/-0.17932	1.463 +/- 0.044	4.732 +/- 0.20184
Prickle1$^{Bj/Bj}$	0.9795 +/-0.110163	1.57466 +/-0.11677	2.03766 +/- 0.01542	1.5844 +/- 0.08080	5.4055 +/-0.28082	1.506 +/- 0.044	3.8665 +/-0.19795
p-value	P=0.0199*	0.5164	0.0003*	0.1268	0.0020*	0.5144	0.0003*

Measurements are mean +/- sd; blue indicate significant.

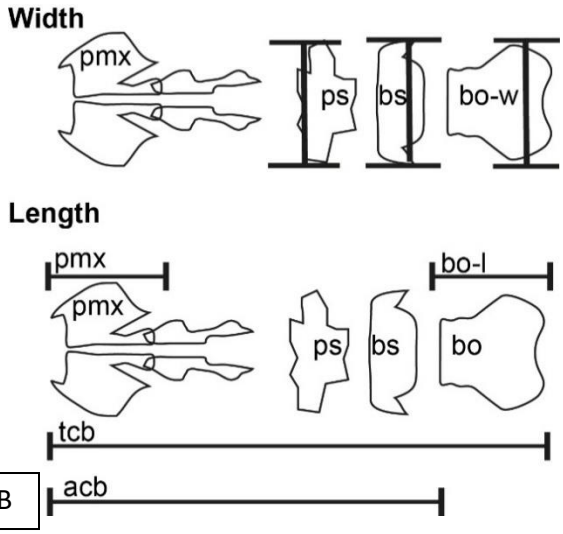
Over all, we saw statistically significant differences for the premaxillary bones, basisphenoid, total cranial base length, and anterior cranial base length. There were no statistically significant differences for presphenoid, and width of the basioccipital.

4.6 Ratio of width to length

In order to delineate regional morphological changes, we took width to length ratios of the measurement and then compared these ratios between the wild and mutant types. We saw statistically significant differences for bs/tcb, bo-w/tcb, bo-l/tcb, and acb/tcb but not for pmx/tcb and ps/tcb.

We determined statistical significance using $p<0.05$. We also took ratio of bs and acb. All the width to length ratios were greater in the mutant type than in the wild type. bs/acb was greater than bs/acb, which signifies that the changes were mostly located to distal region of the skull.

A



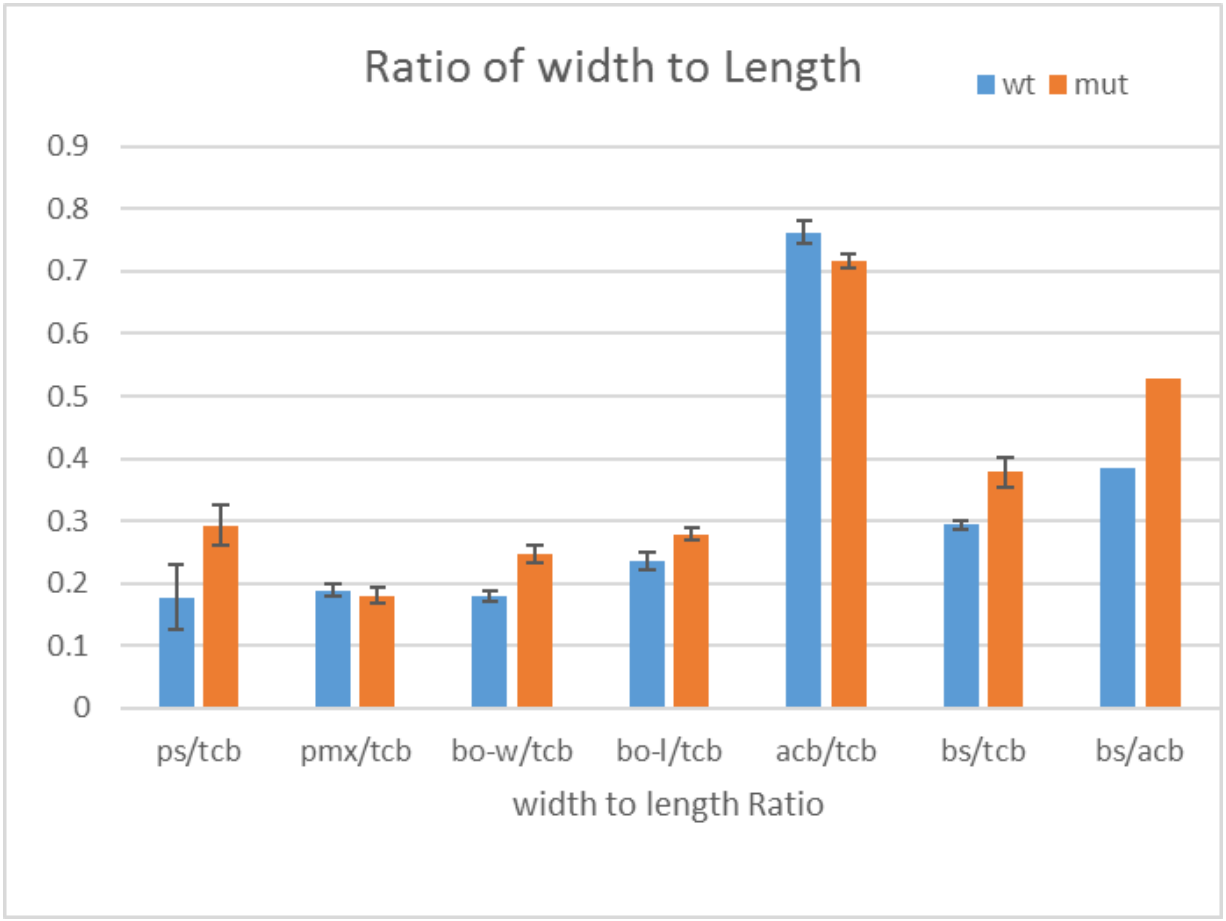


Figure 16.Graph comparing width to Length ratios.
We took the ratio of width to length to identify regional morphological changes.
A) Schematic of measurements. B) BJ Mutants' width: length ratios ps/tcb, bs/tcb, bo-w/tcb, bo-l/tcb and bs/acb of the mutants basicranium are higher than the wild type while ratios acb/tcb and pmx/tcb are lower than the wild type.

Table 2: Width to length ratio of the bones of the basicranium

Table 2 summarizes the width to length ratios						
genotype	pmx/tcb	ps/tcb	bs/tcb	bo-w/tcb	bo-l/tcb	acb/tcb
Prickle1$^{+/+}$	0.188926 +/-0.008943	0.177713 +/-0.109111	0.293341 +/-0.006233	0.178791 +/-0.103389	0.236155 +/-0.012059	0.76265 +/-0.015807
Prickle1$^{Bj/Bj}$	0.18071 +/-0.012635	0.292402 +/-0.029514	0.378124 +/-0.022496	0.247246 +/-0.111116	0.278414 +/-0.009029	0.715403 +/-0.010242
p-value	0.3434	0.0853	0.0002*	0.0005*	0.0005*	0.0009*

Measurements are mean +/- sd; blue indicates statistically significant difference.

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5.0 DISCUSSION

We noticed changes mainly in the anterior region, where the bones had the neural crest origin. No or very little changes in the posterior region. From the bones we looked at the basicranium:

Basisphenoid is neural crest cell, and is wider,

The PMX is neural crest cell and is shorter.

The only bone that has the mesodermal origin is the Basioccipital, and is the same between genotypes. I have found an association with wider anterior cranial (bones derived from the neural crest) and the development of cleft palate.

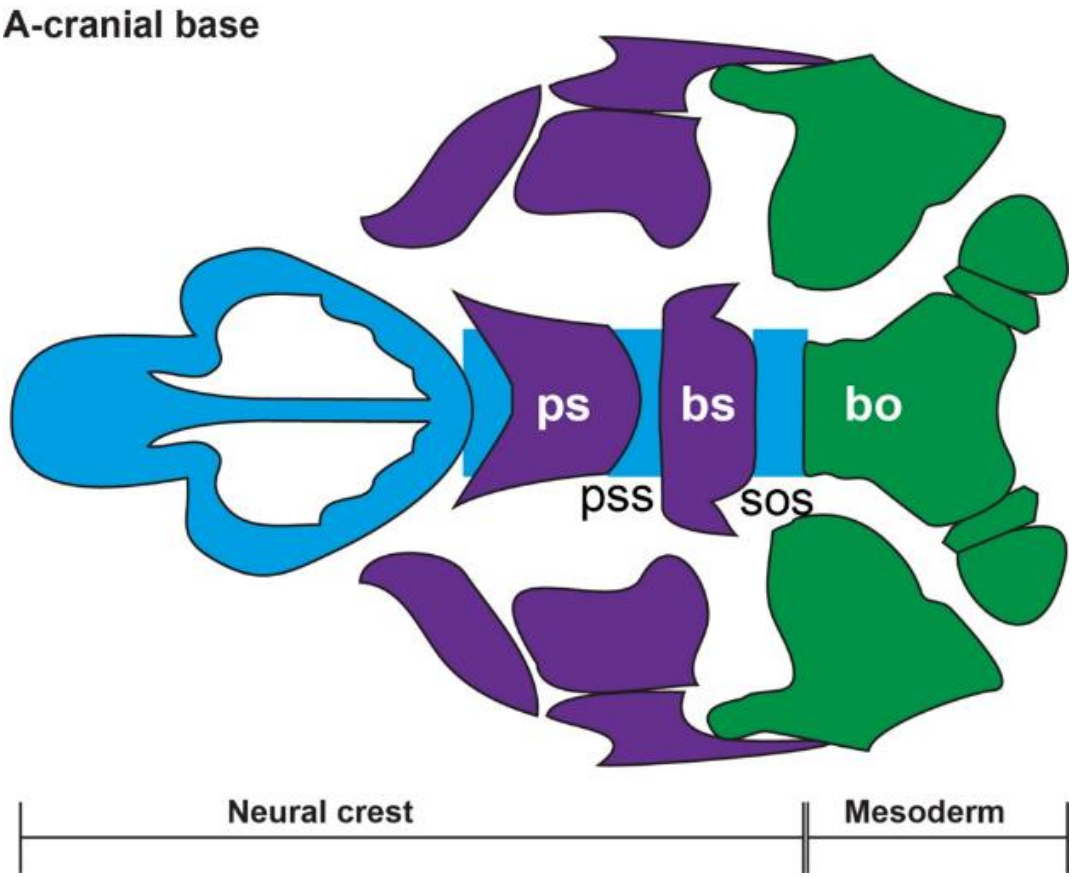

Figure 17.Schematic of the ventral view of mouse cranial base.
bo, basioccipital; bs, basisphenoid; Ps, presphenoid; sos, spheno-occipital synchondrosis; pss, presphenoid synchondrosis; v, vomer; pmx, premaxillary bones

It was hard to measure the width of presphenoid (ps). We could not measure presphenoid width accurately for the all samples because of the palatal bones covering part of the presphenoid bone. Because of this, we had fewer measurements for presphenoid.

There was a difference in basisphenoid width between the wildtype and mutant types and it was statistically significant. The presphenoid width and the ps/tcb ratio were higher in mutant than the wild type but they were not statistically significant, probably due to the above-mentioned reasons.

In this study, we did find significant differences in the craniofacial morphology between the two groups. In our study, the mutation in *Prickle1* changed the phenotypic appearance of these mice. The heads' width of the mutant mice is increased because of the *Prickle1* mutation. We know from

the embryology of lip and palate that the three-dimensional shaped structures (processes and palatal shelves) must meet each other for proper fusion. Increasing the distance between these structures (processes and palatal shelves) may shift over some of these mutants over the threshold of CL and CP.

5.1 Plans for Future:

In this study we only analyzed the cranial base of mutant mice with cleft palate and compared it to the cranial base of the wild type. To fully test our hypothesis we should also compare the cranial base of non-cleft mutants to the cleft mutants. Further studies with increased sample size and histological sections of the head may be even more promising.

6.0 CONCLUSIONS

We observed that at E12.5 the *Beetlejuice* mice have skull that are shorter in the proximal distal axis and expanded in the medio-lateral axis. We confirmed this at later stages and observed that only the anterior most region of the skull is affected in these mice. Therefore, the *Beetlejuice* mutation differently affects the development of the neural-crest derived cranial base. Our data found has found a correlation between the width of the head and the development of cleft palate. More research is needed to establish a firm link between change in morphology of head of the Beetlejuice mice and cleft lip and plate, but we suggest that the increase in width of the skull may predispose these mice to cleft lip and palate. If true, the change in the head morphology would every likely be a relevant etiological factor for cleft lip and palate formation in humans.

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