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Brief communication

Tissue transglutaminase is not a biochemical marker for Alzheimer's disease

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ABSTRACT

Typical hallmarks of Alzheimer's disease (AD) are pathologic deposits in cortical and subcortical regions consisting of self-aggregated proteins such as amyloid-beta (Aβ) or tau. Tissue transglutaminase (tTG) catalyses calcium-dependent cross-linking between proteins (transamidation) resulting in protease-resistant isopeptide bonds. Because of this ability, tTG was suspected to participate in AD pathogenesis. Aβ and tau can be cross-linked by tTG in vitro. In AD neocortex, messenger RNA expression of tTG is increased. However, data on transamidation in AD specimens—activity of not only tTG but also other transglutaminases—are contradictory. The aim of our study was to investigate if tTG is involved in AD development and may be useful as biomarker for AD. We studied human brain samples for tTG concentration, tTG localization, and transamidation activity and cerebrospinal fluid (CSF) for tTG content by novel sensitive and highly specific methods. Neither tTG concentration nor transamidation was increased in AD brain homogenates. Immunohistologically, we found no colocalization of tTG in neocortex sections with tau or Aβ deposits but with blood vessels. Only in rare cases, tTG was detectable in CSF samples. This could be attributed to liberation from erythrocytes. Our data contradict the view that tTG is a potential biochemical marker for AD.

1. Introduction

Alzheimer's disease (AD) is characterized by extensive loss of neuronal cells and synapses. Typically, hallmarks are pathologic deposits in cortical and subcortical regions consisting of self-aggregated amyloid-beta (Aβ) peptides or hyperphosphorylated tau (ptau) protein, which form senile plaques or neurofibrillary tangles, neuritic plaques, and neuropil threads, respectively (Braak et al., 1994; Iwatsubo et al., 1994).

Tissue transglutaminase (tTG) catalyses irreversible cross-linking of polypeptides under the formation of isopeptide bonds (Folk, 1983). Besides a variety of physiological functions, the participation of tTG in the development of neurodegenerative disorders like AD is intensively discussed (Grosso and Mouradian, 2012). Because of its cross-linking ability, tTG is suggested to be causative for self-aggregation process of Aβ and ptau in AD. In fact, messenger RNA levels, tTG protein, and transamidation were found increased in AD brain (Citron et al., 2001; Kim et al., 1999; Wang et al., 2008). An enhanced concentration of tTG was measured in cerebrospinal fluid (CSF) of AD patients (Bonelli et al., 2002). In brain sections, a colocalization of the enzyme with pathologic deposits was observed (Wilhelmus et al., 2008). This led to the assumption that tTG may represent a new biomarker for AD (Bonelli et al., 2002; Citron et al., 2001). We investigated tTG and transamidation activity in postmortem brains and CSF and examined the localization of tTG in neocortex of AD and control brains by immunohistochemistry.

2. Methods

Details of brains, patients, antibodies, measurement of transamidation and tTG, and immunohistochemistry are provided in the Supplementary data. Assay of tTG was performed as (1) sandwich enzyme-linked immunosorbent assay of protein tTG (ptTG, Wolf et al., 2011a); and (2) immunodetection of activated tTG (atTG) after its binding to an acyl donor (Wolf et al., 2011b).

3. Results

Assay of ptTG, atTG, and transamidation in homogenates of frontal cortex revealed no significant differences between controls

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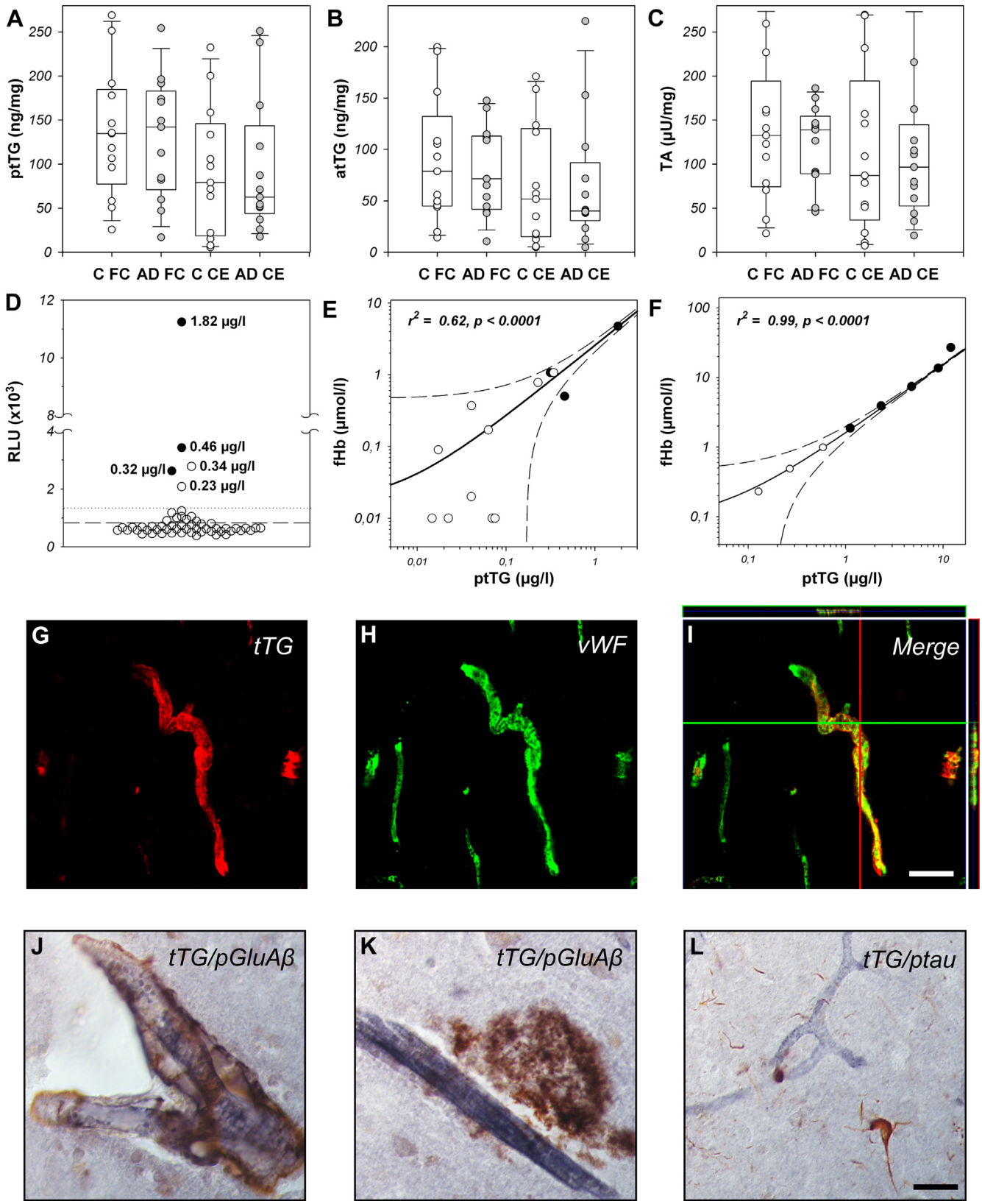

Fig. 1. Determination of tissue transglutaminase (tTG) in neocortex and cerebrospinal fluid (CSF) of controls and Alzheimer's disease (AD) patients. (A–C) Assay of protein tTG (ptTG), activated tTG (atTG), and transamidation activity (TA) in frontal cortex (FC) and cerebellum (CE) of controls (C, white circles, $n = 13$) and AD (gray circles, $n = 13$). There is no significant increase in ptTG, atTG, and TA of the AD group above the control group ($p > 0.87$). (D–E) Concentration of ptTG in CSF of patients with unknown diagnosis. (D) Results are as relative light units (RLU). For samples with signals above the detection limit (background plus 5 times standard deviation, dotted line), the ptTG concentration is indicated. Dashed line indicates the background of the assay. (E) Relation between ptTG and fHb. Regression line and 95% prediction band are displayed. All samples above the background are shown ($n = 13$). (F) Relationship between fHb and ptTG in CSF samples spiked with lysed blood (0.019–2.5 µL per milliliter CSF). Filled symbols (D–F) represent samples with visible