Genotype-phenotype correlation of tracheal cartilaginous sleeves and *Fgfr2* mutations in mice

Austin S Lam MD, C Carrie Liu MD MPH, Gail H Deutsch MD, Joshua Rivera, Jonathan A Perkins DO, Greg Holmes PhD, Ethylin Wang Jabs MD, Michael L Cunningham MD PhD, John P Dahl MD PhD MBA

Seattle Children's HOSPITAL • RESEARCH • FOUNDATION

UW Medicine OTOLARYNGOLOGY HEAD AND NECK SURGERY

INTRODUCTION

Tracheal cartilaginous sleeve (TCS) is a spectrum of life-threatening airway malformations resulting from the vertical fusion of tracheal rings.

TCS is notoriously difficult to diagnose, and early treatment is critical.
A 90% mortality rate has been reported by 2 years of age without tracheotomy.

TCS is associated with craniosynostosis syndromes resulting from gain of function mutations in **FGFR2** (i.e. Apert, Crouzon, Beare-Stevenson, Pfeiffer).

Establishing genotype-phenotype correlation is critical to the early diagnosis, and treatment of the condition.

OBJECTIVES

- **To characterize TCS morphology in mouse models of *Fgfr2* craniosynostosis syndromes.**
- **To establish relationships between specific *Fgfr2* mutations and TCS phenotypes in these mouse models.**

METHODS

P0 knock-in mouse lines with disease specific genetic variations in the *Fgfr2* gene (***Fgfr2*$^{C342Y/C342Y}$**, ***Fgfr2*$^{C342Y/+}$**, ***Fgfr2*$^{+/Y394C}$**, ***Fgfr2*$^{+/S252W}$** and ***Fgfr2*$^{+/P253R}$**) as well as *line-specific controls* were utilized.

Tracheal cartilage morphology as measured by gross analyses (including tracheal ring classifications - **Figure 1)**, microcomputed-tomography, and histopathology were compared.

Assigned Code	Tracheal ring shape	Description	Frequency (%)
A		"Classic ring" – Singular straight band of tracheal cartilage.	100%
B		"Bifurcated ring" – Singular straight band of cartilage that crosses midline and bifurcates into two distinct bands.	61%
C		"Connected rings" – Two distinct rings of cartilage connected by a narrow band of cartilage.	12%
D		"Fused rings" – Two distinct rings of cartilage with wide common connection.	3%
E		"Thickened ring" – One distinct cartilaginous ring with thickened medial segment.	9%
F		"Slitted ring" – Thickened ring of cartilage with slit in middle.	18%
G		"Pentagonal ring" = Distinct ring of cartilage with perpendicularly directed point near the middle.	55%
H		"Ring piece" – Small piece of tracheal cartilage that is distinct from, though may be closely approximated to, a larger ring of cartilage.	24%
I		"Incomplete ring" – Distinct straight band of cartilage that does not span the entire width of the trachea.	49%
J		"Segmented ring" – Straight band of cartilage that has missing segment near the middle.	0%
K		"Merged rings" – Two distinct and complete bands of cartilage that cross midline and merge into a single band on the contralateral side.	49%
L		"Incomplete merged rings" – One distinct incomplete ring that merges with either another incomplete ring or a complete ring.	15%
M		"Laterally connected rings" – Two distinct rings of cartilage that are connected by a thin band at their most lateral aspect.	12%
N		"Double bifurcated ring" – Singular cartilaginous ring of normal thickness that spans the majority of the tracheal width before bifurcating on both ends.	9%
O		"Merged Bifurcated Ring" – Merged ring that bifurcates at the contralateral end.	6%
P		"Complete cartilaginous sleeve" – Uninterrupted sleeve of cartilage spanning the entire width of the trachea, for an entire tracheal segment.	0%
Q		"Partially sleeved rings" – All rings fused with only small areas of non-cartilaginous structures, spanning an entire tracheal segment.	0%
Z	Other	Ring shape that either does not conform to or is a combination of the above listed descriptions.	36%

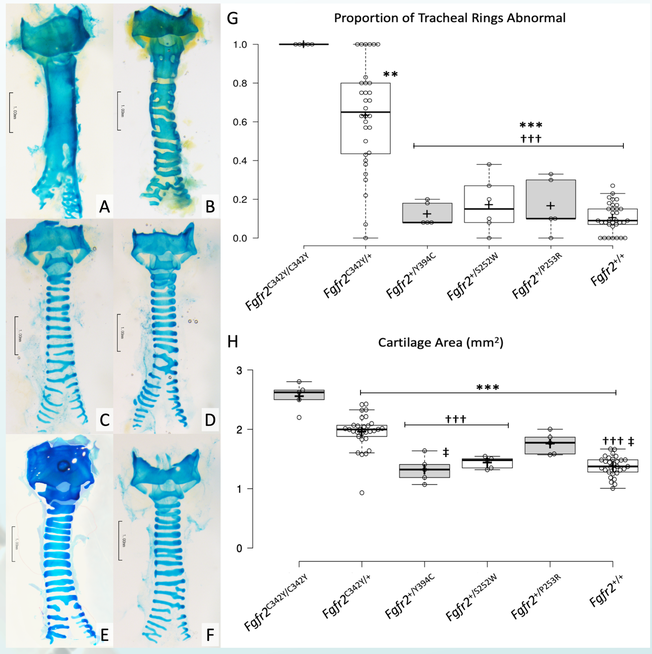

Figure 1 (Left): Tracheal ring types, assigned codes, descriptions and frequencies observed in control specimens. "Normal" tracheal ring types (frequency in controls ≥ 20%) are highlighted in green whereas "Abnormal" and "Sleeve-Type" are highlighted in yellow and red, respectively.

Figure 2 (Above): A-F) alcian blue stained whole-mount specimens, A) *Fgfr2*$^{C342Y/C342Y}$, *B) Fgfr2*$^{C342Y/+}$, C) *Fgfr2*$^{+/Y394C}$, *D) Fgfr2*$^{+/S252W}$, E) *Fgfr2*$^{+/P253R}$ and F) *Fgfr2*$^{+/+}$. G & H) demonstrate box plots representing the proportion of tracheal rings that were abnormal and cartilage area, respectively.
** $p < .01$ vs *Fgfr2*$^{C342Y/C342Y}$ | *** $p < .001$ vs *Fgfr2*$^{C342Y/C342Y}$ | ††† $p < .001$ vs *Fgfr2*$^{C342Y/+}$ | ‡ $p < .05$ vs *Fgfr2*$^{+/P253R}$

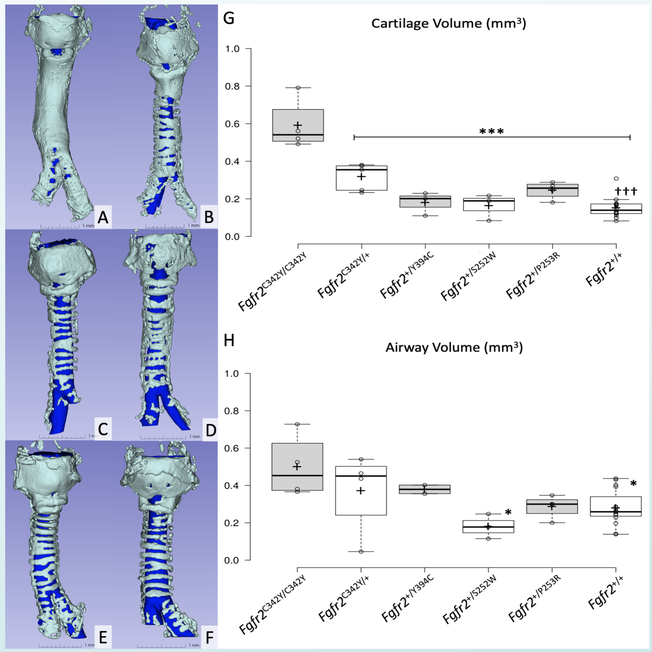

Figure 3 (Above): A-F) 3D-reconstructions of µCT specimens, A) *Fgfr2*$^{C342Y/C342Y}$, *B) Fgfr2*$^{C342Y/+}$, C) *Fgfr2*$^{+/Y394C}$, *D) Fgfr2*$^{+/S252W}$, E) *Fgfr2*$^{+/P253R}$ and F) *Fgfr2*$^{+/+}$. G & H) demonstrate box and whisker plots representing cartilage volume and airway volume, respectively.
* $p < .05$ vs *Fgfr2*$^{C342Y/C342Y}$ | *** $p < .001$ vs *Fgfr2*$^{C342Y/C342Y}$ | ††† $p < .001$ vs *Fgfr2*$^{C342Y/+}$

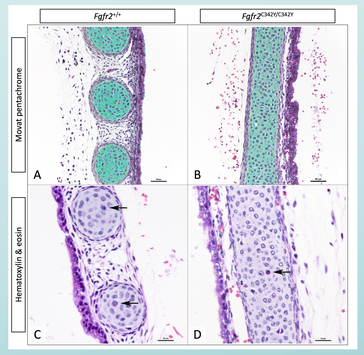

Figure 4 (Left): Representative histologic of A,C) *Fgfr2*$^{+/+}$ and B,D) *Fgfr2*$^{C342Y/C342Y}$ highlight cartilage (green) and mitotic figures (arrows).

RESULTS

- ***Fgfr2*$^{C342Y/+}$ had more abnormal ring morphology** s than all other heterozygous and control groups.
- **TCS segments were found only in *Fgfr2*$^{C342Y/C342Y}$** (100%) and ***Fgfr2*$^{C342Y/+}$** (72%) tracheas.
- **Cricoid and 1st tracheal ring fusion was noted only in *Fgfr2*$^{C342Y/C342Y}$** (100%) and ***Fgfr2*$^{C342Y/+}$** (94%) samples.
- The ***Fgfr2*$^{C342Y/C342Y}$ and *Fgfr2*$^{C342Y/+}$ groups were found to have greater areas and volumes of cartilage** than other lines on gross analysis and microcomputed-tomography.
- Histologic analyses confirmed TCS among the *Fgfr2*$^{C342Y/C342Y}$ and *Fgfr2*$^{C342Y/+}$ groups, with **no appreciable differences in cartilage morphology, cell size or density**.

CONCLUSION

- **This study found TCS phenotypes only in the *Fgfr2*^{C342Y} mouse lines. These lines also had increased tracheal cartilage compared to other mutant lines and controls.**
- **These data support further study of the *Fgfr2* mouse lines and the investigation of other *Fgfr2* variants to better understand their role in tracheal development and TCS formation.**