ApoE4 associated changes in gangliosides in Alzheimer's disease patient iPSC-derived cerebral organoids

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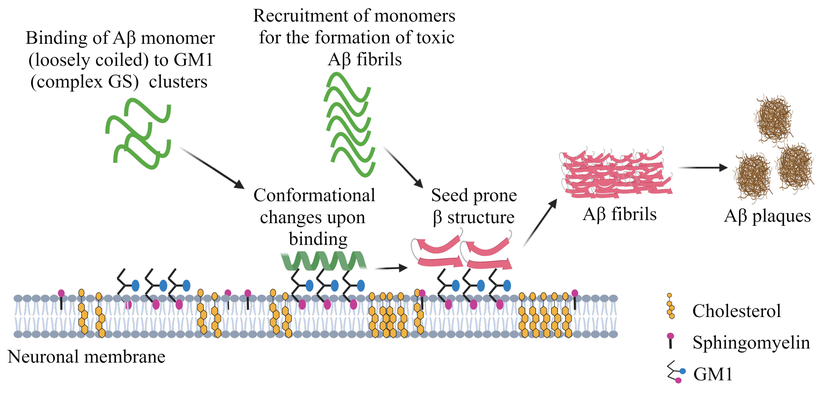
INTRODUCTION



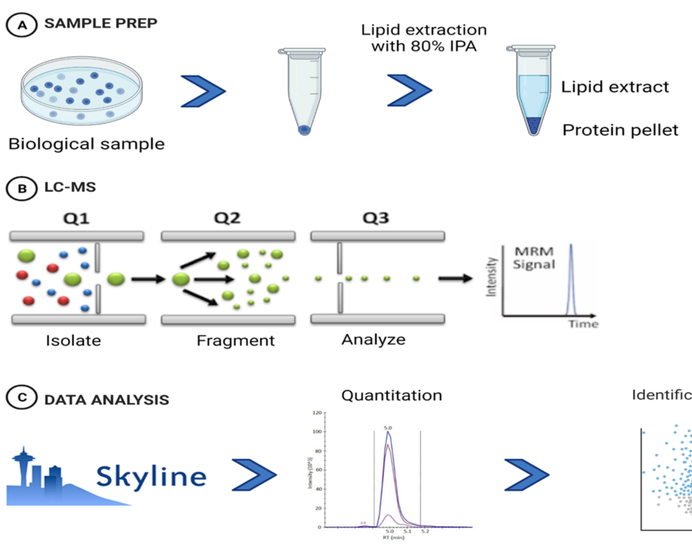
The amyloid β peptide (Aβ) is the key peptide that accumulates and aggregates, leading to Alzheimer's Disease (AD). Aβ is generated from the proteolytic cleavage of APP by β- and γ-secretases[1].

Gangliosides (GSs), sialic acid containing sphingolipids, are commonly present in neuronal membranes. The binding of Aβ to GS enriched clusters could cause the formation of a seed prone β-structure. The seeding can form toxic amyloid fibrils and Aβ plaques[2] (Fig.1).

Our study assessed the levels of GSs in 3D cerebral models to identify factors that elevate the levels of these lipids while playing a role in Aβ formation, namely the β- and γ-secretases and apolipoprotein E (ApoE) phenotypes.

METHODS

The study involved sporadic AD (sAD) cerebral organoids (COs) with ApoE3/3 (N=10) and ApoE4/4 (N=10) phenotypes. The COs were treated with β- and γ-secretase inhibitors to identify the relationship between secretase levels and GSs.



For our study, we utilized ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ) instrumentation in the selective reaction mode (SRM) (Fig. 2).

The molecular species quantitated were - GM1, GM2, GM3, GD1a, GD1b, GD2, and GD3. We also acquired additional structural information by analysing these GSs with different ceramide chain lengths.

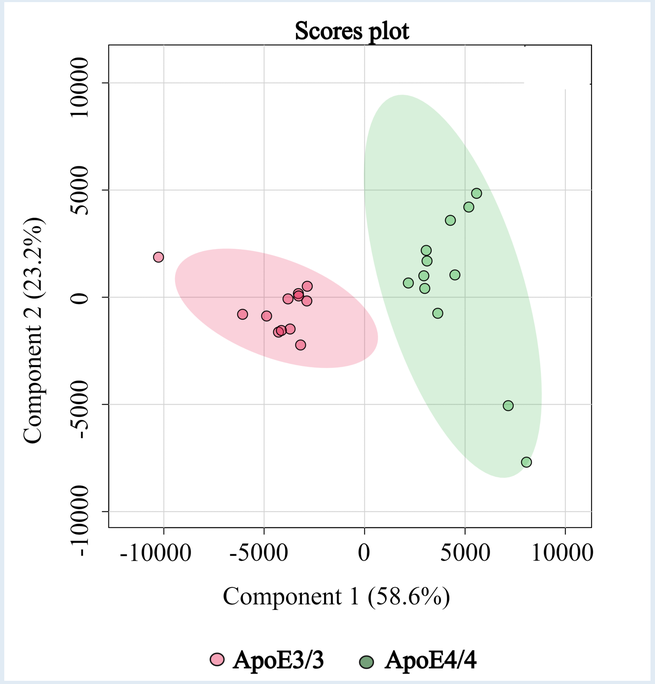
GS levels were normalized to the total protein levels to eliminate variation.

RESULTS

GD2 and GM2 were the most abundant GSs in the COs. The most abundant ceramide chain for GSs were 36:1 and 34:1.

The GSs were analyzed in 110 days old COs with ApoE3/3 and ApOE4/4 phenotype. ApoE4/4 COs had a significantly higher total GSs compared to ApoE3/3 COs.

Prinicipal component analysis (PCA) depicts formation of two distinct clusters for ApoE3 and ApoE4 COs(Fig. 3).



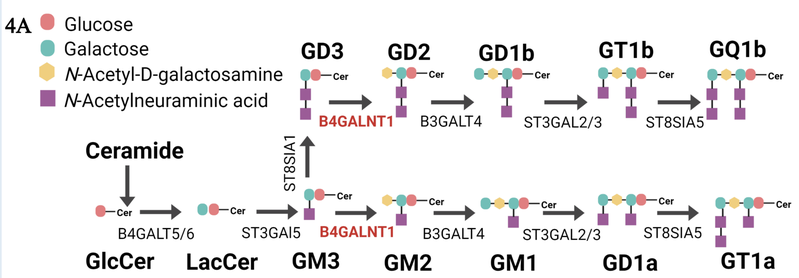


Fig 4A. The ganglioside biosynthetic pathway. GM3 and GD3 are considered simple GSs and are precursors for the complex gangliosides- GM1, GD1a, GD1b, and GT1b.

Biosynthetic enzymes such as **B4GALNT1** (GM2/GD2 synthase) protect secretases from lysosomal degradation[3].

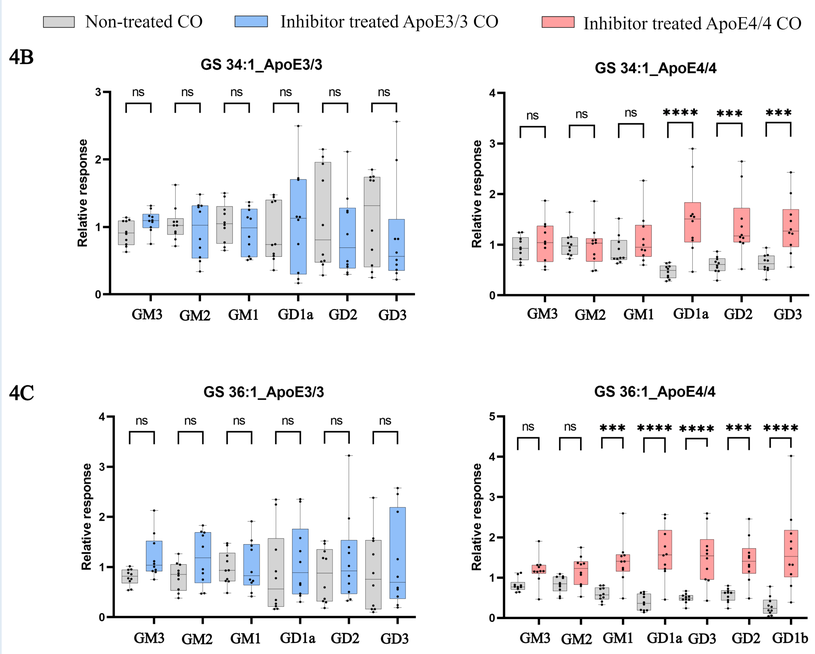


Fig 4B. Box plots depicting GSs (34:1) level in sAD ApOE3/3 and ApoE4/4 COs treated with β- and γ-secretase inhibitors.
34:1 depicts the ceramide chain C18:1/16:0 (sphingoid backbone/fatty acid chain length and saturation).

No changes were observed for ApoE3/3 treated COs. Elevation of GSs - GD1a, GD2, and GD3 were observed in ApoE4/4 COs.

Fig 4C. Box plots depicting GSs (36:1) level in sAD ApOE3/3 and ApoE4/4 COs treated with β- and γ-secretase inhibitors.
36:1 depicts the ceramide chain C18:1/18:0.

No changes were observed for ApoE3/3 treated COs. Elevation of GSs - GM1, GD1a, GD2, and GD3 were observed in ApoE4/4 COs.

CONCLUSION

GSs are abundantly present in the COs. ApoE4/4 phenotype COs have significantly elevated of GSs. This could point to overall dysregulation in lipid transport due to ApoE4.

Treatment with secretase inhibitors elevated the levels of major GSs, which were more pronounced in ApoE4/4 COs.

An increase in levels of GSs after GM3 and GM2 points to the role of the biosynthetic enzymes associated with secretases.

Change in secretase activity can promote the formation of GS clusters in the neuronal membrane, elevating the formation of the GS-Aβ complex, thus, contributing to AD pathology.

REFERENCES
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