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Human iPSC-derived APOE4/4 Alzheimer´s disease astrocytes exhibit a proinflammatory and senescent state that compromise neuronal support

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BACKGROUND

Alzheimer's disease (AD) is characterized by a complex pathology, not fully resolved yet. This fact, together with the lack of reliable models, has impeded the development of effective therapies. Glial cell dysfunction has been proposed to be involved in AD pathogenesis, but this cannot be properly modeled using the available animal models, so we hypothesized that glial cells derived from human induced pluripotent stem cells (iPSCs) from patients can serve as a better platform for studying the disease.

Differentiation of human iPSCs towards the astrocytic lineage

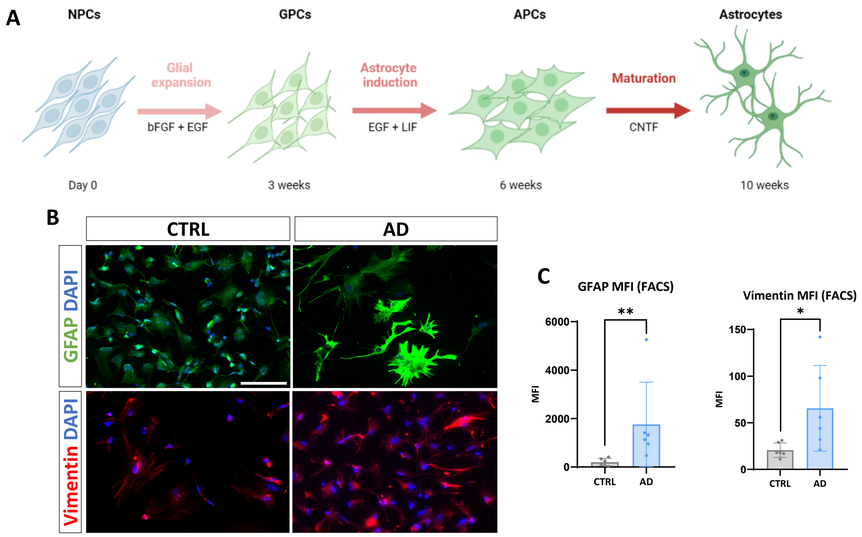

Figure 1. Protocol and characterization of the iPSC-derived astrocytes from CTRL and AD patients. Human iPSC-derived astrocytes from AD APOE4/4 patients and cognitively unimpaired age-matched individuals (CTRL) APOE3/3 were differentiated. **A)** Scheme of the differentiation protocol. **B)** Phenotypic characterization of the astrocytes, which express typical astrocyte markers GFAP and Vimentin. Nuclei stained with DAPI. Scale bar: 50 µm. **C)** FACS analysis show a significant increase in the expression levels of both GFAP and Vimentin markers in AD astrocytes compared to CTRL. Data represented as mean ± SEM, with each dot representing the mean value obtained for a single cell line from at least two independent experiments. **p<0.01 and *p<0.05.

AD astrocytes express a senescence-associated secretory phenotype (SASP)

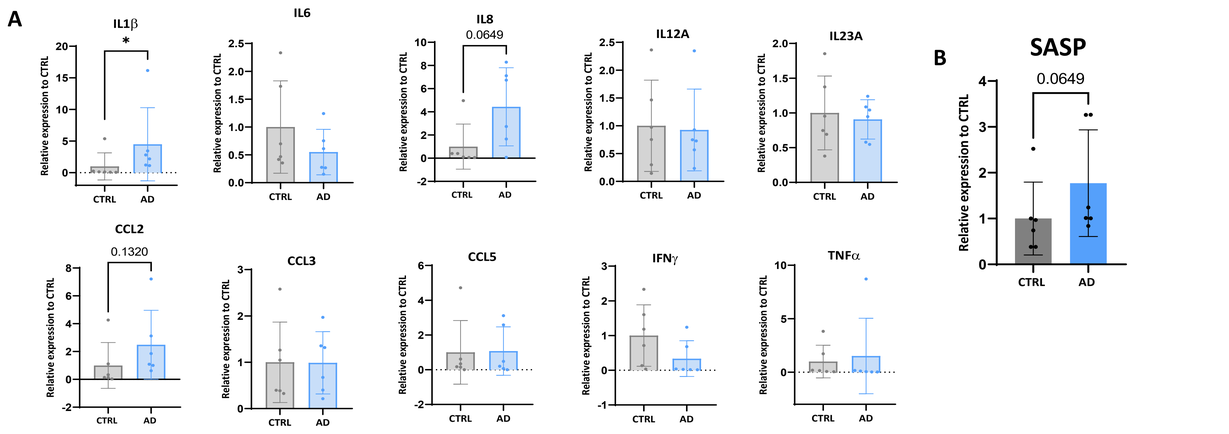

Figure 3. Expression of inflammatory factors of SASP by human iPSC-derived AD APOE4/4 astrocytes. A) Evaluation by qPCR of the gene expression of several pro- and anti-inflammatory cytokines and chemokines under basal conditions in CTRL and AD astrocytes. **B)** Gene score analysis of the SASP genes. Individual values for each cell line are represented, together with the mean ± SD. *p<0.05.

AD astrocytes present features of cellular senescence

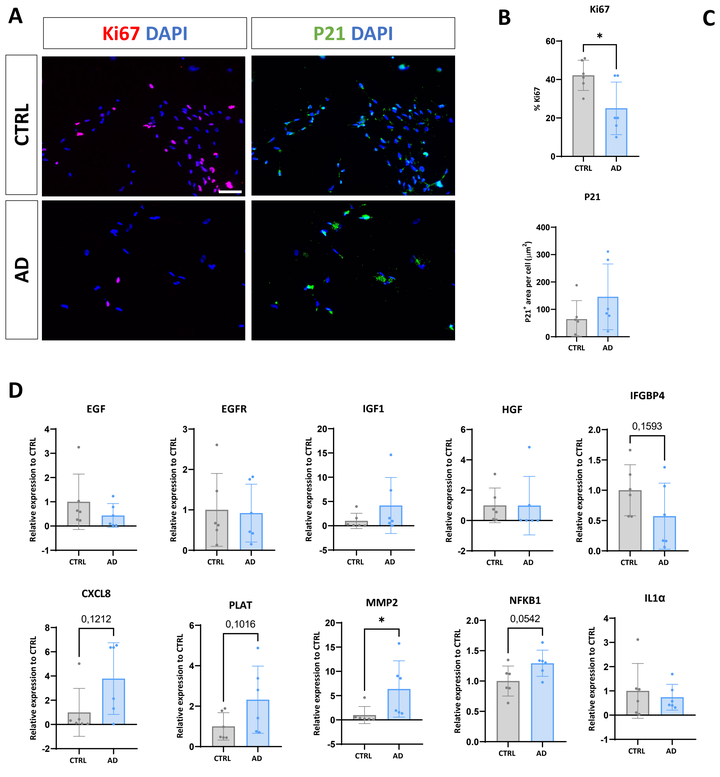

Figure 2. Analysis of senescent characteristics of human iPSC-derived AD and CTRL astrocytes. A) Immunofluorescence images of the proliferation marker ki67 and the senescence marker P21. Nuclei stained with DAPI. Scale bar: 50 µm. **B)** Immunofluorescence quantification of the expression of Ki67 and P21. **C)** MET images of elongated mitochondria in AD astrocytes. **D)** Analysis of the expression of a set of genes associated with senescence by qPCR. **E)** Gene score analysis of the previous senescence genes. Individual values for each cell line are represented, along with the mean ± SD.*p<0.05.

AD senescent astrocytes show impaired neuronal support

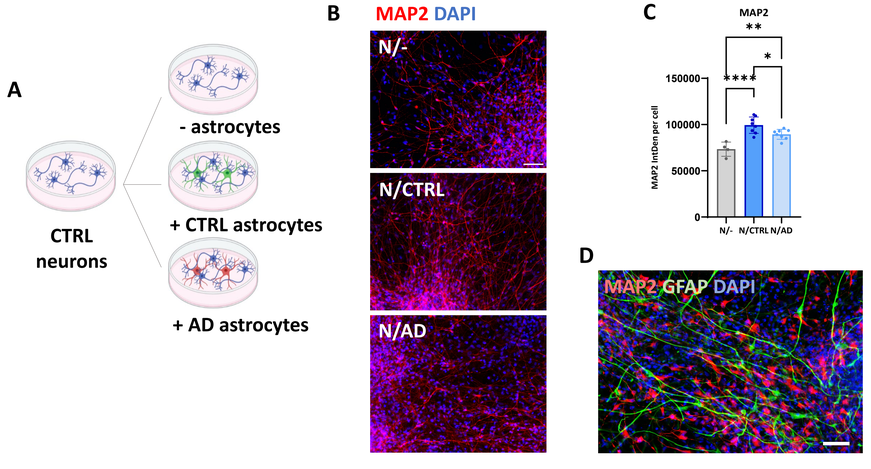

Figure 4. Establishment of astrocyte-neuron co-cultures reveal a deficient neuronal support by AD astrocytes. A) Astrocytes from AD and CTRL subjects were co-cultured with early CTRL neurons for 4 weeks. **B)** Immunofluorescence images of the neuronal marker MAP2 in the different conditions tested. Nuclei stained blue with DAPI. Scale bar: 50 µm. **C)** MAP2 quantification reveals a significant reduction in co-cultures containing AD astrocytes. Data represented as mean ± SD. *p<0.05, **p<0.01, ****p<0.0001. **D)** Phenotypic characterization of a co-culture with MAP2 and GFAP. Nuclei stained blue with DAPI. Scale bar: 50 µm

CONCLUSION

These data suggest that astrocytes derived from APOE4/4 AD patients present an intrinsic senescent phenotype which compromise their functionality. Elucidating the mechanisms inducing these processes and their functional consequences should aid to a better understanding of the role that astrocytes play in AD, by direct functioning and also through their interactions with neurons and the other glial cells. This should lead to the identification of potential therapeutic targets for future AD treatments.

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