

Autism Spectrum Disorder seen through brain organoids - a Portuguese cohort characterization

Ana Manuela Silva^{1,2}, Catarina M. Seabra^{2,3}, Ana Rafaela Oliveira², Mariana Laranjo^{2,3}, Frederico Duque^{5,6}, Cátia Café⁵, João Peça^{2,4}, Guiomar Oliveira^{5,6}

(1) Faculty of Medicine, University of Coimbra, Portugal; (2) Center for Neuroscience and Cell Biology (CNC), Coimbra, Portugal; (3) Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Portugal; (4) Department of Life Sciences, University of Coimbra, Portugal; (5) Neurodevelopmental and Autism Unit, Child Developmental Center, Centro de Investigação e Formação Clínica, Pediatric Hospital, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; (6) University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Background: Autism Spectrum Disorder (ASD) is a severe neurodevelopmental disorder that affects 9,2 in 10 000 children in Portugal¹. There is no treatment for its core features as only symptomatic intervention is available². Brain organoids provide a new window to understand the mechanisms underlying ASD.

Objectives - Characterization of a cohort of ASD patients
- Explore patient-derived brain organoids to assess neurodevelopment

Impact: ASD is a severe disorder which affects not only the patient but the whole family. This study is a step forward towards their quality of life's improvement.

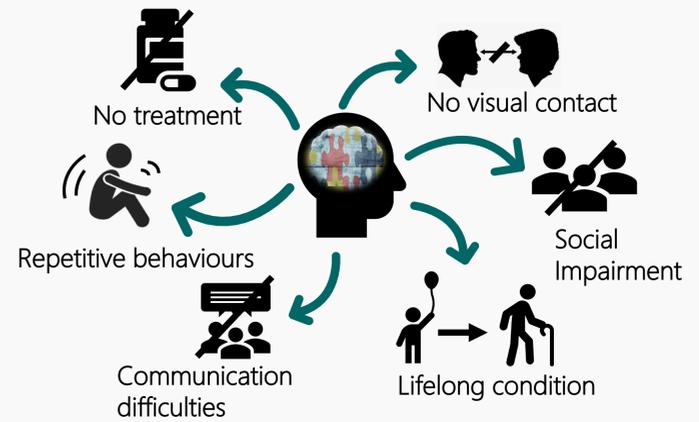


Figure 1. ASD characteristics.

32 Participants

17 Controls

Application of Social Communication Questionnaire

15 Patients

From Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra

ASD diagnosis

Autism Diagnostic Interview-Revised
Autism Diagnostic Observation Schedule
Etiologic genetic study (when indicated)

Data collection

Neurodevelopmental data:

- Cephalic perimeter at birth
- Age of night sphincters control achievement
- First steps' age
- First words' age
- Social smile

Comorbidities
Family History
Age of Diagnosis

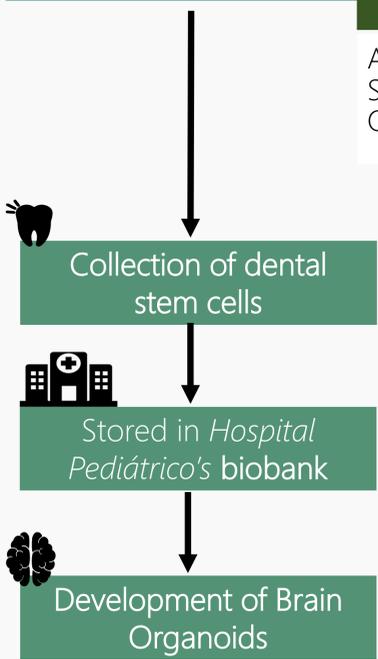


Figure 2. Cell manipulation. Extraction of dental stem cells from third molars (top) or exfoliated teeth (bottom).

Results

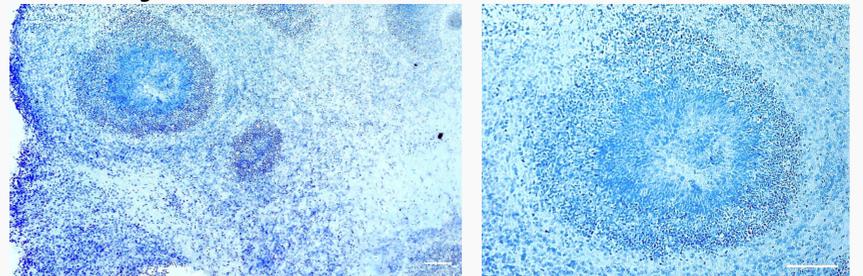
ASD Cohort Characterization

Assessment	Results
Male	57,1%
Female	42,9%
Average age of diagnosis	3,4 years old
First footsteps	15,7 months (M)*
First words	19,9 M*
Night sphincters control	57,6 M*
Macrocephaly or Microcephaly	No cases
Intellectual Disability	35,7%
Positive family history	33,3%
Genetic Diagnosis	40,0%
Variants Identified	SHANK3, MECP2, SLC6A8, CNV loss, CNV gain

Table 1. ASD cohort characterization. Positive family history with cases of ASD. Humor Perturbation or Epilepsy. Social smile and cephalic perimeter were not possible to analyze. *These items correspond to the average age.

Patient-derived Brain Organoids

Nissl Staining



Hematoxylin and Eosin Staining

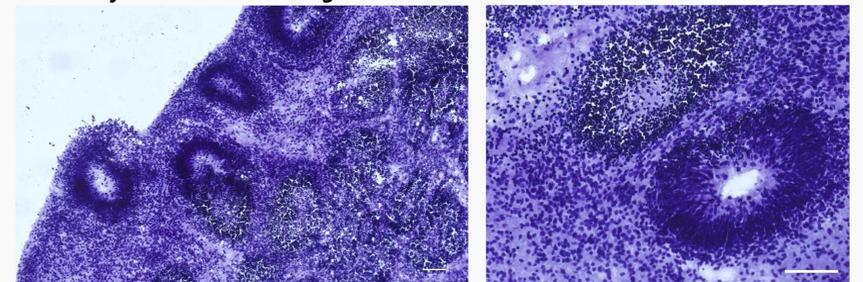


Figure 3. Organoids were sectioned using a cryostat and then stained using two different staining protocols. Top: Nissl Staining in Brain Organoids. Scale Bars, 100 µm. Bottom: Brain Organoids with Hematoxylin and Eosin Staining. Scale Bars, 100 µm. The "ventricle-like" structures mimic the neural tube that is formed during neurodevelopment. These stainings allow the analysis of: neuronal organization, number of ventricles, ventricle width.

Conclusions and Future Work

We established the **first dental stem cells' biobank** in Portugal to study neurodevelopmental disorders and characterized a cohort of ASD patients. We will continue to reprogram these cells and develop **brain organoids** to identify the mechanisms behind ASD, in a **personalized medicine approach**. This way, we are placing the **next piece of the puzzle** in the direction of the understanding of ASD.

References: 1. Oliveira G, Desenvolvimento C De. Epidemiology of autism spectrum disorder in Portugal: prevalence, clinical characterization, and medical conditions. 2007;(Table 1):726-733. 2. Direção Geral de Saúde. Abordagem Diagnóstica e Intervenção na Perturbação do Espectro do Autismo em Idade Pediátrica e no Adulto. 2019:1-31. 3. Lancaster MA, Knoblich JA. Generation of cerebral organoids from human pluripotent stem cells. 2014;9(10):2329-2340. doi:10.1038/nprot.2014.158. 4. Christensen, D. L., Braun, K., Baio, J., Bilder, D., Charles, J., Constantino, J., et al. (2018). Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. *Morbidity and mortality weekly report. Surveillance summaries* (Washington, D.C. : 2002), 65(13), 1-23. doi:10.15585/mmwr.ss6513a1. 5. Duarte A, Jorge A, Leça A, et al. Saúde Infantil e Juvenil Programa Nacional. 2012. 6. Bridgeman CF. Autism Spectrum Disorder. In: *Nelson Textbook of Pediatrics, 2-Volume Set*. Twenty-Fit. Elsevier Inc.; 2020:294-302.e1. doi:10.1016/B978-0-323-52950-1.00054-7