

3D Assessment of Nasopharyngeal and Craniofacial Phenotypes in Ts65Dn Down Syndrome Mice Treated with a Dyrk1a Inhibitor.

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ABSTRACT

Background: Down syndrome (DS) originates from having three copies of chromosome 21 (i.e. Trisomy 21). DS is associated with many detrimental phenotypes including intellectual disabilities, heart defects, abnormal craniofacial development, and obstructive sleep apnea, which develops from restricted nasopharyngeal airways and an underdeveloped mandible. Ts65Dn mice are trisomic for about half of the orthologs on human chromosome 21 and display many phenotypes associated with DS including craniofacial abnormalities. *Dyrk1a* is found in three copies in Ts65Dn mice and individuals with DS, and thought to be a root cause of the craniofacial phenotypes. Epigallocatechin 3-gallate (EGCG) is a green tea polyphenol and inhibitor of Dyrk1a activity.

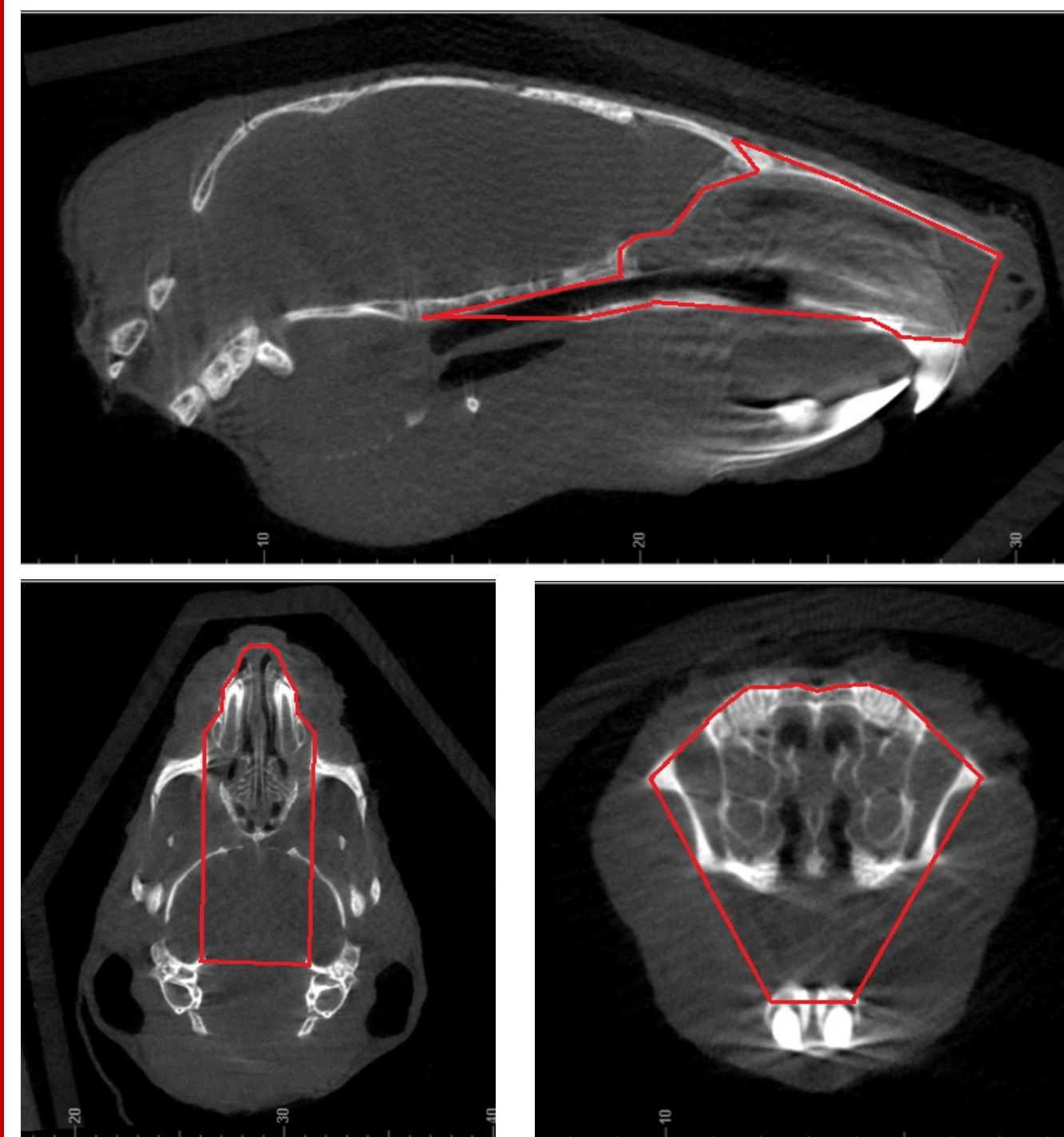
Purpose: We hypothesize that decreased Dyrk1a activity in Ts65Dn mice will ameliorate craniofacial dysmorphology.

Methods: To test our hypothesis we compared Ts65Dn mice with two or three copies of *Dyrk1a* and compared Ts65Dn mice with and without prenatal EGCG treatment. EGCG treated mothers were fed 200mg/kg EGCG on gestational day 7. Six week old mice were sacrificed and their heads imaged using micro-computed tomography (μ CT). From μ CT images, we measured nasopharyngeal airway volume and anatomical landmarks ($n = 54$) from the facial skeleton, cranial vault, cranial base, and mandible. Mean nasopharyngeal airway volumes were graphically compared, and a landmark-based multivariate geometric morphometric approach known as Euclidean Distance Matrix Analysis (EDMA) was carried out to assess local differences in craniofacial morphology between trisomic mouse samples.

Results: Our preliminary results indicate that EGCG treatment and reduced *Dyrk1a* copy number increases mean nasopharyngeal airway volume in Ts65Dn mice. Craniofacial morphometric differences were found among all samples. EGCG treatment increased portions of the mandible and decreased portions of the cranial vault and cranial base.

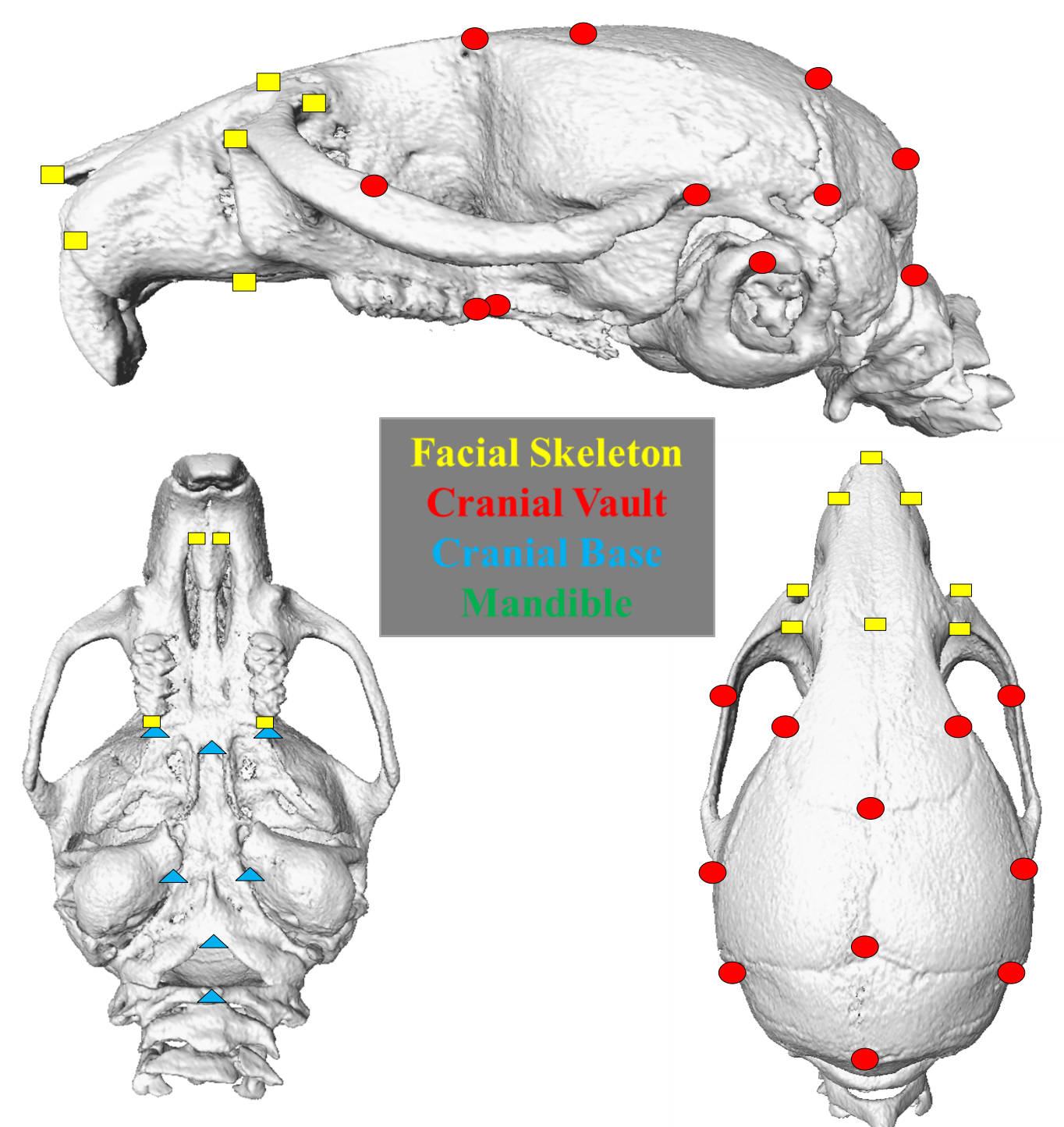
Conclusion: Preliminary analyses suggest that both EGCG treatment and reduced *Dyrk1a* copy number affect craniofacial morphology.

MEASUREMENTS



Left: Boundaries demarcated on μ CT slices to measure nasopharyngeal airway volume. Top – sagittal plane, left – axial plane, right – coronal plane.

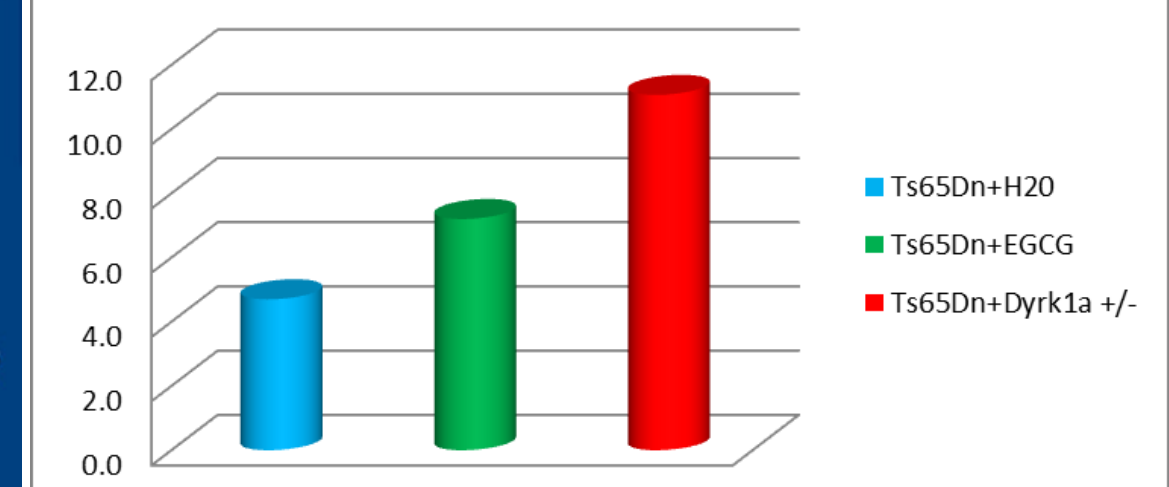
Right: Anatomical landmarks measured from mouse skulls. Landmarks identified for the face are colored yellow, cranial vault in red, cranial base in blue, and mandible in green.



RESULTS: AIRWAYS



Mean nasopharyngeal airway volume (mm³)



Left: Superior and lateral 3D reconstructions of nasopharyngeal airway volumes (pink). Left image is Ts65Dn+H₂O. Middle image is Ts65Dn+EGCG. Right image is Ts65Dn/Dyrk1a^{+/-}.

Right: Comparison of Ts65Dn+H₂O, Ts65Dn+EGCG, and Ts65Dn/Dyrk1a^{+/-} mean nasopharyngeal airway volume. Ts65Dn+EGCG mice exhibit increased nasopharyngeal airway volume relative to Ts65Dn+H₂O mice. Ts65Dn/Dyrk1a^{+/-} mice show increased nasopharyngeal airway volume relative to both Ts65Dn+H₂O and Ts65Dn+EGCG mice.

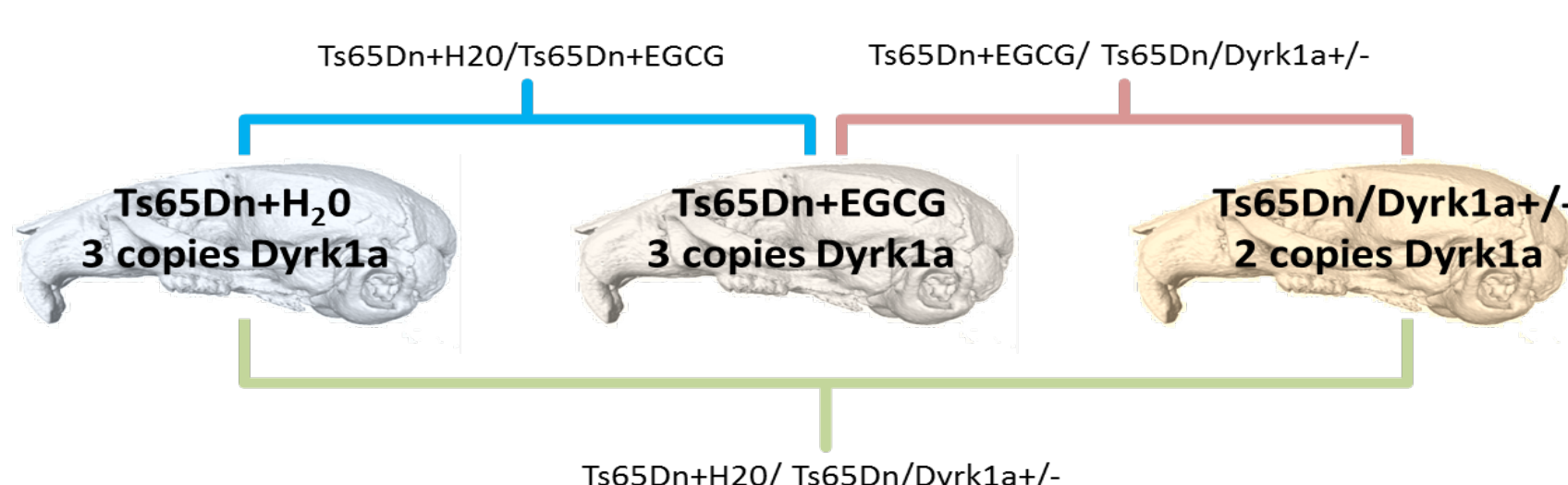
INTRODUCTION

Down Syndrome (DS) is a relatively common genetic disorder found in 1 in 700 live births. DS is caused by trisomy 21. DS is associated with craniofacial abnormalities and airway complications such as obstructive sleep apnea. Dyrk1a is a gene found in three copies in DS. The purpose of this preliminary study is to determine whether reducing copy number or activity of trisomic Dyrk1a will improve craniofacial phenotypes. We have done this by treating DS mice with Epigallocatechin 3-gallate (EGCG), a Dyrk1a inhibitor ($n=4$) or reducing Dyrk1a copy number in Ts65Dn mice ($n=3$) and comparing controls ($n=4$).

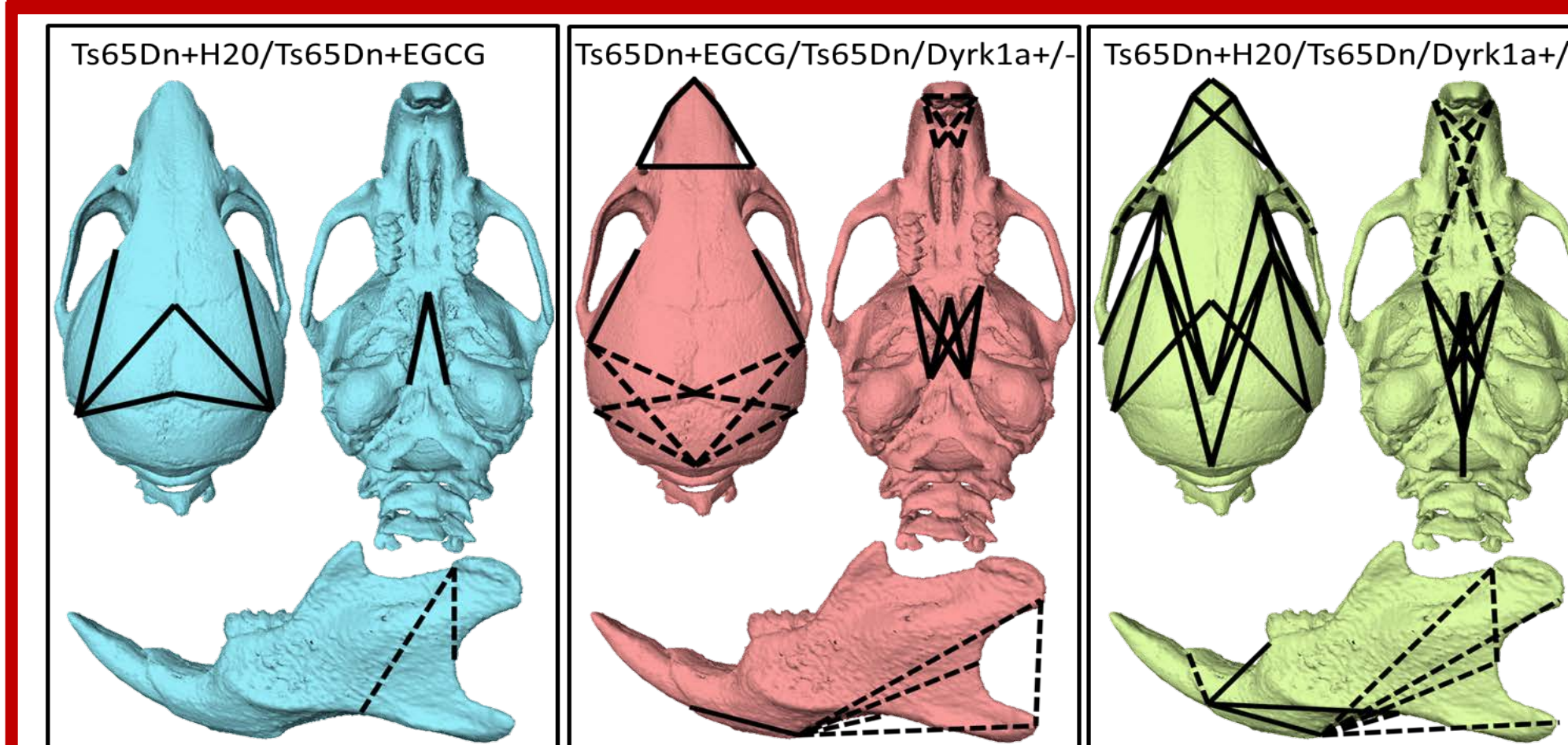
METHODS

EGCG treated mothers were fed 200mg/kg EGCG on gestational day 7. Six week old mice were sacrificed and their heads imaged using micro-computed tomography (μ CT; GE Healthcare, Inc. USA, EVS-R9 μ CT scanner, tube voltage 50kVP, current 1000mA, shutter speed 400ms, 0.043mm resolution/voxel size). Dolphin software (v11.5; Chatsworth, CA) was used to measure nasopharyngeal airway volumes and collect 3D coordinate values of anatomical landmarks ($n = 54$) from mouse skulls and mandibles. Mean nasopharyngeal airway volumes were compared among 3 samples: 1) Ts65Dn mice with 3 copies of Dyrk1a treated with H₂O (Ts65Dn+H₂O); 2) Ts65Dn mice with 3 copies of Dyrk1a treated with EGCG (Ts65Dn+EGCG); and 3) Ts65Dn knockout mice with 2 copies of Dyrk1a (Ts65Dn/Dyrk1a^{+/-}). A multivariate geometric morphometric landmark-based approach known as Euclidean Distance Matrix Analysis (EDMA) was carried out to assess local 2-sample morphological form differences among the 3 groups (see figure below).

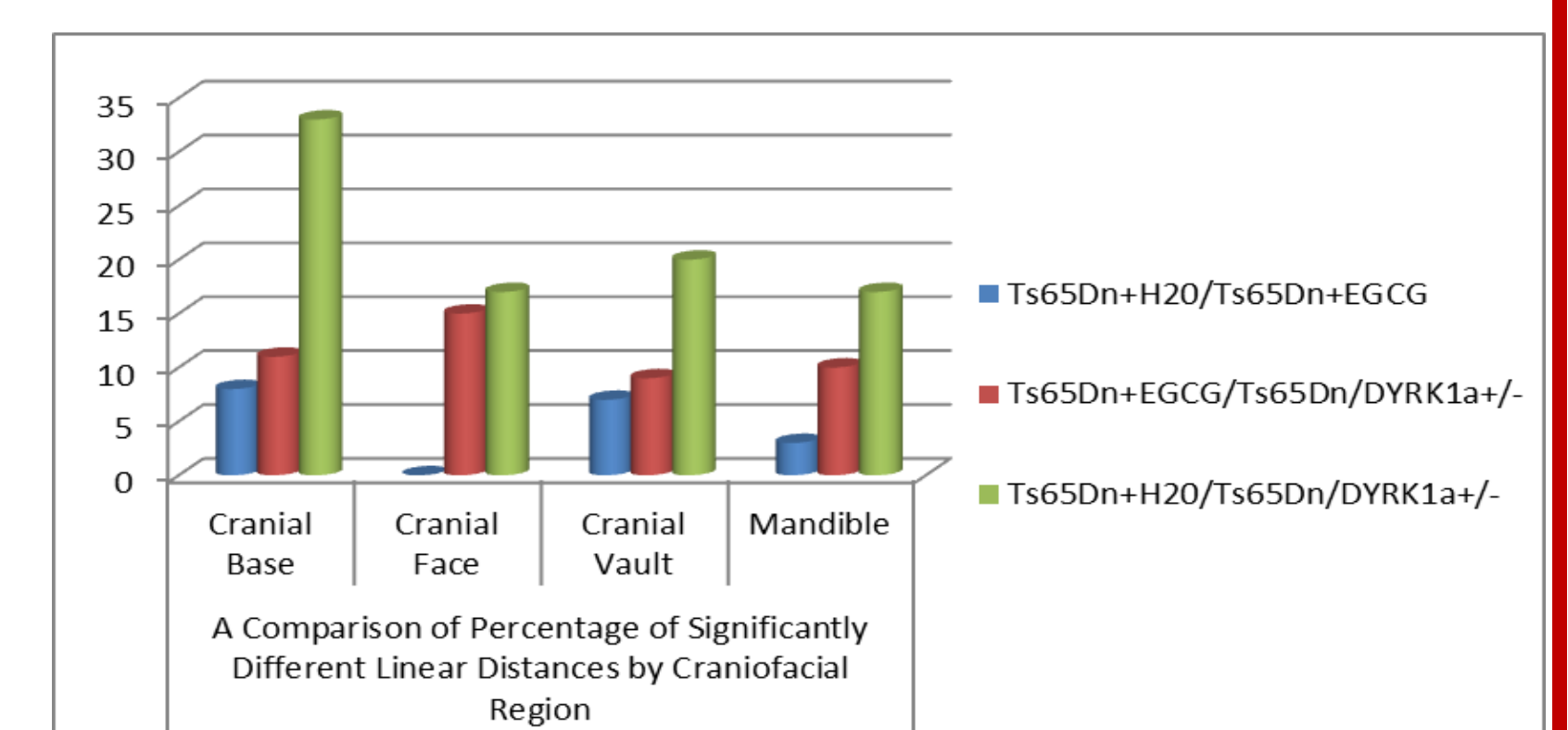
EDMA Comparisons (Face, Vault, Base, Mandible)



RESULTS: MORPHOMETRICS



— Significantly larger in numerator — — — Significantly smaller in numerator



Two-sample morphometric analyses were carried out to compare mouse cohorts: 1) Ts65Dn+H₂O compared to Ts65Dn+EGCG (blue); 2) Ts65Dn+EGCG compared to Ts65Dn/Dyrk1a^{+/-} (red); and 3) Ts65Dn+H₂O compared to Ts65Dn/Dyrk1a^{+/-} (green).

Left: Linear distances that significantly differ between 2-sample comparisons of mouse cohorts.

Right: Percent of significantly different linear distances by craniofacial region for each 2-sample comparison.

CONCLUSIONS

- EGCG treated mice exhibit increased nasopharyngeal airway volumes relative to mice treated with H₂O
- Ts65Dn/Dyrk1a^{+/-} mice have increased nasopharyngeal airway volumes relative to both H₂O and EGCG treated mice.
- H₂O treated mice and Ts65Dn/Dyrk1a^{+/-} mice exhibit the most morphological differences (as expected).
- EGCG treated mice are morphologically different from both H₂O control and Ts65Dn/Dyrk1a^{+/-} mice, and exhibit a craniofacial form that is in between the extreme morphological forms defined by H₂O and Ts65Dn/Dyrk1a^{+/-} mice, which suggests that EGCG does influence morphogenesis.

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