Quantitative Examination of Negative Spaces in a Crouzon/Pfeiffer Mouse Model at Birth Using Multimodal Imaging

Susan M. Motch[1], Thomas Neuberger[2,3], Neus Martínez-Abadías[1], Talia L. Pankratz[1], Yingli Wang[4], Ethylin W. Jabs[4], Timothy M. Ryan[1], and Joan T. Richtsmeier[1]
[1]Department of Anthropology, The Pennsylvania State University, University Park, PA, United States, [2]Huck Institute of the Life Sciences, The Pennsylvania State University, University Park, PA, United States, [3]Department of Bioengineering, The Pennsylvania State University, University Park, PA, United States, [4]Department of Genetics and Genomic Sciences, Mt. Sinai School of Medicine, New York, NY, United States

Objectives

Crouzon syndrome is associated with nearly 50 known *FGFR2* mutations, one of which, the *FGFR2* C342Y mutation, is causative for Crouzon and Pfeiffer craniosynostosis syndromes. Individuals with Crouzon and Pfeiffer syndromes show marked phenotypic variation but usually display premature closure of cranial suture(s), additional craniofacial malformations, as well as defects involving other systems including respiratory disorders and auditory impairments. We used multimodal imaging, µCT and µMRM, of newborn littermates of the *Fgfr2c*$^{C342Y/+}$ mouse model for Crouzon/Pfeiffer syndromes, to investigate the global and regional impact of this mutation on the developing skull and negative spaces of the head at P0. Negative spaces were defined as the air-filled space of the nasopharynx that develop within the intramembranous facial skeleton and fluid filled structures of the cochlea and vestibular canals that develop within the otic skeleton, which is still cartilaginous at birth.

Methods

We obtained µCT images from *Fgfr2c*$^{C342Y/+}$ (N=28) and *Fgfr2c*$^{+/+}$ non-mutant littermates (*1*) (N=31) at birth (postnatal day 0, P0) and µMRM images from a subset of these mice; *Fgfr2c*$^{C342Y/+}$ (N=8), *Fgfr2c*$^{+/+}$ (N=11). Three-dimensional coordinates of 57 cranial landmarks were recorded on the 3D µCT isosurfaces of newborn mouse skulls thresholded for bone and reconstructed using AVIZO 6.3 (Visualization Sciences Group, VSG). Subsets of 3D landmarks were defined to represent the shapes of the global skull and major skull regions: facial skeleton, cranial vault, cranial base. Three dimensional coordinates of 4 neural landmarks located on the 3D µMRM reconstructions were used to create a plane which defined the posterior aspect of the upper airway (Fig. 1). The upper airway and inner ear volumes of each specimen were twice segmented using AVIZO and the averages of the two trials were used in analysis. To extract shape information from all four skull datasets (global skull, facial skeleton, cranial vault, cranial base), we performed a separate General Procrustes Analysis (GPA) (*2*) for each subset of landmarks. To test the effect of allometry, we computed a regression of shape (represented by Procrustes coordinates) on centroid size, computed as the square root of the sum of square distances of a set of landmarks from their centroid (*3*). To compare the mean volumes of the nasopharynx and inner ears, we randomly selected sub-samples from our samples of non-mutant (*Fgfr2c*$^{+/+}$) and Crouzon/Pfeiffer syndrome (*Fgfr2c*$^{C342Y/+}$) mutant mice to compare sample parameters. Differences between sample means were used to make inferences about the difference between the population means.

Results

The Principal Component Analysis (PCA) (*2*) based on the Procrustes coordinates of the subset of landmarks that defined the global skull configuration showed clear separation between Crouzon/Pfeiffer syndrome *Fgfr2c*$^{C342Y/+}$ mice and non-mutant (*Fgfr2c*$^{+/+}$) littermates along PC1, which explained 20.35% of total morphological variation, while PC2 accounted for 9.93% of total shape variation (Fig. 2). The regression of shape on size showed allometry was not a significant factor affecting overall skull shape variation in our sample of Crouzon/Pfeiffer newborn mice, with size only predicting 1.89% of shape variation (P-value=0.3312). This indicates that mutant and non-mutant mice were similar in size. The nasopharynx was quantitatively measured using a novel technique for repeatable segmentation of the negative space of the nasopharynx. *Fgfr2c*$^{C342Y/+}$ mutant mice had distinctly restricted nasal passages and upper airway (2.82 ± 0.12 mm^3) compared to *Fgfr2c*$^{+/+}$ non-mutant littermates (3.21 ± 0.13 mm^3), P=0.032 (Fig. 3). The volume of the cochlea and semicircular canals were quantified similarly. No significant difference in mean inner ear volume was found between mutant (0.97 ± 0.04 mm^3) and non-mutant (1.02 ± 0.03 mm^3) littermates at P0.

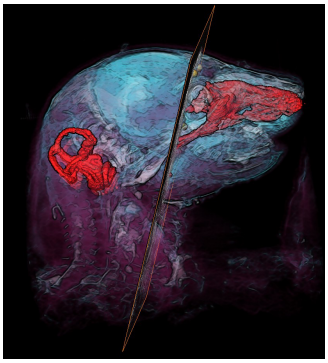

Fig. 1. Plane created using neural landmarks demarcates posterior portion of nasopharynx.

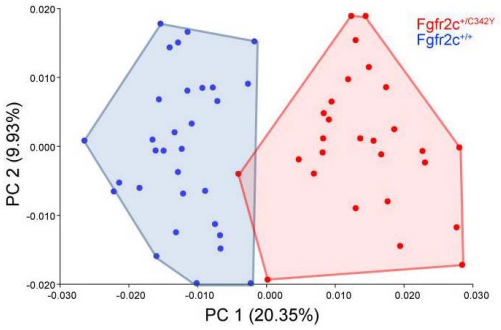

Fig. 2. PCA indicating clear differences in global skull shape of *Fgfr2c*$^{C342Y/+}$ and non-mutant littermates. Mutant mice also exhibited complete or partial fusion of one or both coronal sutures and dysmorphology of the facial skeleton, cranial base, and vault (results not shown).

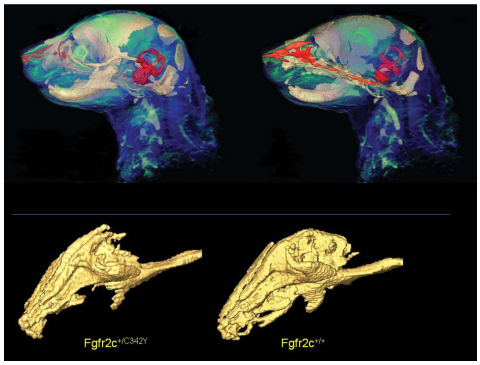

Fig. 3. Top: Superimposition of µCT and µMRM with mid-sagittal view. **Bottom:** Comparison of nasopharynges of mutant and non-mutant at P0.

Conclusions

Our results indicate that while *Fgfr2c*$^{C342Y/+}$mice and non-mutant littermates have similar skull size at birth their skull shapes differ. *Fgfr2c*$^{C342Y/+}$ mice exhibit dysmorphology of the facial skeleton, cranial base and cranial vault. Additionally, the negative space of the nasopharynx of the *Fgfr2c*$^{C342Y/+}$ mice is distinctly restricted compared to non-mutant littermates. No difference in mean inner ear volumes was noted between mutant and non-mutant mice. Future work aims to determine whether differences in the effect of the *FGFR2* C342Y mutation on these negative spaces are due to differential effects of the mutation on endochrondral or intramembranous ossification.

References

1. V. P. Eswarakumar, M. C. Horowitz, R. Locklin, G. M. Morriss-Kay, P. Lonai, *Proc Natl Acad Sci U S A* **101**, 12555 (Aug 24, 2004).
2. F. J. Rohlf, D. Slice, *Systematic Zoology* **39**, 40 (1990).
3. I. Dryden, K. Mardia, *Statistical shape analysis.*, (John Wiley and Sons, Chichester, 1998).

Acknowledgements: This project is supported by the National Institutes of Craniofacial and Dental Research (NIDCR), National Institutes of Health (NIH); Comissionat per a Universitats i Recerca (CUR), Generalitat de Catalunya, Spain; Grant Numbers: R01DE018500; 3R01DE018500-02S1; 2008 BP A 00170