C1 Complement mediates human cord blood serum derived APP α-secretase cleavage activity in vitro

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Abstract

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. In healthy individual amyloid precursor protein (APP) is cleaved by α-secretase generating sAPPα. However, in the neurodegenerative environment of AD patients, Aß peptides of either 40 or 42 residues are generated by increased beta and gamma secretase activity. Human umbilical cord blood cells (UCBC) have proven useful as potential immunomodulatory therapies in various models of neurodegenerative diseases. Our study investigated the impact of UCBS on modulation of sAPPα production. Heat-activated UCBS has significantly promoted sAPPα production indicating presence of heat sensitive α-secretase in CBS. Using LC-MS/MS, We identified the subunits of C1 complex (C1q, C1r and C1s) and α-2-macroglobulin showed significantly greater levels in αCBSF compared with AgBSF. Specifically, C1 markedly increased sAPPα and αCTF production, whereas C1q alone only minimally increased and C3 did not increase sAPPα production in the absence of sera. Furthermore, C1q markedly increased sAPPα and αCTF, while decreasing Aβ, in CHO/APPwt cells cultured in the presence of whole sera. These results confirm that APP α-secretase activity in human blood serum is mediated by C1 opening a potential modality of therapeutic for the future of AD.

Results

1. APP α-secretase activity in CBS

CHO/APPwt cells were treated with 2% CBS for different time. The result indicated the time- dependent production of sAPPα (Fig. 1a) Similarly, CHO/APPwt cells indicated dose-dependent production of sAPPα after treating with different concentrations of CBS (Fig. 1b). CHO/APPwt cells treated with 2% whole or heat-inactivated (56°C) CBS, AgBS or or their puried fraction (αCBSF, αAgBSF) all showed decreased sAPPα levels (Fig. 1c, Fig. 1d)

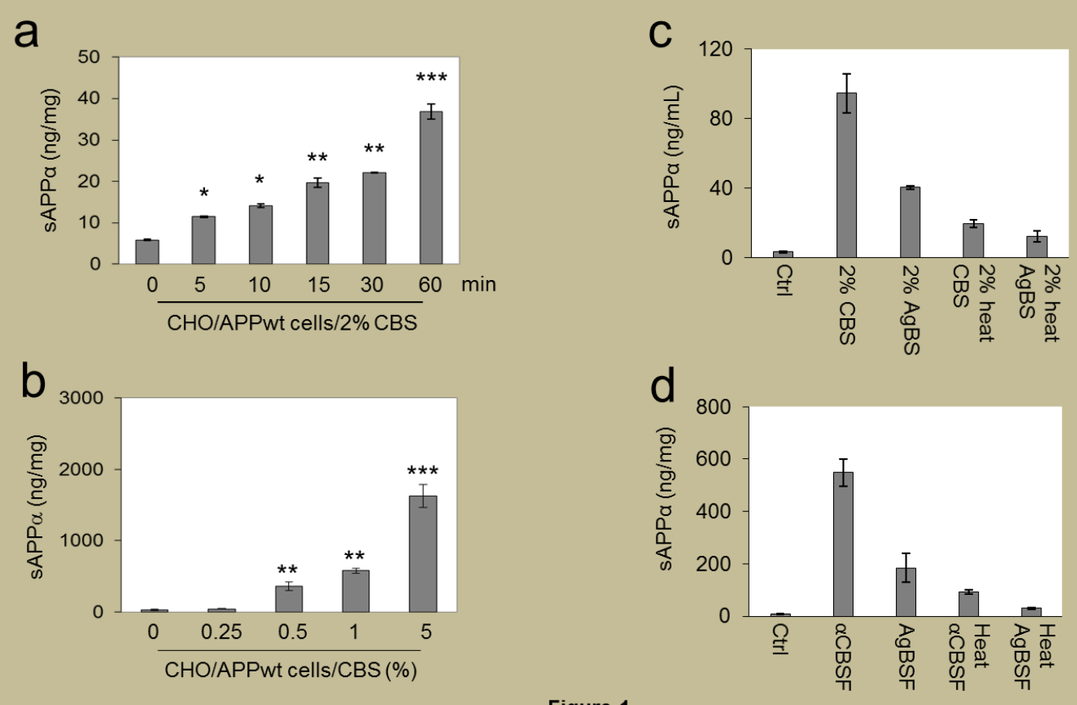

Figure 1

2. APP α-secretase activity in CBS is independent of enzymatic activity complement C3b

We identified 142 the major proteins with differen expression in fraction αCBSF and αAgBSF.. Several of the major proteins identified that most likely to exhibit α-secretase activity are shown in Fig. 2a. we first investigated whether depletion of C3 could limit the activity of CBS α-secretase, and found that compstatin, a C3b inhibitor, did not changes sAPPα production after mixing with1.6% whole (Fig. 2b) as well as C3 depleted (Fig. 2c) CBS at differerent dose in CHO/APPwt. Inactivation of C3b using inhibitor of C3b(iC3b) does not increased sAPPα production markedly (Fig. 3d).

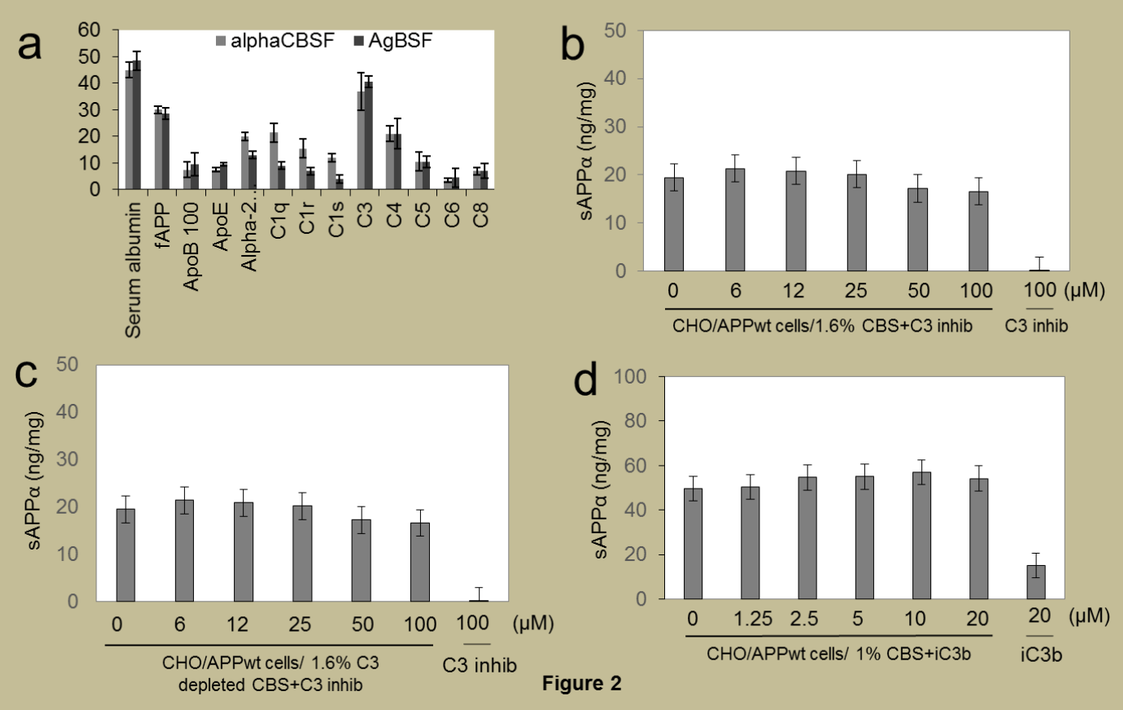

Figure 2

3. APP α-secretase activity in CBS is mediated in part by complement C1 complex

To determine if α-secretase activity in CBS is mediated by C1 complex. CHO/APPwt cells were treated with 1 or 2% CBS that supplemented with C1 inhibitor, and the results showed that the sAPPα production was markedly reduced in a dose dependent fashion(Fig. 3a, Fig. 3b). In order to further confirm that C1 complex mediates α-secretase activity in CBS**.** we determined the effect of purified human C1 on sAPPα production in N2a/APPwt and TgAPPwt/primary neuronal cells in absence of sera. C1 dose-dependently increased sAPPα and αCTF production in N2a/APPwt cells (Fig. 3c-e) and TgAPPwt/primary neuronal cells (Fig. 4f-h).

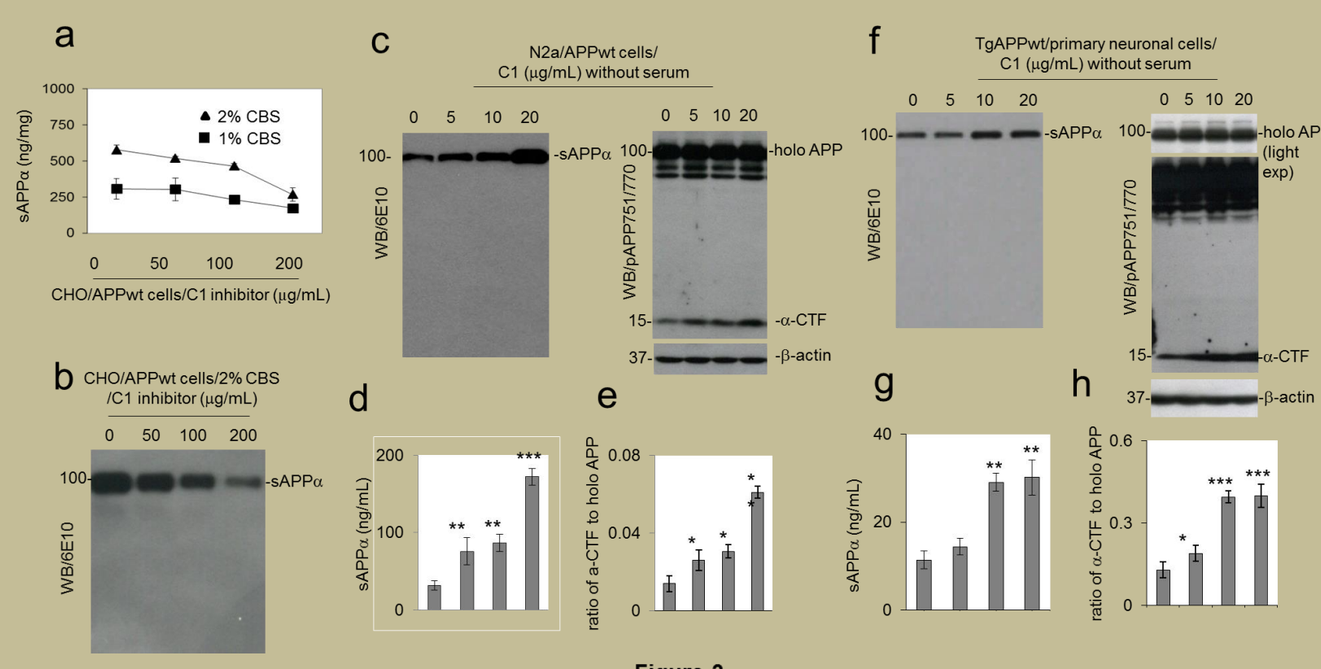

Figure 3

4. C1 complex is necessary for APP α-secretase activity in human blood serum

To further determine the individual component of complement C1 complex involved in APP cleavage, CHO/APPwt cells were treated with purified human C1 or C3, or C1q subunit. As expected, C1 markedly increased sAPPα production, whereas C1q alone minimally increased and C3 did not increase sAPPα production under serum-free condition (Fig. 5a). In contrast, both C1 and C1q markedly increased sAPPα production in CHO/APPwt cells cultured in C1q depleted sera (Fig. 5b), and C1 markedly increased sAPPα and αCTF level, while decreasing Aβ and β-CTF level in presence of whole sera (Fig. 5c). we treated CHO/APPwt cells with plasma obtained from C1qa knockout or age-matched wild-type littermate controls. Interestingly, the production of sAPPα elicited by plasma from male C1qa knockout mice was not significantly different, whereas sAPPα production in female group was significantly greater (Fig. 5d).

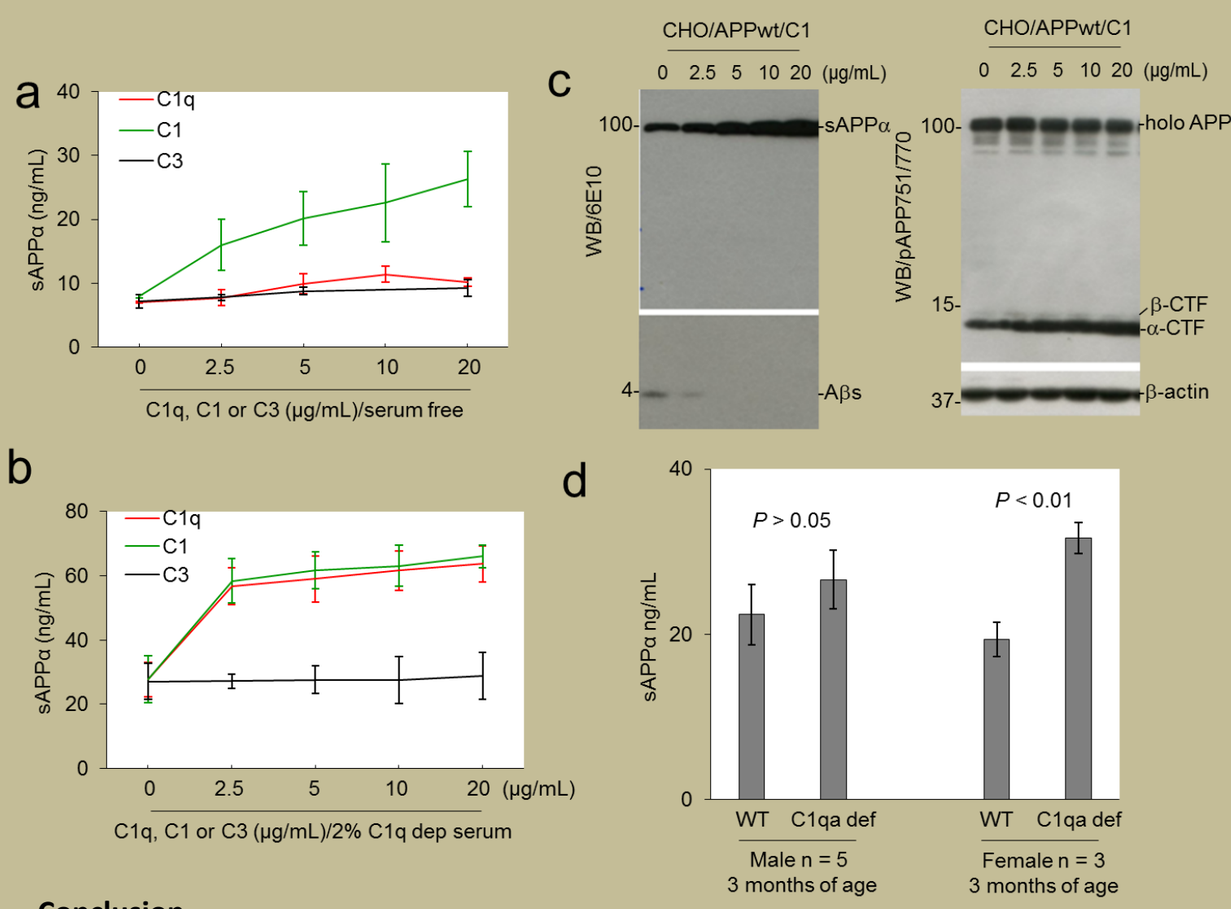

Figure 5

Conclusion

Collectively, our results indicate that CBS contains proteins that promote α-secretase like enzymatic activity. LC-MS/MS analysis in CBSF and AgBSF revealed the presence of 142 proteins of which C1 subunits and alpha-2-macroglobulin showed significantly greater levels in αCBSF compared with α AgBSF. further study showed,C1 subunits can enhance sAPPα production and Aβ reduction in cell culture condition.