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Specific alterations in the circulating levels of the SIRT1, TLR4, and IL7 proteins in patients with dementia

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ABSTRACT

Sirtuins have gained considerable attention as epigenetic regulators for slowing aging and age-related disorders. The growing association between neurodegeneration and inflammation has led researchers to investigate interactions of sirtuins with inflammatory markers in neurodegenerative diseases. We analyzed SIRT1's association with chronic inflammation in dementia as an age-related neurodegenerative condition through Toll-like receptor 4 (TLR4) and interleukin-7 (IL7) for the first time. In the present study, we observed a significant increase in the level of SIRT1 in patients with all types of dementia. Interestingly, the level of TLR4 protein was significantly lower in only the patients with Alzheimer's disease (AD) compared to the healthy elderly subjects. There was no significant change in the level of IL7 between the diseased and healthy elderly subjects. A significant positive correlation between SIRT1 level and age in healthy elderly subjects was evident according to Pearson's correlation test. However, this correlation was not observed in the dementia patients. Furthermore, the positive correlation between the levels of IL7 and TLR4 in the healthy elderly subjects was absent in the dementia patients. However, there was no direct association between the examined single nucleotide polymorphisms (SNPs) and dementia at the molecular level. According to logistic regression analysis, dementia risk increases 1.16 times due to an increase in the SIRT1 level and 24.23 times due to a decrease in the TLR4 level. Interestingly, a high level in the total antioxidant status (TAS) increases the risk of dementia approximately 33.32 times. Therefore, the current study, for the first time, provides a much better molecular understanding of the interaction between decreasing TLR4 levels and increasing SIRT1 levels in dementia, especially in AD. Furthermore, it highlights the importance of epigenetics in several age-related diseases and suggests that developing novel therapies to prevent or slow down the progression of dementia may support healthy aging.

1. Introduction

According to the World Alzheimer Report (2016), approximately 5% of the world's elderly population (46,8 million people) had dementia in 2015, and it is projected that this number will reach 131,5 million people in 2050. Alzheimer's disease (AD) is the most common form of dementia accounting for 60–70% of cases (WHO, 2015). According to the mortality statistics from the Turkish Statistics Institute, the proportion of elderly people who died from AD increased to 4% in 2014 from 2.7% in 2010. In addition, the prevalence rates of probable AD and dementia were calculated to be 11.0% and 20.0%, respectively, in the Turkish population (Gurvit et al., 2008). While AD has emerged

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as a major public health challenge for aging populations all over the world, the underlying molecular mechanisms of disease onset and progression have not yet been unraveled.

Irreversible neuronal loss in critical brain regions is a pathological hallmark of dementia, including AD (Tan et al., 2017). The accumulation of amyloid-beta (Aβ) plaques and the formation of neurofibrillary tangles containing hyperphosphorylated tau protein are thought to be possible causative mechanisms of these neuronal deaths (Hardy and Selkoe, 2002; Šimić et al., 2016). Until quite recently, the treatment strategy for AD pathogenesis has remained limited to the clearance of Aβ plaques to increase neuronal cell survival. However, the consequent lack of definitive therapy for disease progression has directed researchers to develop newer diagnostic and treatment strategies. As they are related to longevity and healthy aging, sirtuin proteins, especially SIRT1, and the other proteins in the sirtuin pathway are thought to be potential diagnostic and therapeutic molecules in dementia.

SIRT1, a NAD-dependent histone deacetylase, has a wide spectrum of metabolic and stress-tolerance properties due to its functions as a transcription factor and cofactor, in addition to being a target for histone and non-histone proteins (Tissenbaum and Guarente, 2001; Salminen et al., 2013; Shimoyama et al., 2011). SIRT1 protects cells against oxidative stress, regulates glucose/lipid metabolism, promotes DNA stability and slows down various age-related disorders, including neurodegenerative conditions, by binding to and deacetylating several substrates (Longo and Kennedy, 2006; Guarente and Franklin, 2011). Chromatin-, stress-, DNA repair-, and/or metabolism-related substrates, such as p53, NF-kB, FOXO, and PARP1, are examples of substrates from which SIRT1 transfers acetyl groups to an ADP-ribose molecule using NAD+ as an enzymatic co-factor (Martínez-Redondo and Vaquero, 2013). Furthermore, in neuronal cultures and animal studies, the neuroprotective role of SIRT1 and its therapeutic potential were previously reported in neurodegenerative diseases, including AD (Patel et al., 2005; Guarente, 2008; Bonda et al., 2011). Molecular studies showed that SIRT1 activation prevents the accumulation of Aβ plaques and tau pathology through the nuclear factor kappa B (NF-kB) signaling pathway by upregulation of the *ADAM10* gene, induction of the Notch pathway, and inhibition of the mTOR pathway (Chen et al., 2005; Song et al., 2011; Braidy et al., 2012). In addition, SIRT1 may regulate Aβ production by modulating BACE1 expression via NF-kB signaling (Kaltschmidt et al., 1999; Bourne et al., 2007; Buggia-Prevot et al., 2008). As shown in previous studies, SIRT1 epigenetically reprograms inflammation taking about AD formation at the earlier stages by altering transcription factors (Yeung et al., 2004; Xie et al., 2013; Hardy and Selkoe, 2002). In addition, it was observed that brains of AD patients have consistently reduced NAD+ levels and SIRT1 transcription and/or protein levels involved in chronic inflammation that can also be altered by increased levels of the activated proinflammatory transcription factor NF-kB (Qin et al., 2006; Serrano-Marco et al., 2012; Vachharajani et al., 2016). This mounting evidence points out the regulatory role of SIRT1 in inflammation during AD progression and/or prevention.

In one of our studies, we found a significant positive correlation between SIRT1 level and age, revealing enhanced SIRT1 expression with increasing age (Kilic et al., 2015). We thought that the high levels of SIRT1 protein might have a role in alleviating the oxidative stress that is significantly increased in the elderly. For the first time, we show in the present study the effects of SIRT1 on chronic inflammation in dementia through Toll-like receptor 4 (TLR4), which activates the NF-kB intracellular signaling pathway (Vaure and Liu, 2014) and interleukin-7 (IL7), an inflammatory cytokine. In addition, we investigated the association between *SIRT1* single nucleotide polymorphisms (rs7895833 A > G in the promoter region, rs7069102 C > G in intron 4 and rs2273773 C > T in exon 5 silent mutation) and the levels of SIRT1, TLR4, and IL7 expression, as well as the total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) with dementia in the Turkish population.

2. Experimental methods

2.1. Study groups

The study groups consisted of 80 randomly selected healthy elderly nursing home residents (age: 73.41 ± 10.02; range: 57–92) and 79 patients suffering from dementia (age: 73.19 ± 7.9; range: 58–94) including AD (n = 49) and other dementia-related conditions, such as progressive nonfluent aphasia (PFNA), progressive supranuclear palsy (PSP), among others (n = 30), who were recruited from the Neurology Department of Bezmialem Vakif University Hospital, Istanbul, Turkey. The parents of patients with a family history of dementia had no consanguinity.

The diagnosis of AD and other dementia-related conditions involved a two-step diagnostic process: screening for cognitive impairment with Folstein's Mini Mental State Examination scale (MMSE; scores #24) and confirmation by a detailed neurological examination, magnetic resonance imagining (MRI), assessment of daily living activities and neuro-psychological testing using the Clinical Dementia Rating Scale and the Blessed Dementia Rating Scale. AD was also diagnosed as per NINCDS-ADRDA criteria. However, the severity of AD was not indicated in the present study.

This study was approved by the Ethical Committee of Bezmialem Vakif University (08.04.2013/No: 36-13). All of the participants, after giving their written informed consent, completed a structured questionnaire in order to collect demographic data. The study was conducted in accordance with the ethical principles described by the Declaration of Helsinki.

2.2. Determination of the SIRT1, TLR4, and IL7 protein levels

After 12 h of fasting, blood samples from subjects were taken into tubes (Vacuette, Greiner Labor technic, Germany) and centrifuged for 5 min at 4 °C. Then, the blood serum and plasma were collected and stored at −20 °C. The plasma/serum samples of the subjects were analyzed for the levels of circulating SIRT1, TLR4, and IL7 proteins using enzyme-linked immunosorbent assay (ELISA) kits from USCN Life Science. Briefly, standards and samples were incubated in antibody-coated 96-well plates for 2 h. After incubation, the substrate solution was added, and the plates were incubated for 15–25 min. After addition of stop solution, the intensity of the color change in each well was measured in a microplate reader at 450 nm (Chromate Manager 4300, Palm City/USA).

2.3. Measurement of the total antioxidant and oxidant status and determination of the oxidative stress index

The total antioxidant status (TAS) and the total oxidant status (TOS) of the serum were determined using an automated measurement method by an automated analyzer (Chromate Manager 4300, Palm City/USA) as described earlier (Erel, 2004, 2005). In the measurement of TAS, the absorbance of colored dianisidyl radicals was monitored to determine the rates of a Fenton reaction, which initiates free-radical reactions resulting in the production of a hydroxyl radical. Then, the antioxidative effect of the sample against potent free-radical reactions was measured in terms of mmol equiv/L Trolox (Rel Assay Diagnostics, Gaziantep/TURKEY).

In the measurement of TOS, the total amount of oxidant molecules in the sample was determined by measuring the intensity of the colored complex produced by the reaction of ferric ions, which were oxidized in a ferrous iono-dianisidine complex due to the presence of oxidants, with xylenol orange in an acidic medium. The calibration of the assay was performed with hydrogen peroxide, and the TOS values are expressed in terms of micromolar hydrogen peroxide (H_2O_2) equivalents per liter (μmol H_2O_2 equiv/L) (Rel Assay Diagnostics, Gaziantep/TURKEY).

The oxidative stress index (OSI) was determined using the formula

Table 1
Primer sequences in *SIRT1* gene polymorphisms.

Polymorphism	Primer sequences
rs7895833 A > G	Forward primer 1: CCCAGGGTTCAACAAATCTATGTTG
	Forward primer 2: GGTGGTAAAAGGCCTACAGGAAA
	Reverse primer 1: GCTTCCTAATCTCCATTACGTTGAC
	Reverse primer 2: CCTCCCAGTCAACGACTTTATC
rs7069102 C > G	Forward primer 1: GTAGCAGGAACTACAGGCCTG
	Forward primer 2: GAGAAGAAAGAAAGGCATAATCTCTGC
	Reverse primer 1: CTATCTGCAGAAATAATGGCTTTTCTC
	Reverse primer 2: GATCGAGACCATCCTGGCTAAG
rs2273773 C > T	Forward primer 1: GTGTGTCGCATCCATCTAGATAC
	Forward primer 2: CTCTCTGTCACAAATTCATAGCCT
	Reverse primer 1: GTAGTTTTCCTTCCTTATCTGACAG
	Reverse primer 2: CTGAAGTTTACTAACCATGACACTG

given in previous literature (Kilic et al., 2014; Sahin-Kavakli et al., 2010).

$$OSI = [(TOS)/(TAS \times 1000)] \times 100$$

2.4. DNA isolation and determination of SIRT1 gene polymorphisms

Genomic DNA was isolated from peripheral blood leukocytes in the blood samples taken from all of the subjects with a DNA isolation kit (Invitrogen, Carlsbad, USA). All of the purified DNA samples were stored at 4 °C until PCR applications were performed (Miller et al., 1988; Salazar et al., 1998).

As described previously, but with minor modifications, the examined *SIRT1* SNPs (rs7895833 A > G in the promoter region, rs7069102 C > G in intron 4 and rs2273773 C > T in exon 5, (Shimoyama et al., 2011)) were analyzed using the PCR-CTPP assay (Atsuta and Hamajima, 2003, 2011).

The *SIRT1* gene segments encompassing the rs7895833 A > G, rs7069102 C > G, rs2273773 C > T polymorphic sites were amplified by PCR using the appropriate primers (Table 1) as described previously (Shimoyama et al., 2011). Briefly, 25 µl total PCR mixtures (100–200 ng DNA, 10.0 pmol of each primer, 1.0 mM dNTPs (deoxynucleotide triphosphates), 25 mM $MgCl_2$ and 2.5 U Taq DNA polymerase) in the supplied reaction buffer (Taq Buffer with $(NH_4)_2SO_4$) were prepared. PCR was performed with an initial denaturation at 95 °C for 10 min; 30 cycles of 95 °C for 1 min, 64 °C for rs7895833 A > G polymorphism, 62 °C for rs7069102 C > G polymorphism, 63 °C for rs2273773 C > T polymorphism for 1 min, and 72 °C for 1 min, with an additional the final step at 72 °C for 5 min. A 2% agarose gel and ethidium bromide staining were used for visualization of the PCR products. Three genotypes for each polymorphism were defined by 3 distinct banding patterns: for the rs7895833 A > G polymorphism: 320, 241 bp for the AA genotype; 320, 241 and 136 bp for the AG genotype; and 320, 136 bp for the GG genotype; for the rs7069102 C > G polymorphism: 391, 277 bp for the CC genotype; 391, 277, 167 bp for the CG genotype; and 391, 167 bp for the GG genotype; for the rs2273773 C > T polymorphism: 314, 228 bp for the CC genotype; 314, 228, 135 bp for the CT genotype; and 314, 135 bp for the TT genotype (Shimoyama et al., 2011).

2.5. Statistical evaluation

The results were presented as the means ± SEM. Statistical analyses of differences in the distribution of the genotypes or alleles in the *SIRT1* gene between the age groups were tested by the Chi-Square ($\chi 2$) test. Levels of proteins, TAS, TOS and OSI were analyzed by one-way ANOVA with dementia types (AD and others) as the independent variable to determine the expression differences between the studied groups. The post hoc comparisons of simple effects were conducted using Tukey's High Significant Difference (HSD) test. In addition,

Table 2
Subject demographics percentages are based on the total number of subjects.

	Elderly control subjects (n = 80)	Patients with dementia (n = 79)
Gender (male/female)(%)	65.0% (n = 52)/35.0% (n = 28)	41.8% (n = 33)/58.2% (n = 46)
Age (years ± SEM)	73.47 ± 10.02	73.19 ± 7.90
MMSE (mean)	NA	15.63
Family history (yes/no) (%)	NA	81.0% (n = 64)/16.5% (n = 13)
Consanguinity (yes/no) (%)	NA	6.3% (n = 5)/93.7% (n = 74)
Concominant diseases (AHT/DM) (%)	NA	27.8% (n = 22)/15.19% (n = 12)

SEM Standard Error of Mean, MMSE Mini Mental State Examination, NA not applicable, AHT arterial hypertension, DM diabetes mellitus.

Pearson's correlation test was used to calculate the linear correlations between the studied proteins with each other and with the other parameters (age, TAS, TOS, and OSI) in each group. Finally, a logistic regression test was applied to calculate the probability of having dementia in terms of the linear combination of studied protein levels. The SPSS 18 for Windows statistical software package was used for all of the statistical analysis of the data (SPSS Inc., Chicago, IL, USA). A p value of < 0.05 was regarded as being statistically significant.

3. Results

The demographics of the patients are summarized in Table 2. Out of 79 dementia patients with a mean 15.63 MMSE score, 58.2% of them were women and 41.8% of them were men. There is no difference between the ages of the control subjects and the dementia patients groups, with mean ages of 73.41 ± 10.02 years and 73.19 ± 7.9 years, respectively. Most of the patients had a family history of AD or dementia (81.0%) and low consanguinity (6.3%). Interestingly, patients mostly have arterial hypertension (AHT) (27.8%) as a concomitant disease (Table 2).

3.1. Expression levels of the SIRT1, TLR4, and IL7 proteins and the levels of TAS, TOS, and OSI and their correlations with each other

The expression levels of the SIRT1, TLR4 and IL7 proteins and the TAS, TOS and OSI levels are given in Figs. 1 and 2, respectively. The level of SIRT1 protein was significantly higher in the AD patients (6.05 ± 0.4 ng/ml) and in patients with other dementia-related conditions (6.46 ± 0.53 ng/ml) compared to the elderly healthy subjects (3.45 ± 0.23 ng/ml) ($p \leq 0.001$) (Fig. 1A). Pearson's correlation test showed that there was a significant positive correlation between SIRT1 level and age in the healthy controls ($p \leq 0.001$); however, there was no correlation between SIRT1 level and age in the subjects with dementia ($p = 0.624$) (Table 3).

The TLR4 protein expression level and its correlation with age, the levels of the SIRT1 and IL7 proteins, and the TAS, TOS, and OSI levels are presented in Fig. 1B and Table 3. The level of TLR4 protein was significantly lower in the AD patients (0.76 ± 0.07 pg/ml) compared to that in the healthy elderly subjects (1.15 ± 0.11 pg/ml) ($p = 0.006$). However, the expression level of TLR4 protein was not different in the subjects with other types of dementia (0.92 ± 0.09 pg/ml) compared to the subjects with no dementia. Pearson's correlation test showed a significant positive correlation between the SIRT1 and TLR4 expression levels in both the control subjects and the dementia patients ($p \leq 0.001$) (Table 3).

The relationship between IL7 concentration and age, the levels of SIRT1 and TLR4 proteins, and the TAS, TOS, and OSI levels are presented in Fig. 1C and Table 3. There was no change in the level of IL7 between the diseased and healthy elderly subjects ($p = 0.483$).

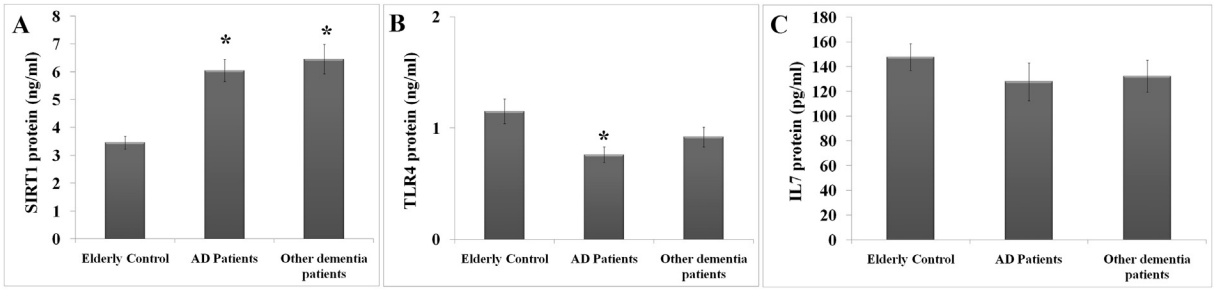

Fig. 1. Levels of SIRT1, TLR4 and OSI in healthy subjects, AD patients, and other dementia patients. The results are shown as mean ± Standart error of mean (SEM). * indicates significant difference against to healthy subjects. *p < 0.05. Statistical evaluation by One-way ANOVA with Post Hoc Tukey's test.

Pearson's correlation test showed that there was a significant positive correlation between the IL7 level and the SIRT1 level in both the control subjects and the dementia patients (p ≤ 0.001) (Table 3). Furthermore, a significant positive correlation was present between the IL7 and TLR4 expression levels in the subjects without dementia (p = 0.004).

The levels of the oxidative stress parameters (TAS, TOS, and OSI) are presented in Fig. 2A, B, and C. In all types of dementia patients, these levels were not significantly different from the healthy subjects. Application of Pearson's correlation test to examine the correlations between the levels of proteins, TAS, TOS, and OSI showed that there was a significant negative correlation between only the TAS and SIRT1 levels, both in the controls (p = 0.012) and in the dementia patients (p = 0.042) (Table 3).

Table 3
The results of Pearson's correlation of SIRT1, TLR4 and IL-7 with age, TAS, TOS and OSI levels.

	Elderly controls (n = 80)		Dementia patients (n = 79)	
	r	p	r	p
SIRT1 correlations with				
Age	0.517*	0.001	0.056	0.624
TLR4 protein (pg/ml)	0.482*	0.001	0.444*	0.001
IL7 protein (ng/ml)	0.425*	0.001	0.407*	0.001
TAS (mmol Trolox Equiv./L)	−0.317*	0.012	−0.230*	0.042
TOS (μmol H_2O_2 Equiv./L)	−0.067	0.634	−0.061	0.624
OSI	−0.017	0.904	−0.127	0.304
TLR4 correlations with				
Age	0.211	0.073	0.017	0.894
SIRT1 protein (ng/ml)	0.482*	0.001	0.444*	0.001
IL7 protein (ng/ml)	0.347*	0.004	0.127	0.326
TAS (mmol Trolox Equiv./L)	−0.058	0.657	0.092	0.472
TOS (μmol H_2O_2 Equiv./L)	−0.024	0.871	0.210	0.123
OSI	−0.058	0.694	0.157	0.251
IL7 correlations with				
Age	0.175	0.139	−0.078	0.503
SIRT1 protein (ng/ml)	0.425*	0.001	0.407*	0.001
TLR4 protein (pg/ml)	0.347*	0.004	0.127	0.326
TAS (mmol Trolox Equiv./L)	−0.204	0.109	−0.069	0.556
TOS (μmol H_2O_2 Equiv./L)	−0.137	0.329	−0.041	0.750
OSI	−0.070	0.618	−0.063	0.623

* p < 0.05.

3.2. Frequencies of the SIRT1 (rs7895833 A > G, rs7069102 C > G, rs2273773 C > T) gene polymorphisms

The frequencies of the genotypes and alleles of the *SIRT1* gene in all of the groups are shown in Table 4. For all of the examined gene polymorphisms (rs7895833 A > G in promoter region, rs7069102 C > G in intron 4, and rs2273773 C > T in exon 5), there was no significant difference between the study groups.

For rs7069102 C > G, the frequency of having the G allele was higher in healthy elderly people compared to the patients with dementia; however, the difference did not reach the accepted level of significance (p = 0.07) (Table 4).

3.3. Relationship between the levels of SIRT1, IL7, TLR4, TAS, TOS, and OSI

Logistic regression analysis showed that if the SIRT1 level increases, the superiority of dementia risk was 1.16 times higher (OR 1.165, 95% CI 1.086–1.249; p ≤ 0.001). Furthermore, an increased TAS level correlated with the severity of dementia (OR 33.32, 95% CI 2.24–495.08 p = 0.011). Interestingly, an increase in the TLR4 level decreases the dementia risk 24.23 times (OR 0.041, 95% CI 0.007–0,258 p = 0.001). In the logistic regressions analyses for age and IL7 level, there was no association between these parameters and the risk of impairment in dementia (OR 1.003, 95% CI 0.913–1.102, p = 0.953 and OR 0.996, 95% CI 0.988–1.003, p = 0.257, respectively). In addition, it was found that females have a 2.46 times higher risk for dementia than males (OR 0.406, 95% CI 0.169–0.973 p = 0.043).

4. Discussion

Aging and age-related disorders are currently an increasing challenge for society due to slowly progressing cognitive declines and organ

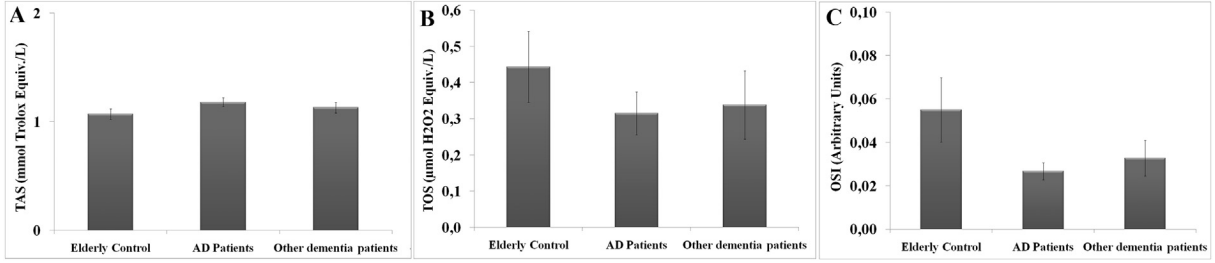

Fig. 2. Levels of TAS, TOS, and OSI in healthy subjects, AD patients, and other dementia patients. The results are shown as mean ± Standart error of mean (SEM). * indicates significant difference against to healthy subjects. *p < 0.05. Statistical evaluation by One-way ANOVA with Post Hoc Tukey's test.

Table 4
Distribution of rs7895833 A > G, rs7069102 C > G and rs2273773 C > T genotypes and alleles in study groups.

	Elderly controls (n = 79)		Patients with dementia (n = 70)		Statistical results	
	(%)	(n)	(%)	(n)	χ^2	p value
rs7895833 A > G						
Genotype						
AA	51.9	41	57.1	40		
AG	48.1	38	42.9	30	0.325	0.569
GG	0.0	0	0.0	0		
Allele						
A	75.9	120	78.6	110	0.230	0.630
G	24.1	38	21.4	30		
rs7069102 C > G						
Genotype						
CC	8.9	7	18.6	13		
CG	55.7	44	57.1	40	4.135	0.127
GG	35.4	28	24.3	17		
Allele						
C	36.7	58	47.1	66	3.270	0.070
G	63.3	100	52.9	74		
rs2273773 C > T						
Genotype						
CC	0.0	0	0.0	0		
CT	98.7	78	100.0	70	–	1.000
TT	1.3	1	0.0	0		
Allele						
C	49.4	78	50.0	70	0.012	0.910
T	50.6	80	50.0	70		

n, number of individuals. Statistical evaluation by the Chi-square test.

dysfunctions. Moreover, the correlation between aging and neurodegeneration has significant clinical importance and has led investigators to search for candidate signaling proteins related to dementia. Previous studies revealed roles for sirtuin proteins, especially SIRT1, in aging and age-related disorders, such as AD and dementia, using in vivo and in vitro models (Song et al., 2011; Braidy et al., 2012). In the present study, we aimed to make a contribution to the global understanding of the underlying mechanisms of dementia and AD pathology by studying SIRT1 and its relationship with the inflammation markers TLR4 and IL7, in addition to the known mechanisms of neurodegeneration in AD, such as amyloid beta aggregation and neurofibrillary tangle formation. Furthermore, we investigated polymorphisms in the *SIRT1* gene in patients with dementia to demonstrate the association between the genetic variation and the phenotype, which is important for a much better molecular understanding of the role of the longevity gene *SIRT1* and its protein product, especially in light of the importance of epigenetics. This information will help to develop essential life-coaching strategies to increase the quality of life of patients with dementia-related diseases.

Previous studies performed in animals and cell culture have shown an association between increased SIRT1 activity and the alleviation of an Alzheimer's disease-like disorder through reduction of oligomerized Aβ via an increased level of alpha-secretase (Song et al., 2011; Braidy et al., 2012; Yuan et al., 2012; Qin et al., 2006) or through induction of a defensive response against toxins and free radicals that slowed down apoptosis and increased cell repair (Canto and Auwerx, 2009; Cohen et al., 2004; Ungvari et al., 2008). In contrast to a previous human study (Kumar et al., 2013), in the current study, we observed a significant increase in the SIRT1 protein level in patients with dementia, including AD, compared to healthy elderly people, as was previously shown in some AD animal models (Kim et al., 2007). Previous animal studies also showed that instead of a decline in SIRT1 activity, aging increases the amount of available SIRT1 protein in mice, which could be related to oxidative damage (Braidy et al., 2011; Koltai et al., 2010). An induction of SIRT1 expression occurs when the organism encounters a biological stress, such as aging or an age-related disorder, due to the function of SIRT1 as an important stress sensor molecule. Therefore, the upregulation of SIRT1 in dementia patients may be interpreted as a neuroprotective adaptive response since in our study, we paid attention to the increase in the total antioxidant status in the AD patients.

In previous literature, it was shown that overexpression of SIRT1 also prevented the activation of microglia, which are found around senile plaques in the brains of AD patients (Chen et al., 2005). Indeed, the activation of microglia results in the production of proinflammatory cytokines through activation of NF-kB, which is triggered by the activation of TLR4, which itself modulates neuronal survival in the brain by inducing the innate immune response (Carty and Bowie, 2011; Kawai and Akira, 2007; Mollen et al., 2006; Tang et al., 2008). In the presence of Aβ, the TLR4 concentration increases, triggering Aβ induction of NF-kB-dependent genes, thereby showing the role of TLR4 in sensing and responding to Aβ (Frank et al., 2009; Carty and Bowie, 2011). In our study, we observed a significant decrease in the TLR4 concentration only in the AD patients, who have, importantly, more Aβ plaques than patients with other dementia-related diseases. In a previous study, the authors showed that inhibition of TLR4 also prevented the production of Aβ-induced inflammatory molecules, such as IL-6, and TNF-α (Walter et al., 2007; Jana et al., 2008; Udan et al., 2008). Indeed, recent evidence showed that increased SIRT1 expression decreases the TLR4 concentration (Cheng et al., 2015) and that the SIRT1 activator, resveratrol, has potent anti-inflammatory effects in the AD brain (Moussa et al., 2017). Consistent with our study, these findings suggest a compensatory mechanism against inflammation-induced brain injury. In addition, increased SIRT1 activity interferes with TLR4 oligomerization upon receptor stimulation by the suppression of NF-kB to decrease disease progression (Nabavi et al., 2015).

In addition to the innate immune system, changes in adaptive immunity in dementia were also investigated in this project by monitoring the concentration of IL-7, which is an extrinsic signal that regulates early B-cell development (Corfe and Paige, 2012) via a crosstalk with LPS-induced TLR4 signaling (Ruprecht and Lanzavecchia, 2006; Li et al., 2014). It was previously shown that B cell-mediated immune responses were induced by misfolded Aβ peptides due to the presence of autoantibodies against Aβ (Kellner et al., 2009; Weksler et al., 2002). However, in our study, we observed insignificant changes in the levels of IL7 between dementia patients and control subjects, similar to a recent study performed using resveratrol to investigate changes in neuro-inflammation in AD (Moussa et al., 2017).

Demonstration of genetic variations may give people hints about how they can change their lifestyles to decrease the risk of neurodegenerative disease. Despite several *SIRT1* polymorphism studies on BMI, obesity, diabetes and cardiovascular diseases (Botden et al., 2012; Clark et al., 2012; Cui et al., 2012; Dong et al., 2011; Figarska et al., 2013; Peeters et al., 2008; Shimoyama et al., 2011, 2012; Zarrabeitia et al., 2012; Zillikens et al., 2009), there are only a few studies on *SIRT1* SNPs in AD in humans (Morgan et al., 2007; Helisalmi et al., 2008; Halaschek-Wiener et al., 2009; Hohman et al., 2016). Whereas we found a strong relationship between rs7895833 and aging in our recent study (Kilic et al., 2015), in the present study we did not find any molecular relationship between the studied *SIRT1* gene polymorphisms and dementia, consistent with the studies done by Helisalmi and his colleagues in the Finnish population (Helisalmi et al., 2008).

According to the “free-radical theory of aging”, oxidative stress results in damage to the organism due to an imbalance between the concentrations of free radicals and antioxidants, leading to advanced aging (Harman, 1956). Therefore, we investigated the levels of TAS, TOS and OSI to determine the relationship between these oxidative stress parameters and AD. In this study, we observed a strong relationship between the TAS level and the risk of dementia. This relationship may be connected to the high levels of SIRT1 protein in older people because of SIRT1's role in the induction of antioxidants (Olmos et al., 2013; Tanno et al., 2010).

To summarize, we observed a significant increase in the SIRT1 levels in AD and other dementia patients, but a significant decrease in the TLR4 levels only in the AD patients. These results showed that the role of the SIRT1 protein in the inflammation in dementia may occur through the TLR4 protein, which is an intracellular signaling molecule for immune functions and the inflammatory response. In addition, the lack of an effect on IL7 expression in dementia patients could show that the compensatory rise in the SIRT1 concentration may not affect the level of IL7. According to our results, the increase in the SIRT1 levels and the decrease in the TLR4 levels increase the risk of dementia 1.16 times and 24.23 times, respectively. Also consistent with the literature (Viña and Lloret, 2010), females are 2.46 times more susceptible than males for the risk of dementia in our study. As previously mentioned above, the increase in the SIRT1 level might be a compensatory mechanism for age-related damage and oxidative stress for neuroprotection in both healthy aging and in dementia. However, this compensational increase in SIRT1 levels may disrupt the compensational inflammatory response that increases the risk of dementia in elderly people.

In conclusion, we aimed to determine a biomarker for AD in the blood, with a focus on SIRT1 protein and its activity-related proteins (IL7 and TLR4), to overcome limitations for early detection and effective differential diagnosis of this neurodegenerative diseases, as previously done by investigators for different biomarkers (Molochnikov et al., 2012; Agrawal and Biswas, 2015; Li et al., 2017; Mapstone et al., 2014). Specifically, the levels of these markers in the blood do not reflect their levels in the brain/brain parenchyma, whereas some for proteins, such as APOE and amyloid beta, their blood levels truly reflect their levels in the brain (Bu et al., 2017; Hwang et al., 2015). Therefore, additional research with experimental animal models is needed to investigate changes in the levels of the examined proteins in brain tissue to make more accurate conclusions to explain the relationship between SIRT1, TLR4 and IL7 and the causes and progression of AD.

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