American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Mini Review

Advances in Alzheimer's Disease Research: Human Cerebral Organoids

Patricia Mateos Martínez[1,3], Rosa González Sastre[1,2,3], Raquel Coronel[1], Andreea Rosca[1], Sabela Martín Benito[1,2,4], Adela Bernabeu Zornoza[1], Victoria López Alonso[2] and Isabel Liste[1]*

[1]*Neural Regeneration Unit, Carlos III Health Institute (ISCIII), Spain*

[2]*Computational Biology Unit, Carlos III Health Institute (ISCIII), Spain*

[3]*PhD researcher of the National University of Distance Education (UNED), Spain*

[4]*PhD researcher of the National Distance Education University (UNED), Spain*

***Corresponding author:** Isabel Liste, Neural Regeneration Unit, functional unit of Chronic Disease Research (UFIEC), Carlos III Health Institute (ISCIII), Majadahonda 28220, Madrid, Spain.

To Cite This Article: *Patricia Mateos Martínez, Rosa González Sastre, Raquel Coronel, Andreea Rosca, Sabela Martín Benito, Adela Bernabeu Zornoza, Victoria López Alonso, Isabel Liste . Advances in Alzheimer's Disease Research: Human Cerebral Organoids. Am J Biomed Sci & Res. 2023 18(1) AJBSR.MS.ID.002421,* DOI: 10.34297/AJBSR.2023.18.002421

Received: January 28, 2023; **Published:** February 02, 2023

Abstract

Alzheimer's disease (AD) is the main neurodegenerative disorder in old age, causing memory impairment and dependency. The histopathology of AD is characterized by the presence of amyloid plaques and neurofibrillary tangles formed by Aβ peptide and hyperphosphorylated Tau, respectively.

There is still no cure or effective treatment for AD. This could be due, in part, to the lack of suitable research models since animal models do not recapitulate the full physiological complexity of the human brain. With the development of induced pluripotent stem cells (iPSCs), these limitations could be overcome. Even so, the bi-dimensional (2D) culture models still do not allow to recapitulate all types of brain cells and do not show a three-dimensional (3D) arrangement.

Since obtaining 3D cultures called organoids, a new opportunity arises to overcome the limitations of previous models. Human Cerebral Organoids (hCOs) represent a pioneering model, in which part of the complexity of the human brain is present. For this reason, they are fast becoming a very remarkable model for the study of the evolution of the molecular and cellular pathology of AD.

This review provides a brief overview of AD research, focusing on the most recent advances achieved through the development of stem cell and cerebral organoid technology.

Keywords: Alzheimer disease, Pluripotent stem cells, Organoids, Cerebral organoids, 3D models

Background on Alzheimer 's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the main cause of dementia in older people. AD is characterized by a progressive decline in cognitive functions, memory loss, and motor impairments due to widespread loss of neurons and synaptic connections in the cortex, hippocampus, amygdala, and basal forebrain [1-3].

Post-mortem analysis of the brains of AD patients has identified two main features associated with this disorder, consisting of aggregates of misfolded proteins: neurofibrillary tangles (NFTs) and amyloid plaques (Figure 1) [4]. The NFTs are generated by the abnormal intracellular deposition of hyperphosphorylated Tau protein. The Amyloid plaques consist of extracellular fibrillar deposits of abnormally folded amyloid-β (Aβ) peptide with 40 or 42 amino acids (Aβ40, Aβ42), two byproducts of amyloid precursor protein (APP) metabolism [2,4-7].

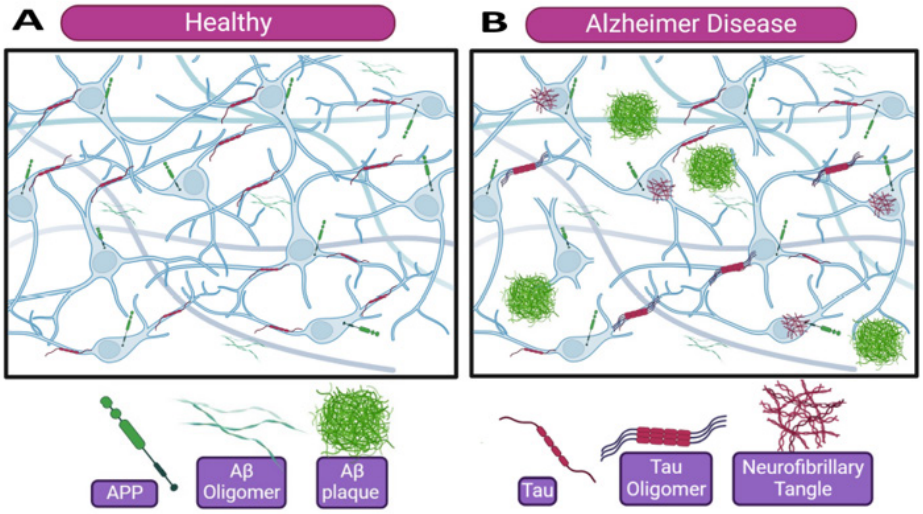

Figure 1: Main histopathological alterations observed in the brain of Alzheimer's disease (AD) patients. A. Healthy Brain. B. AD brain with neurofibrillary tangles inside of neurons and amyloid plaques outside.

The causes that trigger AD are not yet known, although age is considered the greatest risk factor, since its incidence doubles every 5 years after the age of 65. Approximately 95% of cases are sporadic or late-onset AD. The APOE4 gene is currently known to be the strongest genetic risk factor associated with late-onset AD [8]. In addition, there is a strong genetic component that represents 5% of all cases, known as familial or early-onset AD. Familial AD is caused by autosomal dominant mutations (or duplications) in the gene encoding APP, and in the genes encoding presenilin 1 (PSEN1) and presenilin 2 (PSEN2), catalytic components of the γ-secretase complex that processes APP [9-10]. These mutations are responsible for triggering early-onset AD due to overproduction of the Aβ peptide, leading to early deposition of Aβ plaques in the brain [11-13].

Currently, there is no effective cure for AD. Over the last decades only a few pharmacological treatments have been approved, such as acetylcholinesterase inhibitors and N-methyl d-aspartate receptor antagonists. They alliviate some symptoms, but do not stop the neurodegeneration [14,15]. Despite substantial knowledge about the pathologic features, the mechanism that initiates or leads to the development of AD remains poorly understood [16]. This could be mainly due to the lack of relevant in vivo and in vitro models that recapitulate the disease and that allow understanding the molecular mechanisms of AD, as well as testing the designed drugs.

Early studies used animal models to understand the pathology of AD. Different species such as Drosophila melanogaster, Caenorhabditis elegans and Dario rerio were used, although the mouse is the main specie used as AD model, since mice present an effect like that observed in humans [17]. Mouse models with familial mutations of AD exhibit Aβ-induced synaptic and memory deficits, but do not fully recapitulate other key pathological events of AD, including pathology other than NFTs [17,18].

Currently, due to the high rate (~99.6%) of clinical trial failure, there is debate about the usefulness of the animal models [19]. They are considered not realistic enough since we cannot see neurodegeneration in their brains, such as neuronal loss or the development of NFTs. In addition, there are anatomical differences between mice and human brain morphology and physiology [20,21]. Animal models have a bias in the information they provide, since the genes that are modified only represent a minority of cases (fAD) and do not represent the totality of the pathology [14]. Similarly, human neurons derived from AD patients (post-mortem) have shown elevated levels of toxic Aβ species and phosphorylated Tau, but no Aβ plaques or NFTs [22].

In this regard, stem cell technology, together with advances in 2D and 3D neural differentiation (in the case of human Pluripotent Stem Cells (hPSCs)), offers a unique opportunity to overcome this challenge and generate an unlimited supply of neurons and human brain cells for in vitro studies [23].

Stem Cell Technology and Cerebral Organoids

Recent advances in the field of stem cells and new knowledge related to cell reprogramming have opened a new world of opportunities and the creation of more reliable models. During the last decades, the study of stem cells and their characteristics has been deepened, as well as their possible therapeutic applications as an alternative method to procedures developed in vivo [24]. Therefore, the use of derived models of the iPSC neural cells helped us to have a better understanding of the disease [25-27]. By definition, stem cells are those cells that remain in an undifferentiated state, have the ability of self-renewing and they can proliferate and differentiate to give rise to different cell lines according to their potency. The most used nowadays are pluripotent stem cells (PSCs) and multipotent stem cells (MSCs) [28].

PSCs are characterized by their potential to generate, after their differentiation, specialized cells corresponding to the three germ lines (ectoderm, mesoderm, and endoderm). PSCs mainly include two types of cells:

i. Embryonic stem cells (ESCs). These cells are obtained from the inner cell mass of the embryonic blastocyst [29].

ii. Induced pluripotent stem cells (iPSCs). These cells are generated by cellular reprogramming of somatic cells through the introduction of transcription factors (Oct4, Sox2, Klf4 or Nanog and Lin28) [30,31] and show morphological, molecular, and functional like ESCs.

In general, these versatile cells are proving to be a very useful tool both for the study of human embryonic and brain development and for the molecular and pathological pathways associated with different neurological diseases, including AD [32-35].

The vast knowledge about PSCs arose from monolayer (2D) cell cultures, which lack the three-dimensional

(3D) component essential for organogenesis. This is especially important in the human brain, which is characterized by its complexity, expansion, and interplay of neural ramifications [36]. Recent technological advances have made it possible to obtain 3D cultures, derived from hPSCs that are called organoids. These organoids offer the real possibility of growing 3D structures in culture that closely resemble human tissue and are rapidly becoming a new research approach for disease modeling [37,38]. This potential, combined with the availability of human iPSCs (hiPSCs) from patients, makes it possible to model tissues with different genetic loads, mutations, etc. In addition, in the case of brain organoids, they also allow the study of human neurodevelopment [39,40].

In recent years, the work of Drs. Lancanster and Knoblich have established the methodology that has made possible to generate cerebral organoids (COs) from hPSCs (also called mini-brains or brain organoids) (Figure 2) [41,42]. The protocol has been modified to obtain more specific brain structures, such as cortical organoids [43]. These differentiation protocols offer great opportunities for studies of neurodegenerative diseases, such as AD [44,45]. Since 2D culture models do not have the complex extracellular environment necessary for extracellular protein aggregation, 3D culture conditions generated from hPSCs hold more promise for recapitulating key in vitro features of AD pathophysiology, such as Aβ peptide production and its aggregation in amyloid plaques, Tau protein hyperphosphorylation and NFT-like structures, synaptic dysfunction and neuronal degeneration, among others.

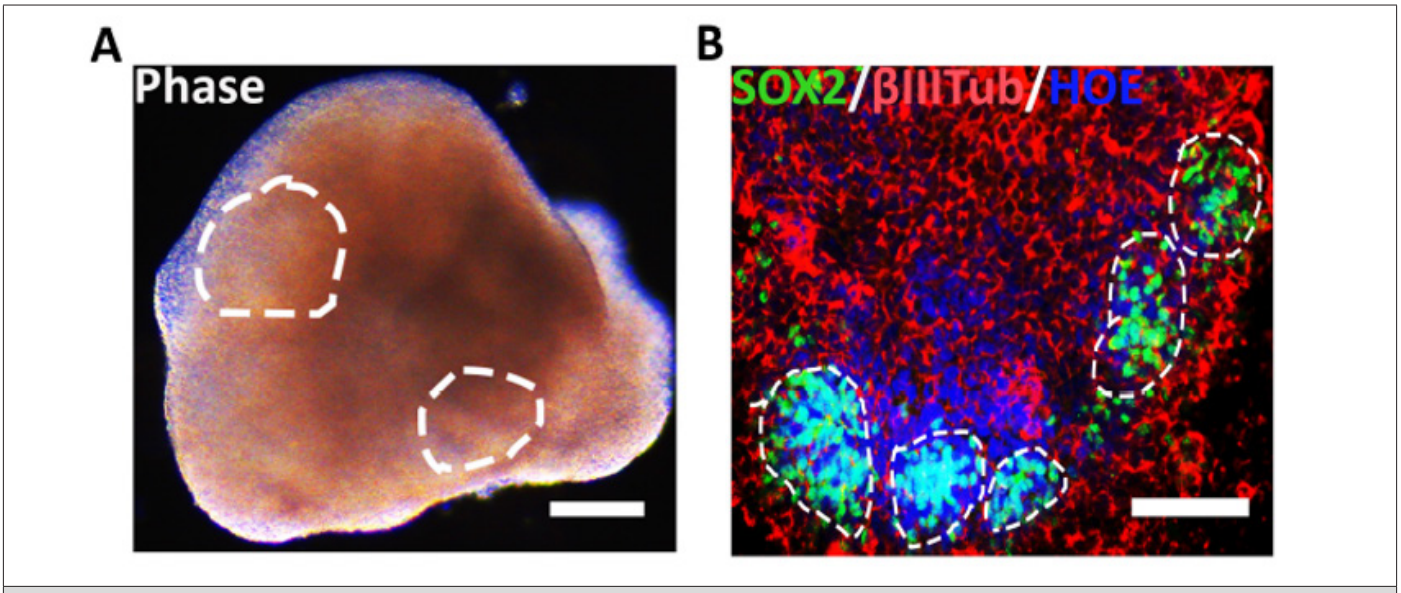

Figure 2: Cerebral Organoid (CO). A. Bright-field representative image of one CO. B. Immunohistochemistry showing proliferative zones positive for SOX2 (green), a neural precursor marker, and differentiated neurons stained for βIIITub (red). Scale bar= 100μm. Dashed lines mark the proliferative zones. βIIITub: Tubulin β 3 Class III. Hoe: Hoechst.

And indeed, some recent studies have shown that COs generated from hiPSCs derived from familial AD patients harboring APP duplications or PSEN1 mutations can reproduce some of the main AD-like pathologies, including hyperphosphorylated Tau, amyloid aggregation, endosomal abnormalities, and cellular apoptosis [46-48]. In addition, some of these COs showed alterations in the number of glutamatergic neurons, lactate dehydrogenase activity and gene expression of inflammatory factors like cytokines [49]. These findings are significant as the phenotypes have never been reported in mouse models with familial AD mutations and could be an excellent opportunity to study the molecular and cellular processes that take place during the early steps and evolution of AD.

At present, it has been possible to model sporadic AD as well, by using Aftin-5 (an agonist of Aβ42) to induce the production and secretion of Aβ42 peptide in COs [50]. Another group has produced sporadic AD COs using hiPSCs derived from AD patients with APOE gene mutations. These organoids presented high levels of both Aβ peptide and hyperphosphorylated Tau, neuronal apoptosis, and synaptic loss, suggesting that APOE4 can be independently associated with increased levels of hyperphosphorylated Tau in these COs [51].

Conclusions

As has been commented through this review, 3D models, especially COs, seem to be, currently, the best model to advance in the research of AD. Compared with the previous models (in vivo animals and 2D cultures in vitro), COs, have been shown not only to recapitulate some of the most important features of the AD pathology, but also to be more efficient. This has allowed tremendous progress to be made in the field in the last decade.

Human COs represent an innovative model in which part of the complexity of the human brain is present. For this reason, they are quickly becoming a very interesting model for the study of the molecular and cellular pathology characteristic of AD at the brain level.

These organoids can be generated from control iPS cells from patients with familial or sporadic AD, to which mutations in genes of interest can be introduced or deleted by genetic editing. This could accelerate the discovery of new therapeutic targets for the possible treatment of this disorder. In addition, COs provides a very appropriate technology to deepen the study of the interactions of the different brain cell phenotypes (neuron-astrocytes-oligodendrocytes-microglia) in physiological and pathological conditions.

However, deepening the use of these models and perfecting them is the challenge that the scientific community now faces. Even with the limitations that they still present, they are already giving great results, allowing not only to better understand the AD, but also to delve into possible therapies.

Acknowledgements

The authors would like to thank to financing entities:

i. State R+D+i Program Oriented to the Challenges of Society. Ministry of Science and Innovation (PID2021-126715OB-I00)

ii. Strategic Action in Intramural Health (PI22/00055)

iii. Ministry of Science and Innovation and Universities, within the program "R&D Projects «Retos Investigación» (RTI2018-101663-B-100)

Conflict of Interest

The authors declare no conflict of interest.

References

1. Lane CA, Hardy J, Schott JM (2018) Alzheimer's disease. Eur J Neurol 25(1): 59-70.
2. Burns A, Byrne EJ, Maurer K (2002) Alzheimer's disease. Lancet 360(9327): 163-165.
3. Amemori T, Jendelova P, Ruzicka J, Urdzikova LM, Sykova E (2015) Alzheimer's Disease: Mechanism and Approach to Cell Therapy. Int J Mol 16(11): 26417-26451.
4. Serrano Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 1(1): a006189.
5. Selkoe DJ, Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med 8(6): 595-608.
6. Bernabeu Zornoza A, Coronel R, Palmer C, Monteagudo M, Zambrano A, et al. (2019) Physiological and pathological effects of amyloid-β species in neural stem cell biology. Neural Regen Res 14(12): 2035-2042.
7. Bernabeu Zornoza A, Coronel R, Palmer C, López Alonso V, Liste I (2021) Oligomeric and Fibrillar Species of Aβ42 Diversely Affect Human Neural Stem Cells. Int J Mol Sci 22(17): 9537.
8. Zhao J, Fu Y, Yamazaki Y, Ren Y, Davis MD, et al. (2020) APOE4 exacerbates synapse loss and neurodegeneration in Alzheimer's disease patient iPSC-derived cerebral organoids. Nat Commun 11(1): 5540.
9. Kim DH, Yeo SH, Park JM, Choi JY, Lee TH, et al. (2014) Genetic markers for diagnosis and pathogenesis of Alzheimer's disease. Gene 545(2): 185-193.
10. Armstrong RA (2019) Risk factors for Alzheimer's disease. Folia Neuropathol 57(2): 87-105.
11. Schindler SE, Fagan AM (2015) Autosomal Dominant Alzheimer Disease: A Unique Resource to Study CSF Biomarker Changes in Preclinical AD. Front Neurol 6: 142.
12. Bekris LM, Yu CE, Bird TD, Tsuang DW (2010) Genetics of Alzheimer disease. J Geriatr Psychiatry Neurol 23(4): 213-227.
13. Kondo A, Albayram O, Zhou XZ, Lu KP (2017) Pin1 Knockout Mice: A Model for the Study of Tau Pathology in Alzheimer's Disease. Methods Mol Biol 1523: 415-425.
14. Yang J, Li S, He XB, Cheng C, Le W (2016) Induced pluripotent stem cells in Alzheimer's disease: applications for disease modeling and cell-replacement therapy. Mol Neurodegener 11(1): 39.
15. Kumar A, Singh A, Ekavali (2015) A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacol Rep 67(2): 195-203.
16. Tincer G, Mashkaryan V, Bhattarai P, Kizil C (2016) Neural stem/progenitor cells in Alzheimer's disease. Yale J Biol Med 89(1): 23-35.
17. Qin T, Prins S, Groeneveld GJ, van Westen G, de Vries HE, et al. (2020) Utility of animal models to understand human Alzheimer's disease, using the mastermind research approach to avoid unnecessary further sacrifices of animals. Int J Mol Sci 21(9): 3158.
18. Malm T, Magga J, Koistinaho J (2012) Animal Models of Alzheimer's Disease: Utilization of Transgenic Alzheimer's Disease Models in Studies of Amyloid Beta Clearance. Curr Transl Geriatr Exp Gerontol Rep 1(1): 11-20.
19. Drummond E, Wisniewski T (2017) Alzheimer's disease: experimental models and reality. Acta Neuropathol 133(2): 155-175.
20. Choi SH, Kim YH, Quinti L, Tanzi RE, Kim DY (2016) 3D culture models of Alzheimer's disease: a road map to a "cure-in-a-dish". Mol Neurodegener 11(1): 75.
21. Tong G, Izquierdo P, Raashid RA (2017) Human Induced Pluripotent Stem Cells and the Modelling of Alzheimer's Disease: The Human Brain Outside the Dish. Open Neurol J 11: 27-38.
22. Choi SH, Kim YH, Hebisch M, Sliwinski C, Lee S, et al. (2014) A three-dimensional human neural cell culture model of Alzheimer's disease. Nature 515(7526): 274-278.
23. Arber C, Lovejoy C, Wray S (2017) Stem cell models of Alzheimer's disease: progress and challenges. Alzheimers Res Ther 9(1): 42.
24. Engle SJ, Blaha L, Kleiman R J (2018) Best Practices for Translational Disease Modeling Using Human iPSC-Derived Neurons. Neuron 100 (4): 783-797.
25. Baldassari S, Musante I, Iacomino M, Zara F, Salpietro V, et al. (2020) Brain Organoids as Model Systems for Genetic Neurodevelopmental Disorders. Front Cell Dev Biol 8: 590119.
26. Nascimento JM, Saia Cereda VM, Sartore RC, da Costa RM, Schitine CS, et al. (2019) Human Cerebral Organoids and Fetal Brain Tissue Share Proteomic Similarities. Front Cell Dev Biol 7: 303.

27. Penney J, Ralvenius WT, Tsai LH (2020) Modeling Alzheimer's disease with iPSC-derived brain cells. Mol Psychiatry 25(1): 148-167.

28. Rosca A, Coronel R, Moreno M, González R, Oniga A, et al. (2020) Impact of environmental neurotoxic: current methods and usefulness of human stem cells. Heliyon 6(12): e05773.

29. Thomson JA, Itskovitz Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, et al. (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391): 1145-1147.

30. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5): 861-872.

31. Yu J, Vodyanik MA, Smuga Otto K, Antosiewicz Bourget J, Frane JL, et al. (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318(5858): 1917-1920.

32. Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, et al. (2012) Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. Nature 482(7384): 216-220.

33. Yang J, Li S, He XB, Cheng C, Le W (2016) Induced pluripotent stem cells in Alzheimer's disease: applications for disease modeling and cell-replacement therapy. Mol Neurodegener 11(1): 39.

34. Revilla A, González C, Iriondo A, Fernández B, Prieto C, et al. (2016) Current advances in the generation of human iPS cells: implications in cell-based regenerative medicine. J Tissue Eng Regen Med 10(11): 893-907.

35. Garcia Leon JA, Caceres Palomo L, Sanchez Mejias E, Mejias Ortega M, NuñezDiaz C, et al. 2020) Human Pluripotent Stem Cell-Derived Neural Cells as a Relevant Platform for Drug Screening in Alzheimer's Disease. Int J Mol Sci 21(18): 6867.

36. Qian X, Song H, Ming GL (2019) Brain organoids: advances, applications and challenges. Development 146(8): dev166074.

37. Lancaster MA, Knoblich JA (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. Science 345(6194): 1247125.

38. Papaspyropoulos A, Tsolaki M, Foroglou N, Pantazaki AA (2020) Modeling and Targeting Alzheimer's Disease With Organoids. Front Pharmacol 11: 396.

39. Di Lullo E, Kriegstein AR (2017) The use of brain organoids to investigate neural development and disease. Nat Rev Neurosci 18(10): 573-584.

40. Trujillo CA, Muotri AR (2018) Brain Organoids and the Study of Neurodevelopment. Trends Mol Med 24(12): 982-990.

41. Lancaster MA, Knoblich JA (2014) Generation of cerebral organoids from human pluripotent stem cells. Nat Protoc 9(10): 2329-2340.

42. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, et al. (2013) Cerebral organoids model human brain development and microcephaly. Nature 501(7467): 373-379.

43. Paşca AM, Sloan SA, Clarke LE, Tian Y, Makinson CD, et al. (2015) Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. Nat Methods 12(7): 671-678.

44. Baldassari S, Musante I, Iacomino M, Zara F, Salpietro V, et al. (2020) Brain Organoids as Model Systems for Genetic Neurodevelopmental Disorders. Front Cell Dev Biol 8: 590119.

45. Park JC, Jang SY, Lee D, Lee J, Kang U, et al. (2021) A logical network-based drug-screening platform for Alzheimer's disease representing pathological features of human brain organoids. Nat Commun 12(1): 280.

46. Hernández Sapiéns MA, Reza Zaldívar EE, Cevallos RR, Márquez Aguirre AL, Gazarian K, et al. (2020) A Three-Dimensional Alzheimer's Disease Cell Culture Model Using iPSC-Derived Neurons Carrying A246E Mutation in PSEN1. Front Cell Neurosci 14: 151.

47. Gonzalez C, Armijo E, Bravo Alegria J, Becerra Calixto A, Mays CE, et al. (2018) Modeling amyloid beta and Tau pathology in human cerebral organoids. Mol Psychiatry 23(12): 2363-2374.

48. Raja WK, Mungenast AE, Lin YT, Ko T, Abdurrob F, et al. (2016) Self-Organizing 3D Human Neural Tissue Derived from Induced Pluripotent Stem Cells Recapitulate Alzheimer's Disease Phenotypes. PLoS One 11(9): e0161969.

49. Yan Y, Song L, Bejoy J, Zhao J, Kanekiyo T, et al. (2018) Modeling Neurodegenerative Microenvironment Using Cortical Organoids Derived from Human Stem Cells. Tissue Eng Part A 24(13-14): 1125-1137.

50. Pavoni S, Jarray R, Nassor F, Guyot AC, Cottin S, et al. (2018) Small-molecule induction of Aβ-42 peptide production in human cerebral organoids to model Alzheimer's disease associated phenotypes. PLoS One 13(12): e0209150.

51. Zhao J, Fu Y, Yamazaki Y, Ren Y, Davis MD, et al. (2020) APOE4 exacerbates synapse loss and neurodegeneration in Alzheimer's disease patient iPSC-derived cerebral organoids. Nat Commun 11(1): 5540.