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Gender-related increase of tropomyosin-1 abundance in platelets of Alzheimer's disease and mild cognitive impairment patients

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ABSTRACT

The incidence of Alzheimer's disease (AD) is higher in elderly women than in men. The molecular background of this gender-related risk, however, is largely unknown. In a previous proteomics study, we identified significantly elevated levels of monoamine oxidase-B and tropomyosin-1 in AD patients, together with significant changes of the genetic AD risk factors apolipoprotein E4 (*APOE4)* and glutathione S-transferase omega 1 (*GSTO1)*, in platelets - a promising source for AD blood biomarkers. The present study aimed to investigate the gender-specificity as well as the disease-stage dependency of these biomarkers in AD patients and those with mild cognitive impairment (MCI). Tropomyosin-1 and monoamine oxidase-B protein levels were quantified by 2-D DIGE and 1-D Western blotting. Here, for the first time, we revealed a significant increase of 38&39 kDa tropomyosin-1 protein levels in female but not male AD (+ 56%; $p = 0.008$) and MCI patients (+ 46%; $p = 0.041$) measured by 1-D WB. In contrast, levels of monoamine oxidase-B were, independently of gender, elevated in AD patients (+ 52%; $p = 0.009$) but unaltered in MCI compared to control subjects. Moreover, we confirmed that *APOE4*-positive females are at a higher risk (OR = 18.7; $p = 9.7E-09$) of developing AD compared to *APOE4*-positive males (OR = 6.5; $p = 5.9E-04$). No gender-related effects were observed for *GSTO1*.

Significance: Platelet tropomyosin-1 constitutes a gender-related and stage-dependent protein in cognitive impairment. In contrast, platelet monoamine oxidase-B, frequently described to be increased in platelets and brains of AD patients, shows a gender-independent but stage-related increase since it is unaltered in MCI subjects. A blood biomarker test for this preceding stage of AD that considers gender-specificity is not yet available. The newly described AD-related platelet protein profiles might refine and facilitate routine diagnosis and enable early as well as tailored interventions.

1. Introduction

As biomarkers keep getting discovered for diagnosing diseases affecting patients in their later years, the question of whether these tools are equally reliable for use in both men and women becomes more pertinent. One such example is the use of blood biomarkers to monitor Alzheimer's disease (AD) or mild cognitive impairment (MCI). Thus far, only a few AD-predictive markers have been identified, and it remains to be elucidated how accurate they are between the genders. Hence, in light of the rapid emergence of predictive tools in modern medicine, there is an urgent need to appraise them for gender reliability.

Globally, nearly 44 million people suffer from late-onset AD or a related type of dementia. AD is characterised by a progressive impairment in memory caused by neurodegeneration starting in the entorhinal cortex and hippocampus [1,2]. The hallmarks of AD-related brain pathology are extracellular senile plaques containing amyloid β (Aβ) and intracellular neurofibrillary tangles consisting of hyperphosphorylated tau protein. The prodromal state of AD is defined as mild cognitive impairment (MCI). The description of the MCI cognitive state is below normal age-calibrated memory and cognitive functions, but sufferers are not yet demented [3].

So far, there is no therapy to prevent progression of the cognitive decline once symptoms have become apparent. Since therapies have to start before excessive cerebral atrophy takes hold, there is great demand for identifying preclinical stages of AD [1]. Currently, diagnosis of AD and MCI is based on neuropsychological testing and brain

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imaging, e.g. computer tomography, magnetic resonance imaging, and positron emission tomography. By now, the best-validated biomarkers for diagnosing AD are low concentrations of amyloid β (Aβ1–42) peptide and high total- and phosphorylated tau [4] in the cerebrospinal fluid (CSF). However, collection of CSF requires invasive lumbar puncture and, thus, is not feasible in the initial step of routine screening. To date, no blood biomarker panels are available that can reliably differentiate between either cognitively healthy individuals or those with MCI and AD [5,6]. The use of blood biomarkers would facilitate early medical consultation and timely treatment. Since platelets are easily accessible from blood and share biochemical characteristics with neurons [7], they are considered as a promising biomarker source for the development of an AD blood test [8]. For instance, human platelets contain tau protein and high levels of APP in their proteome, both proteins that have been described to have different isoform patterns in AD [8]. In addition, platelets are able to store neurotransmitters (serotonin, glutamate and dopamine) and they possess α-, β- and γ-secretase proteins, thus, are capable to generate all APP metabolites [8].

In our previous platelet proteomics study [9], we characterised a panel of proteins which might be useful as diagnostic targets for AD. We identified significantly elevated levels of monoamine oxidase-B (MAOB) and two protein species of tropomyosin-1 (TPM1) in AD patients. Additionally, we determined augmented protein levels of apolipoprotein E4 (APOE4), representing the major genetic risk factor for late-onset AD. Moreover, stratification by *APOE4* revealed a significant increase in the intensity of a protein spot corresponding to the predominant GSTO1*Ala variant of glutathione S-transferase omega 1 (rs4925) in *APOE4*-negative AD patients [9]. Since this increase in GSTO1*Ala was also verifiable in MCI patients, the biomarker might have a predictive value [10].

The main risk factor for AD is age; however, gender might also have an important influence since women over the age of 85 years have an increased incidence of AD compared to men of the same age [11]. With regard to additional well-known risk factors for AD, it has now become apparent that the *APOE4* allele increases the AD risk not only dose-dependent but also gender-related. Intriguingly, a single *APOE4* allele increases the risk of AD in healthy older women more eminently than in men [12,13].

In the present study, we investigated whether the proteomic AD platelet biomarkers tropomyosin-1 and monoamine oxidase-B might be affected by gender. In addition, we also determined their protein profile in a preceding stage of AD, by studying MCI patients also divided into gender groups. At first, a gender-related influence on tropomyosin-1 and monoamine oxidase-B was evaluated by increasing our existing 2-D DIGE platelet proteome database from 17% to 40% male study subjects. Then, using fluorescence 1-D Western blotting (WB) quantification, the stage-dependency of these platelet biomarkers was assessed in male and female AD and MCI patients.

2. Material and methods

2.1. Study population

Study participants were recruited in Austria and were all of Caucasian origin. AD patients were from geriatric-, retirement-, and nursing homes. Cognitively healthy controls and MCI patients were selected from spouses and care givers of AD patients, in retirement homes and by word-of-mouth recommendation. The Ethics Committee of Vienna approved the study protocol (EK 04-070-0604 and EK 09-219-1209) and the trial was conducted in accordance with the Declaration of Helsinki. All study participants or their trustee signed an informed consent before study entry. Only non-smoking individuals were recruited and none of the study participants received any antipsychotic drugs or antidepressants. The cognitive performance of all participants (86 AD patients, 30 MCI patients, and 110 cognitively healthy controls) was assessed as previously described [10] by the German neuropsychological CERAD test battery. A clinical examination, including an anamnesis, was made to exclude any other systemic disorders which might affect the cognitive status.

The 86 clinically suspected AD patients were also examined by structural brain scanning using MRI to exclude any other brain pathology accounting for cognitive impairment, like stroke or tumors. Finally, diagnosis of probable AD was made by a physician and a psychologist according to the criteria by the US National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [14]. Approximately 20% of the recruited AD patients had been treated with AD-related medication such as acetylcholinesterase inhibitors (e.g. donepezil) or NMDA-receptor antagonist (e.g. memantine), whereby this medication intake was equally distributed between genders. A total of 30 MCI patients were diagnosed by a psychologist and a physician according to the guidelines of the consensus conference in Stockholm [15]. Clinical criteria for MCI were a MMSE ≥ 24, not demented, intact activities of daily living, and impairment in at least two domains of memory (CERAD) using diagnostic comprehensive criteria [16]. None of the MCI patients received any AD-related medication. The 110 controls were classified as cognitively healthy by a physician and psychologist when MMSE ≥ 26 and neuropsychological measure of the CERAD test battery corresponded to age-appropriate norms in all cognitive domains [17]. Demographic characteristics of the Western blot study population are shown in Table 1, the characteristics of the entire 2-D DIGE cohort are outlined in Supplementary Table 1.

Table 1
WB study group characterisation.

	i) All			ii) Female			iii) Male		
	AD	MCI	Co	AD	MCI	Co	AD	MCI	Co
N	30	30	60	15	18	33	15	12	27
Mean age ± SD	78.2 ± 6.3	76.7 ± 7.8	75.9 ± 7.4	80.3 ± 4.1	79.3 ± 8.2	77.8 ± 7.6	76.6 ± 7.5	73.6 ± 6.4	73.8 ± 6.7
Mean MMSE ± SD	18.8 ± 7.2	27.6 ± 1.6	28.9 ± 1.2	17.2 ± 9.8	27.1 ± 1.7	28.8 ± 1.1	20.0 ± 4.8	28.2 ± 1.2	29.0 ± 1.3
Education (years)	10.1 ± 1.8	11.7 ± 2.1	12.1 ± 2.9	9.7 ± 1.6	11.5 ± 2.4	11.0 ± 2.2	10.4 ± 1.9	12.0 ± 1.8	13.5 ± 3.1
Plts $*10^3$/μL ± SD	245 ± 120	212 ± 52	228 ± 77	286 ± 175	211 ± 40	241 ± 67	215 ± 50	214 ± 65	212 ± 86
% *APOE4/*[a]	76.7	33.3	18.4	86.7	27.8	6.1	66.7	41.7	33.3
% *APOE4/4*	16.7	3.3	0	6.7	0	0	26.7	8.3	0

Legend: 30 AD patients, 30 MCI patients and 60 sex- and matched controls were analysed by 1-D Western blotting. *APOE4* allele distribution is depicted as i) % of all ii) % of all females iii) % of all males. Platelet counts were determined in whole blood anti-coagulated with EDTA.

[a] Percentage of APOE4-positivity (homo- or heterozygous), AD – Alzheimer's disease, MCI – mild cognitive impairment, Co – Controls, MMSE – Mini mental state examination, SD – Standard deviation; Plts – platelets.