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Analysis of the craniofacial phenotype of Down Syndrome rodent models

Introduction

Down syndrome (DS) also called Trisomy 21 (T21) is the most common chromosomal disorder and the principal cause of intellectual disability worldwide (EUROCAT, 2019). In most cases, it is due to an extra copy of chromosome 21 (Hsa21). It covers a complex set of pathologies that involve practically all organs and systems (Lyle et al. 2009). The most frequent and distinctive alterations are learning disability and craniofacial dysmorphism (Lana-Elola et al. 2011). The objective of this study is to understand the pathways and genes that are altered during the development of craniofacial abnormalities in T21 (Figure 1).

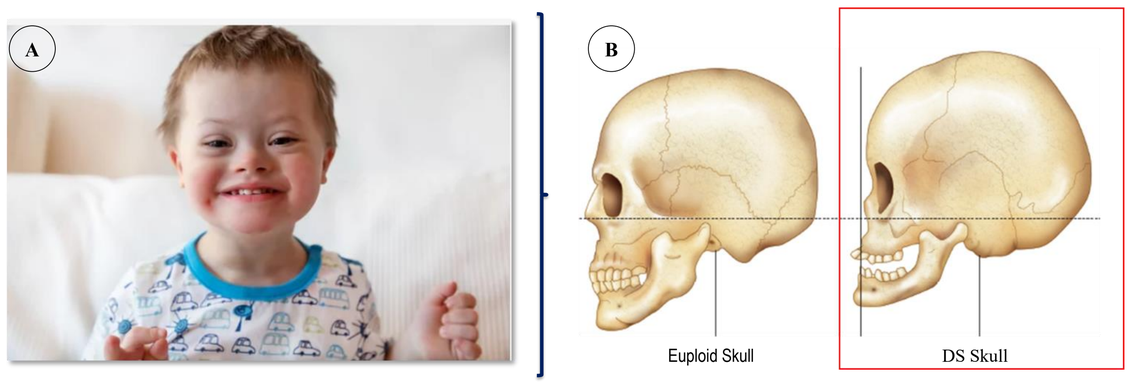

Figure 1 DS Craniofacial Phenotype. (A) Facial gestalt in DS individual: Flattened nasal bridge, almond-shaped eyes, short neck, small ears. (B) Comparison between the skull of an euploid versus a DS human skull. Craniofacial phenotype: Overall reduction in head dimensions (Microcephaly), Small midface, reduced facial height, orbital region reduced mediolaterally, reduced bizygomatic breadth, small maxilla, brachycephaly (relatively wide neurocranium), small mandible.

Image: Down Syndrome Child, images, photos et images vectorielles de stock | Shutterstock. Image Skeletal structure: Dierssen, Hérault et Estivill 2009)

Methods and Results

We combined a new genetically modified rat model (with segmental duplications in rat chromosome 11 and 20) recently created, thanks to CRISPR / Cas9 technology, at the Institut Clinique de la Souris (ICS)- Illkirch, and new mice models with different segmental duplications (Figure 2). We performed comparative 3D morphometric analysis (Figure 3), with Euclidean distance analysis, Procrustes general analysis and Principal component analysis, to evaluate the craniofacial bone structures of these models, compared them with the human DS phenotype and information from already known mice models. (Figure 4, 5).

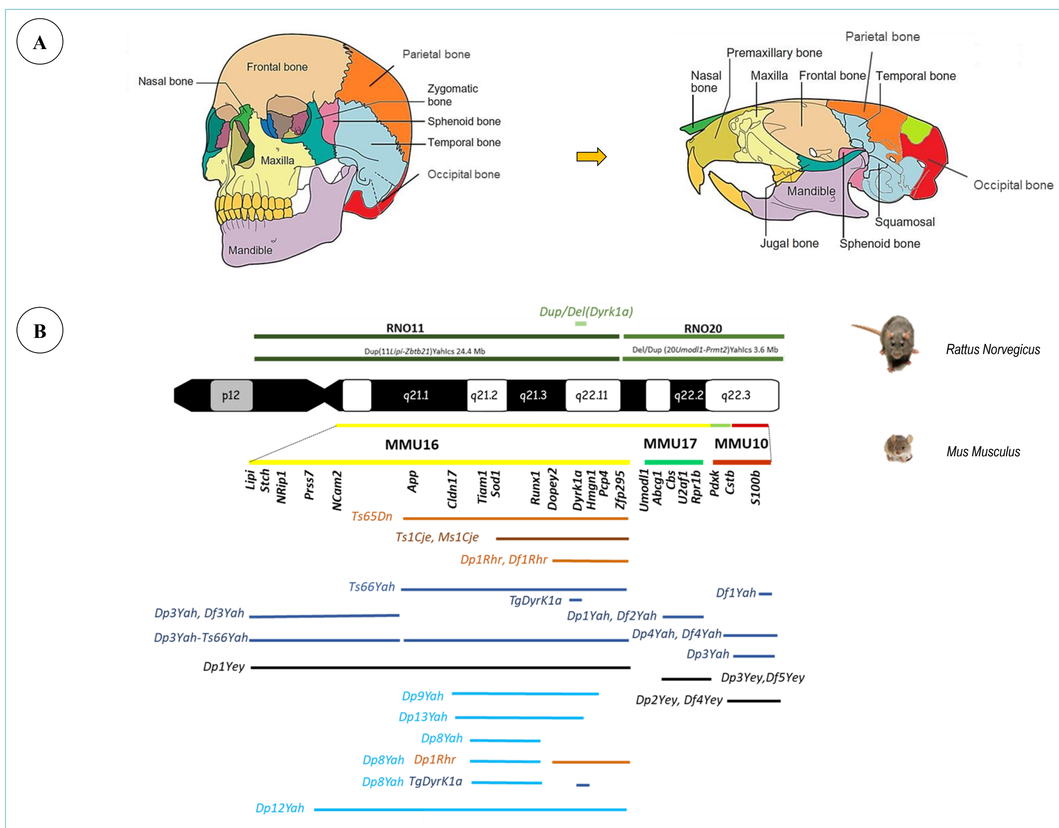

Figure 2 DS Rodent Models. (A) Representation of the high correspondence between the human skull structures and rodent skull structures. (B) Homologous region of the human chromosome 21 (HSA21) are located on rat chromosomes 11 and 20 (RNO), and on mouse chromosomes 16, 17 and 10 (MMU). In upper part, rat models with segmental duplications of Dup(RNO11) and Dup(RN020). Down part, mouse models with different segmental duplications in chromosome 16, 17 and 10.

Image skeletal structures:. Mouse Models of Rare Craniofacial Disorders. Current Topics in Developmental Biology, 2015. Scheme rodent model and homologous region of HSA21: Dr. Yann Herault

Figure 3 3D Morphometric analysis. Methodology for quantification and statistical analysis of form: micro-CT scan, 3D modelisation, location of landmarks, Euclidean Distance Matrix Analysis (Form difference matrix / Shape difference matrix), General Procrustes Analysis and Principal Component Analysis (Hallgrimsson et al. 2015). Population average methodology (with New software Stratovan Checkpoint): From a volume data of a specimen with surfaces and a homologous set of 3D landmarks, landmarks data is used to represent the population (Orientation and scale may be different among specimen), rigid alignment to remove orientation differences, average pointset is computed using Generalized Procrustes Alignment (to remove the scale difference), resulting in the target average pointset. All volumes are then merged resulting in a population average volume (User manual Stratovan Checkpoint, 2020).

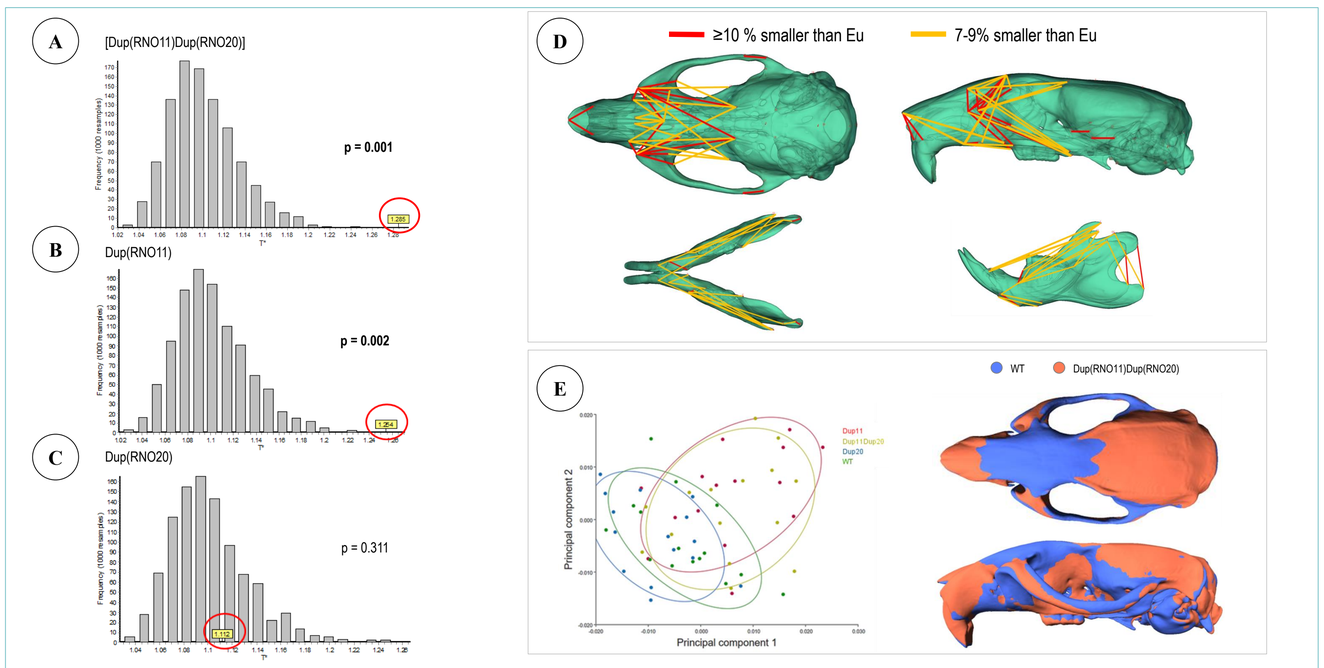

Figure 4 Result based on new DS rat models. (A) Bootstrap of the FDM, Wt vs [Dup(RNO11)Dup(RNO20)], significant difference. (B) Bootstrap of the FDM, Wt vs Dup(RNO11), significant difference. (C) Bootstrap of the FDM, Wt vs Dup(RNO20), no significant difference. (D) Percentage of decrease of the Relative Euclidean distance (RED) in the population with more significant changes ([Dup(RNO11)Dup(RNO20)]), in red the RED smaller in 10% or more, in orange the RED smaller in 7-9%. (E) Results of GPA, PCA and Population average (Warping). In the left, PCA graphic, comparison between the principal components of all genotypes, the distribution show no difference between WT and Dup(RNO20) and significant differences in Dup(RNO11) and [Dup(RNO11)Dup(RNO20)]. In the right, Warping technique WT vs [Dup(RNO11)Dup(RNO20)] of average population models, revealing the form differences and the regions and bones affected.

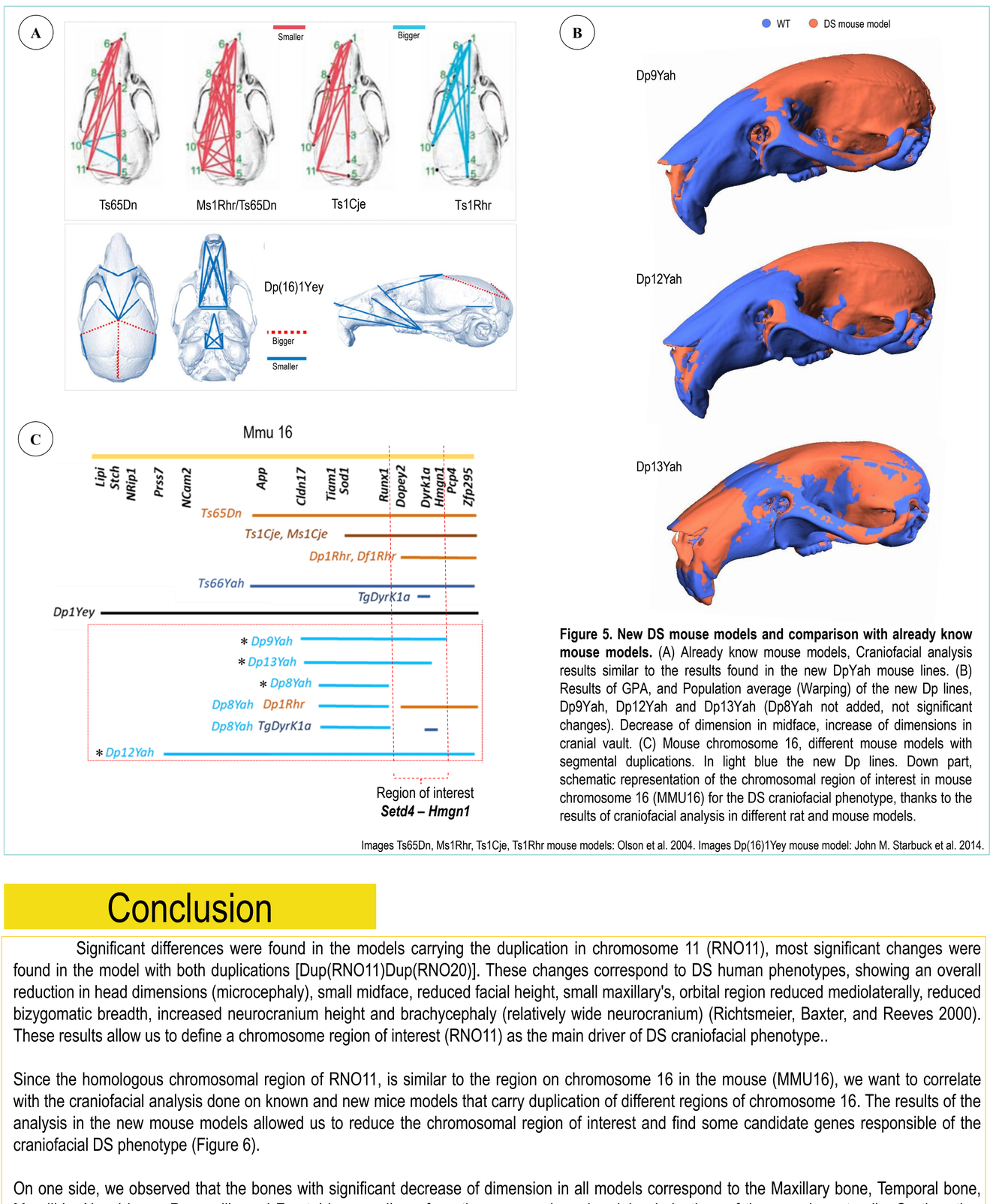

Figure 5. New DS mouse models and comparison with already know mouse models. (A) Already know mouse models, Craniofacial analysis results similar to the results found in the new DpYah mouse lines. (B) Results of GPA, and Population average (Warping) of the new Dp lines, Dp9Yah, Dp12Yah and Dp13Yah (Dp8Yah not added, not significant changes). Decrease of dimension in midface, increase of dimensions in cranial vault. (C) Mouse chromosome 16, different mouse models with segmental duplications. In light blue the new Dp lines. Down part, schematic representation of the chromosomal region of interest in mouse chromosome 16 (MMU16) for the DS craniofacial phenotype, thanks to the results of craniofacial analysis in different rat and mouse models.

Images Ts65Dn, Ms1Rhr, Ts1Cje, Ts1Rhr mouse models: Olson et al. 2004. Images Dp(16)1Yey mouse model: John M. Starbuck et al. 2014.

Conclusion

Significant differences were found in the models carrying the duplication in chromosome 11 (RNO11), most significant changes were found in the model with both duplications [Dup(RNO11)Dup(RNO20)]. These changes correspond to DS human phenotypes, showing an overall reduction in head dimensions (microcephaly), small midface, reduced facial height, small maxillary's, orbital region reduced mediolaterally, reduced bizygomatic breadth, increased neurocranium height and brachycephaly (relatively wide neurocranium) (Richtsmeier, Baxter, and Reeves 2000). These results allow us to define a chromosome region of interest (RNO11) as the main driver of DS craniofacial phenotype..

Since the homologous chromosomal region of RNO11, is similar to the region on chromosome 16 in the mouse (MMU16), we want to correlate with the craniofacial analysis done on known and new mice models that carry duplication of different regions of chromosome 16. The results of the analysis in the new mouse models allowed us to reduce the chromosomal region of interest and find some candidate genes responsible of the craniofacial DS phenotype (Figure 6).

On one side, we observed that the bones with significant decrease of dimension in all models correspond to the Maxillary bone, Temporal bone, Mandible, Nasal bone, Premaxilla and Frontal bones; all are from the same embryonic origin, derivatives of the neural crest cells. On the other side, we found bones with an increase in their dimensions such as the Parietal bone, Intraparietal bone and occipital bone, with another embryonic origin, Mesoderm.

This allows us to postulate that within our region of interest we have candidate genes that generate a decrease in the dimensions of the midface and candidate genes that generate an increase in the size of the cranial vault. For this, molecular studies will be carried out considering this new region of interest.

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