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# Analysis of the craniofacial phenotype of Down Syndrome rodent models

## Introduction

Down syndrome (DS) also called Trisomie 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).

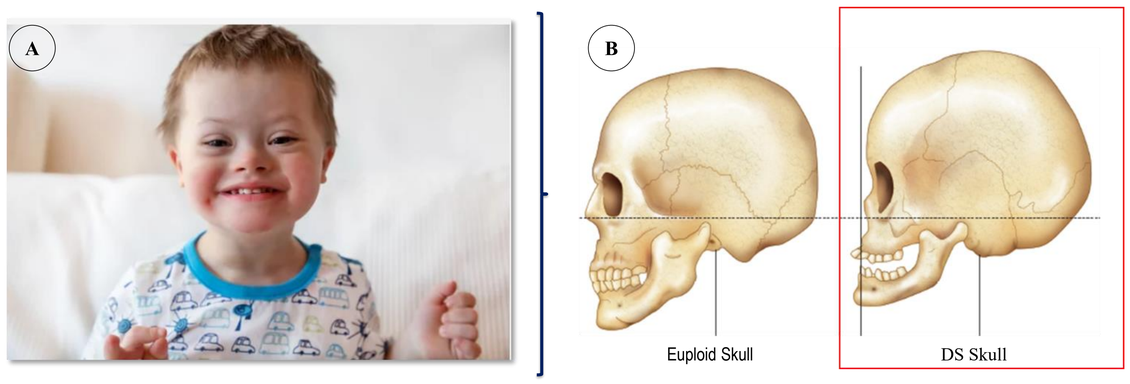



**Figure 1 DS Craniofacial Phenotype.** (A) Facial gestalt in DS individual: Flattened nasal bridge, almond-shaped eyes, short neck, small ears. (B) Comparison between the skull of an euploid versus a DS human skull. Craniofacial phenotype: Overall reduction in head dimensions (Microcephaly), Small midface, 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

Using a new genetically modified rat model recently created thanks to CRISPR / Cas9 technology at the Institut Clinique de la Souris (ICS)- Illkirch, two regions homologous to human chromosome 21 were duplicated on rat chromosomes 11 and 20 (Dup(RNO11), and Dup(RNO20) respectively) (Figure 2). Comparative 3D morphometric analysis (performing Euclidean distance analysis and Procrustes general analysis) (Figure 3) was used to evaluate the craniofacial bone structures of these rat models, understand where are replace by the localization of significant changes and correlate them with the DS human phenotype and already known and new mice models (Figure 4, 5).

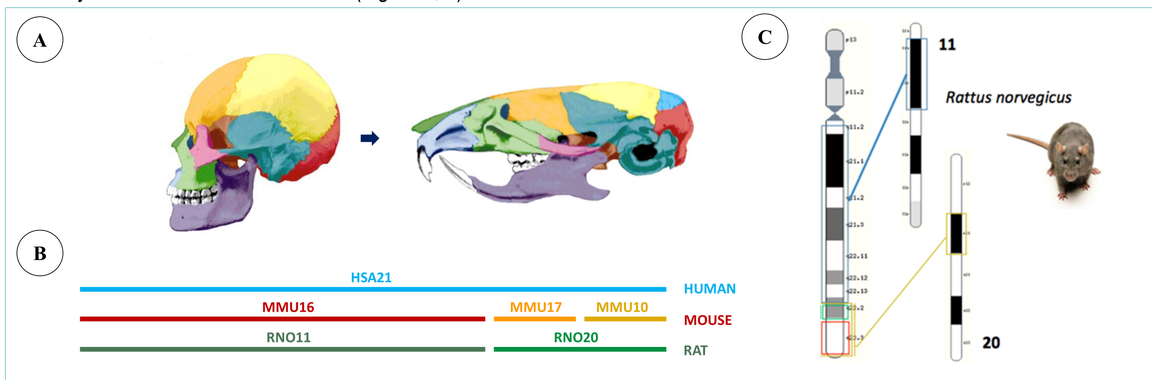



**Figure 2 Rodent Models**. (A) Representation of the high correspondence between the human skull structures and rodent skull structures. (B) Homologous region of the human chromosome 21 (HSA21) are located on mouse chromosomes 16, 17 and 10 (MMU), and on rat chromosomes 11 and 20 (RNO). (C) Segmental duplications of Dup(RNO11) and Dup(RN020) lines.

Image skeletal structures: Richtsmeier, 2000. Image rat model and homologous region of HSA21: Dr. Yann Herault

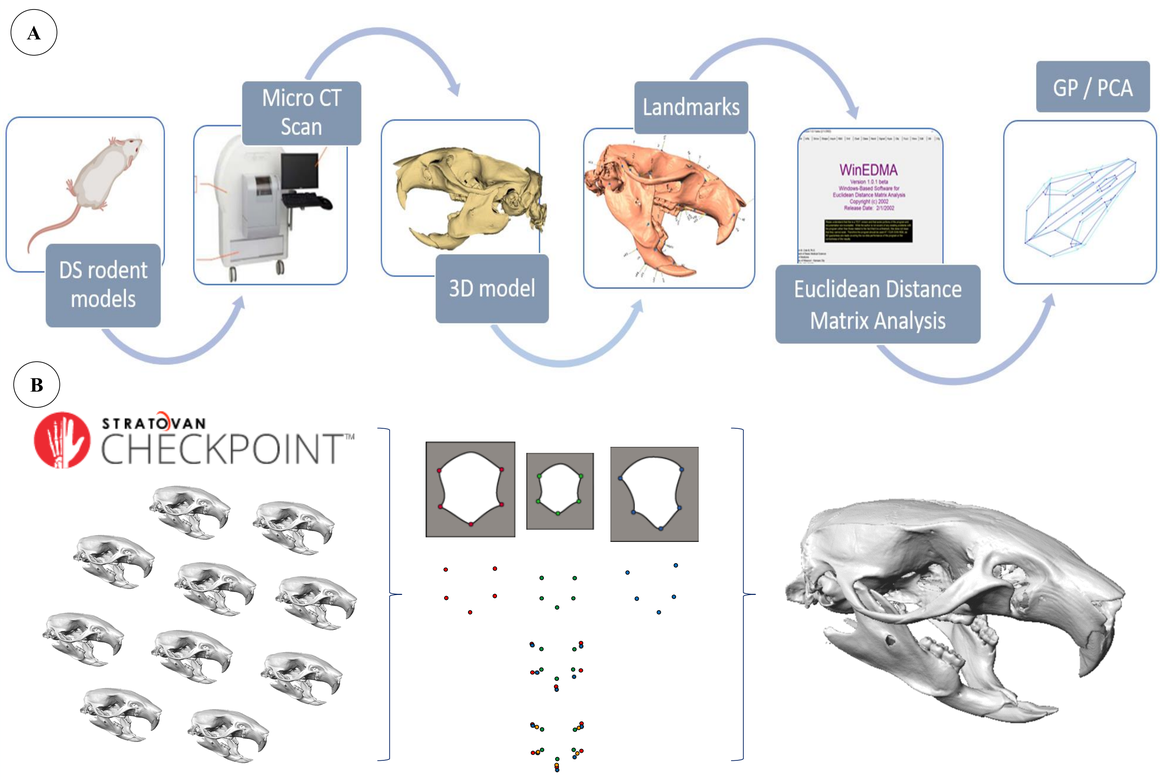
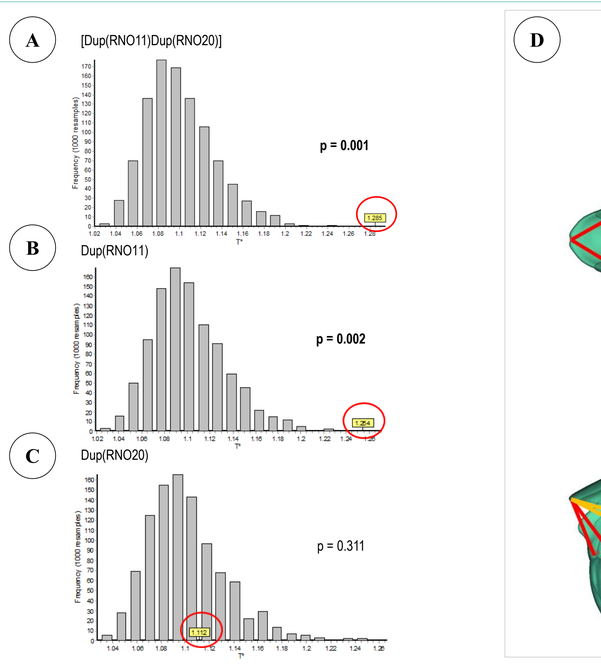



**Figure 3 3D Morphometric analysis.** (A) Methodology for quantification and statistical analysis of form: micro-CT scan, 3D modelisation, location of landmarks, Euclidean Distance Matrix Analysis (Form difference matrix / Shape difference matrix), General Procrustes Analysis and Principal Component Analysis (Hallgrimsson et al. 2015). (B) Population average methodology (with New software Stratovan Checkpoint): Start 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 together resulting in a population average volume (User manual Stratovan Checkpoint, 2020).



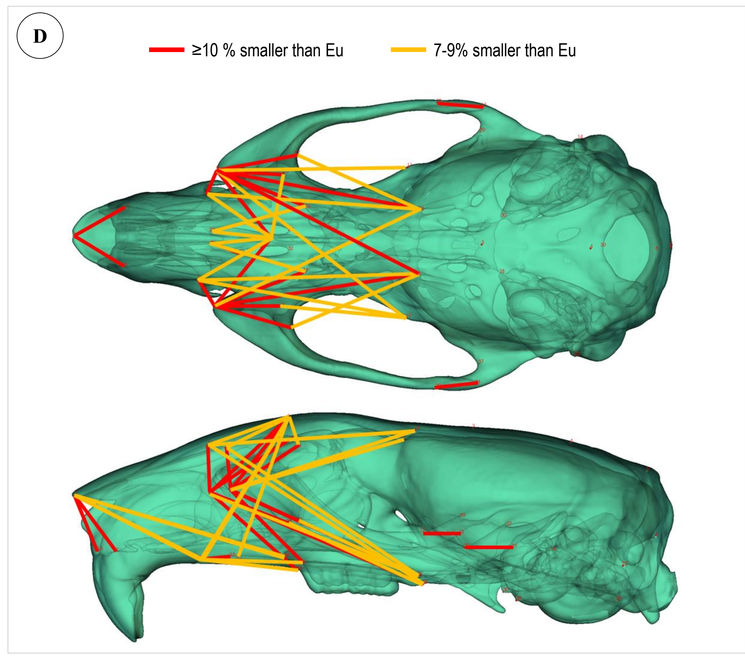




**Figure 4 EDMA Analysis.** (A) Bootstrap of the FDM, Wt vs [Dup(RNO11)Dup(RNO20)], significant difference. (B) ) Bootstrap of the FDM, Wt vs Dup(RNO11), significant difference. (C) ) Bootstrap of the FDM, Wt vs Dup(RNO20), no significant difference. (D) Percentage of decrease of the Relative Euclidean 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%.

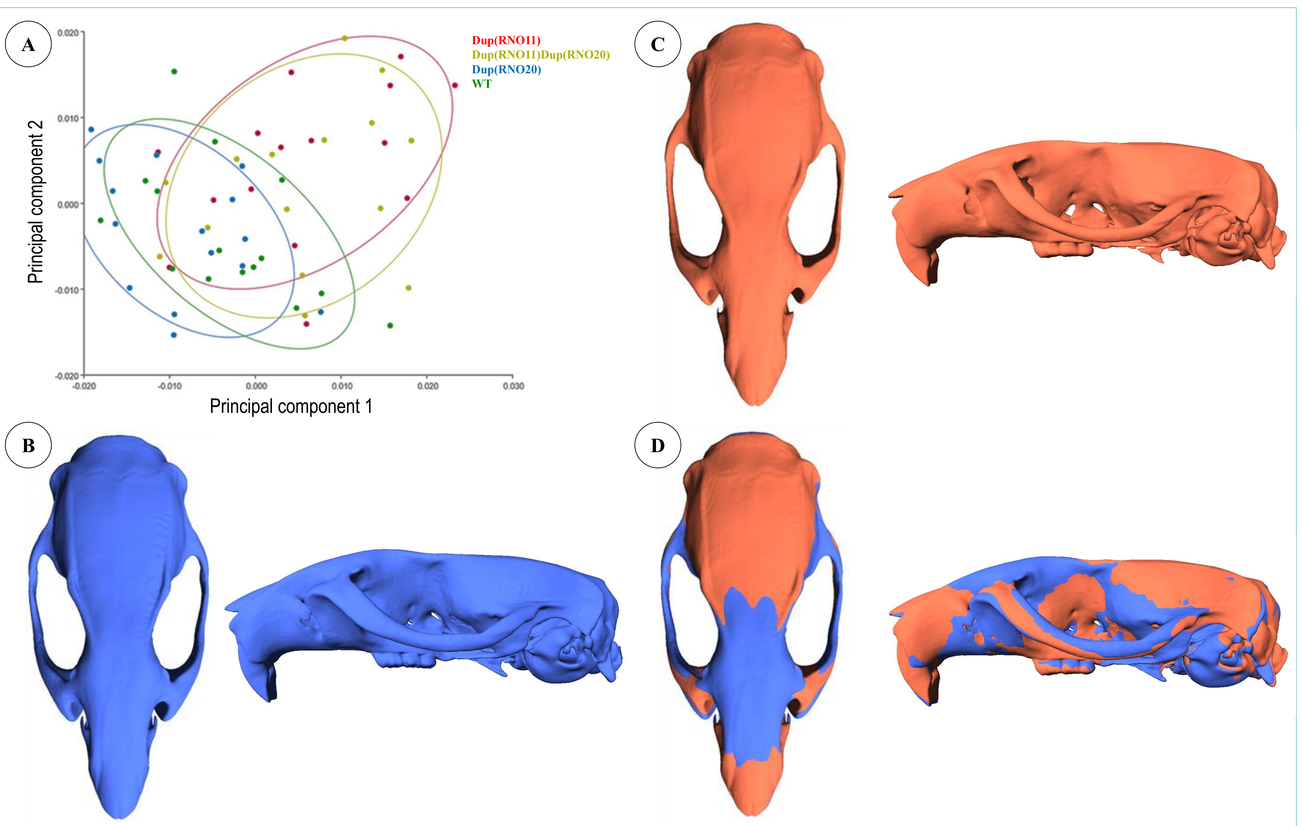



**Figure 5 General Procrustes Analysis, Principal Component Analysis and Population average (Warping).** (A) Principal Component graphic, color correspondence: Green/WT, Blue/Dup (RNO20), Red/Dup (RNO11), Yellow/[Dup(RNO11)Dup(RNO20)]. Comparison between the principal components of all genotypes, the distribution show no difference between WT and Dup(RNO20) and significant differences in Dup(RNO11) and [Dup(RNO11)Dup(RNO20)]. (B) Average Model of WT population (Blue). (C) Average model of the population with more significant changes [Dup(RNO11)Dup(RNO20)] (Orange). (D) Warping technique WT vs [Dup(RNO11)Dup(RNO20)] average models. Revealing the regions and bones affected and size differences.

## 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)]. This changes correspond to a DS human phenotype, 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 responsible for the DS craniofacial phenotype. These results could be correlated with the craniofacial analysis in different already know and new mice models that carry a duplication on different regions of chromosome 16, since the homologous chromosomal region of RNO11, is found on the chromosome 16 in the mouse (MMU16), this allow us to reduce the chromosomal region of interest and find some candidate genes responsible of the craniofacial DS phenotype (Figure 6).

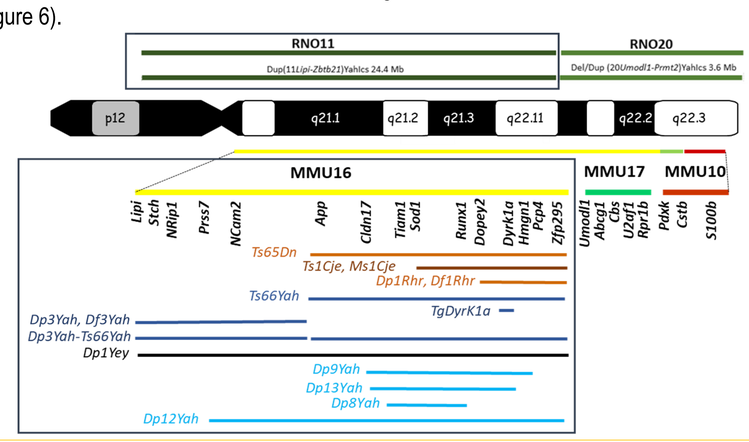



**Figure 6 Mouse models.** Schematic representation to show that homologous chromosomal region of RNO11, is found on chromosome 16 in the mouse (MMU16). Mice models with duplications of different regions of Chromosome 16 (Mmu16).

Scheme DS rodent model: Dr. Yann Herault

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