Clusterin, TNF-α, and IL-6 polymorphism and implications on Alzheimer's disease risk determination in Saudi population

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Abstract

Background: In the wake of the warning by WHO that the prevalence of dementia may rise by 125% in Middle East by 2050, identification of genetic risk factors in Arab population is urgent.

Methods: To genotype the Single Nucleotide Polymorphisms (SNPs) in clusterin (CLU) rs11136000, rs1532278; tumor necrotic factor (TNF-α) -308 rs1800629 A/G and -857 rs1799724 T/C; interleukin-6 (IL-6) rs1800796 G/C (-572 G/C) and rs1800795 G/C (-174 G/C), and to determine their association with Alzeimer's Disease (AD), DNA was isolated from the blood of 42 elderly Saudi AD patients (19 male, 23 female) and 23 healthy controls (11 male, 12 female), recruited for this study. Total serum cholesterol, LDL-C, HDL-C, and triglyceride levels were measured using an autoanalyzer. Serum concentrations of beta-amyloid 1–40 (Aβ1-40), beta-amyloid 1–42 (Aβ1-42), CLU, and inflammatory biomarkers (IL-6, TNF-α, and the C-reactive protein) were assessed by ELISA. The gene polymorphisms were analyzed by RT-PCR using the TaqMan assay.

Results: The results show that in the rs1532278 SNP of CLU gene, GA heterozygous allele was significantly higher in AD patients (57.1%) than in the control subjects (26.1%; OR = 3.67, CI = 1.10-12.32; p=0.036), thus it may be a risk factor for AD. On the other hand, the AD patients who carried genotype GG for TNF-α SNP rs1800629 showed significantly higher levels of serum IL-6 (p = 0.04), and hence may increase susceptibility to AD in Saudi population.

Keywords: Polymorphism, IL-6, TNF-α, Clusterin, dementia, Saudi Arabia.

Background

According to Alzheimer's Disease International (ADI) Delphi consensus study, almost 70% of the population in the developing world will be affected by neurocognitive disorders by 2040 [1]. Despite the alarming warning of the World Health organization (WHO), that Alzheimer's Disease (AD) related neurodegenerative disorder will increase by >100% in Arab countries by 2050, it is still considered as a normal aging process [2]. The number of primary studies conducted to estimate its existing burden in Arab populations is only marginal. However, international studies demonstrate a prevalence that varies among different social classes with age and genetic contributions representing the major risk factors. Other associated risks for AD include diabetes mellitus, cardiovascular disease risk factors and gender (females at high risk) [3-5]. A systematic review published in 2019 covered 18 studies conducted between 1990 to 2018 in the Arab region on the prevalence of AD and dementia [6].The overall prevalence in Saudi Arabia was around 3.85-6.4%, in Egypt around 2-2.26%; 3.34-7.4% in Lebanon, 1.1% in Qatar and 3.6% in United Arab Emirates [7-11].

Genetic susceptibility at multiple genes and the interactions among them as well as environmental factors are likely to influence the risk of AD. Several studies suggest that AD involves polygenic risk factors; however, the precise etiology of the disease remains unclear [12-13] Genome-wide association studies (GWAS) have resulted in the identification of numerous loci that are associated with the risk of developing Late Onset Alzheimer Disease (LOAD) (14). These loci can be classified broadly into genes that are involved in lipid metabolism, the inflammatory response, and endocytosis [15]. Despite the recognized association between the ApoE genotype and the risk of AD, only 50% of LOAD patients are carriers of the *APOE*ε4 allele, which indicates that additional genetic factors may contribute to

the risk of LOAD [16].The involvement of neuroinflammation and oxidative stress has gained much attention based on strong evidence Inflammation is implicated in the etiology and pathophysiology of several brain pathologies, including Alzheimer's disease. The presence of Aβ plaques activate the production of inflammatory mediators to remove Aβ accumulation. Aggregated proteins and chronic inflammatory reactions contribute to neuronal death through the production of hyperactive inflammatory mediators such as the reactive oxygen species (ROS) and nitric oxide (NO) [17].

The present study attempted to assess the relationship between the activity of inflammatory biomarkers CRP, cytokines IL-6 and TNF-α with AD and to know if their levels can be used as biomarkers of AD. Furthermore, the possible associations between selected polymorphisms in the IL-6 and TNF-α genes and AD in Saudi subjects were evaluated similarly, numerous studies have demonstrated an association of LOAD with other genetic risk factors, but the results are inconsistent for subjects belonging to different ethnic and geographical groups. Despite the fact that genetic information on AD patients in western countries is abundant, such information on patients of Arabic ethnicity is scarce [18]. Thus, the present study aimed to establish genetic-biochemical interactions of select gene single nucleotide polymorphisms (SNPs) that may affect the risk of AD in a sample of Saudi Arabians.

Method

Participants

Participants enrolled in this study were recruited from the neurology clinic at King Saud University Medical City. Patient participants were examined by specialist in geriatric neurology and diagnosed with dementia of the Alzheimer's type according to the DSM-V criteria for major neurocognitive disorder (DSM-V reference) and the National Institute on

Aging and the Alzheimer's Association criteria for Alzheimer's disease [19]. Patient encounters typically included a review of the history of illness, cognitive assessments, complete neurological examination, basic laboratory testing, and imaging with either magnetic resonance imaging (MRI) or computed tomography (CT). Patients were reassessed on subsequent visits over the year to reaffirm the diagnosis. Excluded from the study were patients with coexisting cerebrovascular disease, autoimmune disorders or disorders of neuroinflammation, malignancy, active psychiatric disorders or taking psychiatric medication prior to the diagnosis of AD, anyone who did not meet probable AD criteria or situations where consent could not be obtained. Control participants were assessed for history of cognitive decline with the aid of an informant. Only those with no history of cognitive symptoms or functional impairment were included. Controls were obtained from primary care clinics, or were acquaintances from the community, or unrelated companions of the patients. Those with any conditions related to inflammatory disorders were also excluded such as autoimmune diseases, malignancy, using immunosuppressants, recent surgery, or recurrent or ongoing infections were excluded. The study was approved by the Ethical Committee, at the College of Science and the internal review board at the college of medicine, King Saud University (# KSU-SE-18-24). The Control group consisted 23 participants, 11 males, and 12 females, with a mean age 67.8 ± 7.0 years. The AD group consisted of 42 patients 42, 19 males, and 23 females, with a mean age 74.2 ± 8.9 years. Information regarding the demographic variables, age, family history of AD, and disease status was collected. The anthropometry and other clinical data was obtained from the clinical records when needed. Anthropometry included height (cm), weight (Kg), BMI (Kg/m^2), and blood pressure (mmHg). The clinical data included lipid profiles (mmol/l) (total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides.

Genotyping

DNA extraction from the blood was performed using DNeasy® Blood & Tissue Kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA purity (260:280 ratio) and concentrations were detected by NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) Genotyping was done by evaluating all SNPs (Table 1) in TNF-α, IL-6 and CLU gene, using allelic discrimination CFX96 real-time polymerase chain reaction (PCR) with pre-designed TaqMan genotyping assays (Applied Biosystems, Foster City, CA, USA). All genotyping was performed in 10 µL reactions, using TaqMan Genotyping Master Mix in 96-well plates in an ABI 7000 instrument (Applied Biosystems). Genotypes and allele frequency were analyzed by Bio-Rad CFX Manager Software. Thermal cycling was initiated with a denaturation step of 10 min at 95°C, followed by 48 cycles of 15 sec at 95°C and 60 sec at 60°C. Fluorescence detection occurred at 60°C. IL-6 gene (rs1800796 G/C (-572 G/C) and rs1800795 G/C (-174 G/C), in the interleukin-6 promoter TNF- α (-308 rs1800629 A/G and -857 rs1799724 T/C).

Table 1: SNP information

Gene	rs no.	Position	Common Allele	Variant Allele	SNP's Location
CLU	rs 11136000	Chr8:27607002	C	T	Intron 3
	rs1532278	Chr8:27608798	C	T	Intron 3
IL-6	rs1800796	Chr7:22726627	G	C	Promoter
	rs1800795	Chr7:22727026	G	C	Promoter
TNF-α	rs1799724	Chr6:31574705	C	T	Promoter

	rs1800629	Chr6:31575254	A	G	Promoter

Analysis of CLU, IL-6 and TNF- α and CRP

The serum levels of IL-6, TNF-α, CLU, and the CRP were determined by using ELISA (Quantikine® ELISA Kit, R&D Systems, USA). For inter- and intra-assay, the percentage coefficient variation (CV%) for IL-6 was 3.6 % and 1.6%; for TNF-α, it was 7.3 % and 2.2%; for CRP, it was 7% and 3.8%; and for CLU, it was 3.7% and 8.4%, respectively. Measurements of serum Aβ1–42 and Aβ1–40 were performed by using an ELISA Kit (CUSABIO, USA). The CV% values for the Aβ1-42 concentrations for all subjects were <8% and <10% for the intra-assay and the inter-assay measurement. Meanwhile, the CV% values for the Aβ1–40 concentrations for the intra-assay and inter-assay measurements were <8% and <10%, respectively.

Statistical analysis

All statistical analyses were performed using IBM SPSS (version 21) statistical package (IMB, Armonk, NY, USA). Biochemical parameter data were presented as the mean ± standard deviation (SD) for normal variables, whereas the median (1^{st} Quartile to 3^{rd} Quartile) was given for non-normal variables. A p-value ≤ 0.05 was considered to be significant. *P*-values were obtained using the Mann-Whitney U test when two groups were compared, and the Kruskal-Wallis test when three groups were compared. Data were presented using Graph Pad Prism 8. For genotyping, data were presented as N (%), odds ratio (OR), and 95% confidence intervals (CIs), with a p-value ≤ 0.05 considered to be significant. *P*-values were obtained from logistic regression analyses. The Hardy-Weinberg equilibrium was tested using the Chi-square test.

Results

Participant characteristics

A significantly ($p = 0.004$) enhanced expression of CLU was detected in the AD patients (340.4 µg/ml ± 74.6) compared with that of the control group (265.0 µg/ml ± 80.9). The serum levels of other biochemical markers of Alzheimer's disease, such as Aβ1-42, Aβ1-40, CRP, and cytokines TNF-α and IL-6 were comparable between the two tested groups. No significant differences could be observed except in case of CLU (Table 2A).

Table. 2 A. Descriptive statistics according to status of Disease

Parameters	Control	Patients	P-value	
N	23	42		
Age (Years)	67.8 ± 7.0	74.2 ± 8.9	0.006	
Male/Female	11/12	19/22	0.909	
BMI (Kg/m^2)	29.4 ± 6.7	27.2 ± 5.5	0.283	
Systolic Blood Pressure (mmHg)	130.4 ± 14.2	133.6 ± 17.7	0.538	
Diastolic Blood Pressure (mmHg)	68.1 ± 7.9	71.3 ± 12.2	0.366	
Total cholesterol (mmol/l)	4.8 ± 1.2	4.6 ± 1.1	0.485	
HDL-cholesterol (mmol/l)	1.0 ± 0.2	1.0 ± 0.3	0.850	
LDL-cholesterol (mmol/l)	3.1 ± 1.0	2.9 ± 0.9	0.623	
Triglycerides (mmol/l) #	1.4 (1.1 - 2.2)	1.4 (1.0 - 2.1)	0.606	
Glucose (mmol/l)	8.8 ± 3.8	7.4 ± 3.4	0.131	
Clusterin (ug/ml)	265.0 ± 80.9	340.4 ± 74.6	0.004	
Aβ1-42 (ng/ml) #	0.7 (0.4 – 1.5)	0.8 (0.4 – 1.5)	0.570	
Aβ1-40 (ng/ml) #	75.1 (50.1 – 106.6)	56.1 (44.9 – 165.0)	0.503	
TNF-α (pg/ml)#	8.7 (6.2 - 11.3)	7.9 (6.4 - 9.7)	0.467	
IL-6 (pg/ml) #	7.9 (7.1 - 12.7)	7.2 (3.3 - 9.6)	0.114	
CRP (ng/ml) #	35.4 (14.7 - 71.7)	35.5 (18.0 - 103.2)	0.411	

Note: Data presented as Mean ± SD for normal variables whereas Median (1^{st} Quartile – 3^{rd} Quartile) for non-normal variables. # indicates non-normal variables; P<0.05 considered as significant. * indicates P-value adjusted for age. P-value are obtained from Mann-Whitney U test.

Table 2 B. Descriptive statistics according to Severity of Disease

Parameters	Severity			P-value
	Mild	Moderate	Severe	
N (%)	15(35.7)	5(11.9)	22((52.3)	
M/F	11/4	¼	7/12	0.042
Age	73.7 ± 6.6	80.0 ± 8.9	73.8 ± 9.6	0.333
BMI (Kg/m2)	28.9 ± 4.4	28.6 ± 4.5	26.3 ± 6.2	0.554
Systolic BP (mmHg)	130.9 ± 13.3	134.0 ± 13.0	137.5 ± 19.1	0.609
Diastolic BP (mmHg)	69.1 ± 10.6	68.6 ± 12.1	74.1 ± 13.2	0.508
T-cholesterol (mmol/l)	4.2 ± 1.0	4.3 ± 1.3	4.8 ± 1.0	0.458
HDL-cholesterol (mmol/l)	0.9 ± 0.2	0.8 ± 0.2	1.0 ± 0.4	0.534
LDL-cholesterol (mmol/l)	2.6 ± 1.0	2.6 ± 0.9	3.3 ± 0.9	0.318
Triglycerides (mmol/l) #	1.4 (1.4 - 2.1)	2.0 (1.6 - 2.2)	1.2 (0.9 - 1.9)	0.383
Glucose (mmol/l)	7.6 ± 4.4	7.6 ± 2.4	7.5 ± 3.0	0.998
Clusterin (ug/ml)	342.3 ± 83.4	368.2 ± 41.9	324.9 ± 70.7	0.537
Aβ142 (ng/ml) #	1.1 (0.6 - 1.5)	0.7 (0.6 - 0.8)	0.9 (0.3 - 1.7)	0.837
Aβ140 (ng/ml) #	165.0 (50.2 - 239.9)	66.1 (50.1 - 165.2)	57.7 (44.9 - 86.0)	0.525
TNF-α (pg/ml)	8.0 (7.3 - 10.7)	8.4 (5.8 - 8.4)	7.7 (6.4 - 9.7)	0.754
IL-6 (pg/ml)	7.1 (4.6 - 9.1)	9.6 (9.6 - 14.0)	6.0 (3.0 - 7.3)	0.317
CRP (ng/ml)	37.9 (32.5 - 70.2)	41.3 (18.0 - 103.2)	55.1 (17.0 - 168.1)	0.926

Note: Data presented as Mean ± SD for normal variables whereas Median (1st Quartile – 3rd Quartile) for non-normal variables. # indicates non-normal variables; P<0.05 considered as significant. P-values are obtained from Kruskal-Wallis Test.

Furthermore, the clinical data suggests no significant difference between the Alzheimer's patients and the control group in the measurements of BMI, blood pressure, total cholesterol, or triglycerides. Similarly, no significant differences could be observed in descriptive parameters or biochemical markers in three groups according to the severity of disease described as mild, moderate or severe (Table 2B).

Biochemical parameters in relation to genotypic characteristics of participants

No significant differences were observed for any measured biochemical parameters in relation to the CLU gene SNP rs1532278 or the CLU gene SNP rs11136000 genotypes in the two tested groups of participants (Table 3; Table 4).

Table 3. Descriptive statistics according to CLU gene SNP rs11136000

Parameters	Control				Patients			
	GG	GA	AA	P-value	GG	GA	AA	P-value
N	9	9	5		8	20	13	
Age (Years)	68.6 ± 7.4	67.8 ± 6.0	66.6 ± 9.2	0.82	68.1 ± 7.5	76.0 ± 9.0	75.0 ± 9.1	0.19
BMI (Kg/m2)	27.9 ± 6.6	26.5 ± 4.2	38.0 ± 4.5	0.07	26.9 ± 6.5	26.8 ± 5.3	29.1 ± 5.5	0.59
Systolic BP (mmHg)	126.7 ± 17.2	131.5 ± 14.2	135.7 ± 9.6	0.89	120.8 ± 18.7	136.4 ± 16.2	135.7 ± 19.3	0.31
Diastolic BP (mmHg)	67.2 ± 7.2	68.3 ± 9.2	69.7 ± 9.3	0.89	70.8 ± 10.3	70.4 ± 12.3	73.4 ± 14.0	0.86
T-cholesterol (mmol/l)	4.5 ± 1.1	5.0 ± 1.4	5.1 ± 1.3	0.63	4.9 ± 0.4	4.3 ± 1.2	4.8 ± 1.0	0.53
HDL-cholesterol (mmol/l)	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.4	0.85	1.0 ± 0.2	1.0 ± 0.4	0.9 ± 0.1	0.72
LDL-cholesterol (mmol/l)	2.7 ± 0.9	3.4 ± 1.1	3.3 ± 1.1	0.18	3.2 ± 0.5	2.6 ± 1.0	3.3 ± 1.0	0.33
Glucose (mmol/l)	7.7 ± 3.6	8.8 ± 4.1	12.2 ± 2.8	0.38	5.6 ± 1.6	7.9 ± 4.1	7.9 ± 3.0	0.31
Triglycerides (mmol/l) #	2.0 (1.2 - 2.2)	1.2 (1.0 - 1.9)	1.5 (1.3 - 1.9)	0.52	1.5 (1.1 - 1.9)	1.4 (0.9 - 1.9)	1.7 (1.2 - 2.2)	0.89
Clusterin (ug/ml)	290.9 ± 73.0	244.7 ± 59.4	250.8 ± 122.2	0.49	330.9 ± 101.1	353.2 ± 80.0	325.1 ± 68.9	0.78
Aβ1-42 (ng/ml) #	0.8 (0.4 - 1.8)	0.6 (0.4 - 0.7)	1.0 (0.7 - 1.3)	0.58	0.8 (0.7 - 1.5)	0.8 (0.3 ± 1.6)	0.8 (0.4 - 2.9)	0.76
Aβ1-40 (ng/ml) #	57.2 (34.6 - 109.9)	100.4 (74.1 - 249.0)	57.7 (52.1 - 75.4)	0.33	110.6 (56.1 - 165.0)	47.5 (37.0 ± 86.0)	57.7 (50.1 - 248.3)	0.38
TNF-α (pg/ml)	8.6 (7.2 - 9.4)	11.1 (6.2 - 13.5)	8.9 (8.6 - 9.3)	0.88	11.9 (8.8 - 15.0)	7.8 (6.2 ± 9.5)	7.5 (6.4 - 9.1)	0.28
IL-6 (pg/ml)	8.6 (7.1 - 11.7)	7.9 (2.7 - 18.9)	8.8 (7.0 - 14.1)	0.98	10.3 (8.7 - 14.0)	7.2 (2.9 ± 7.3)	6.3 (3.3 - 9.6)	0.07
CRP (ng/ml)	33.9 (14.8 - 42.2)	43.5 (17.3 - 66.1)	45.1 (8.6 - 90.4)	0.91	70.2 (41.3 - 129.0)	32.7 (14.7 ± 73.5)	32.5 (18.0 - 103.2)	0.35

Note: Data presented as Mean ± SD for normal variables whereas Median (1st Quartile – 3rd Quartile) for non-normal variables. P<0.05 considered as significant. P-values are obtained from Kruskal -Wallis Test.

For the control group, the carriers of GG genotype for IL-6 gene SNP_rs1800796 had significantly lower systolic blood pressure (p = 0.009) and triglycerides (p = 0.04) compared to carriers of CC genotype for this SNP (Table 5).

Table 4. Descriptive statistics according to CLU gene SNP rs1532278

	Control				Patients			
Parameters	GG	GA	AA	P-value	GG	GA	AA	P-value
N	12	6	5		12	24	5	
Age	68.3 ± 6.3	68.0 ± 7.4	66.6 ± 9.2	0.86	71.6 ± 8.6	76.8 ± 8.4	68.0 ± 9.7	0.16
BMI (Kg/m2)	27.9 ± 5.7	25.7 ± 4.9	38.0 ± 4.5	0.06	28.8 ± 5.5	27.6 ± 5.7	23.9 ± 3.5	0.38
Systolic BP (mmHg)	130.0 ± 16.0	127.3 ± 15.7	135.7 ± 9.6	0.72	123.3 ± 17.9	136.6 ± 16.2	139.4 ± 21.8	0.25
Diastolic BP (mmHg)	69.5 ± 7.5	64.3 ± 8.5	69.7 ± 9.3	0.54	70.6 ± 11.8	70.9 ± 12.6	74.8 ± 14.3	0.88
T-cholesterol (mmol/l)	4.4 ± 1.0	5.4 ± 1.4	5.1 ± 1.3	0.22	4.9 ± 1.0	4.2 ± 1.1	5.3 ± 0.8	0.15
HDL-cholesterol (mmol/l)	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.4	0.78	1.1 ± 0.4	0.9 ± 0.2	1.0 ± 0.2	0.41
LDL-cholesterol (mmol/l)	2.7 ± 0.9	3.8 ± 1.1	3.3 ± 1.1	0.06	3.2 ± 0.9	2.6 ± 0.9	3.6 ± 0.9	0.17
Glucose (mmol/l)	7.5 ± 3.1	10.3 ± 5.1	12.2 ± 2.8	0.36	6.1 ± 1.8	8.6 ± 4.2	6.0 ± 1.2	0.24
Triglycerides (mmol/l) #	1.3 (1.0 - 2.1)	1.7 (1.2 - 2.2)	1.5 (1.3 - 1.9)	0.99	1.4 (1.1 - 1.9)	1.6 (0.9 - 2.0)	1.6 (1.0 - 2.2)	0.98
Clusterin (ug/ml)	284.6 ± 69.9	232.1 ± 56.6	250.8 ± 122.2	0.45	310.0 ± 70.4	373.2 ± 65.5	295.4 ± 77.6	0.14
Aβ1-42 (ng/ml) #	0.7 (0.4 - 1.8)	0.6 (0.4 - 0.7)	1.0 (0.7 - 1.3)	0.47	1.3 (0.8 - 2.0)	0.7 (0.3 ± 1.2)	0.6 (0.3 - 2.1)	0.21
Aβ1-40 (ng/ml) #	69.7 (36.2 - 144.7)	95.1 (56.1 - 102.6)	57.7 (52.1 - 75.4)	0.88	57.7 (44.9 - 165.0)	66.1 (37.1 ± 200.9)	50.1 (45.1 - 57.7)	0.79
TNF-α (pg/ml)	8.7 (6.8 - 13.0)	8.6 (2.7 - 11.3)	8.9 (8.6 - 9.3)	0.85	7.2 (6.9 - 8.8)	8.2 (5.9 ± 9.5)	8.7 (6.8 - 13.2)	0.84
IL-6 (pg/ml)	9.5 (7.1 - 12.7)	7.8 (4.9 - 12.3)	8.8 (7.0 - 14.1)	0.77	7.3 (6.3 - 10.3)	7.2 (2.9 ± 8.5)	3.3 (0.6 - 9.6)	0.42
CRP (ng/ml)	35.4 (16.1 - 52.1)	42.0 (13.9 - 75.9)	45.1 (8.6 - 90.4)	0.96	70.2 (41.3 - 77.3)	33.0 (17.0 ± 103.2)	23.0 (13.1 - 98.0)	0.39

Note: Data presented as Mean ± SD for normal variables whereas Median (1^{st} Quartile – 3^{rd} Quartile) for non-normal variables. P<0.05 considered as significant. P-values are obtained from Kruskal-Wallis Test.

Table 5. Descriptive statistics according to IL-6 gene SNP_rs1800796

	Control			Patients		
	CC	GG	P-values	CC	GG	P-values
N	5	17		4	35	
Age (Years)	67.4 ± 8.8	67.9 ± 6.7	0.94	73.0 ± 4.8	74.9 ± 9.2	0.63
BMI (Kg/m2)	35.3 ± 5.4	27.9 ± 6.3	0.10	32.5	27.8 ± 5.2	0.48
Systolic Blood Pressure (mmHg)	145.7 ± 3.5	126.6 ± 13.3	0.009	122.3 ± 19.1	134.6 ± 18.2	0.35
Diastolic Blood Pressure (mmHg)	67.3 ± 7.4	68.3 ± 8.3	0.63	60.7 ± 5.0	72.4 ± 12.8	0.12
Total cholesterol (mmol/l)	5.5 ± 0.6	4.7 ± 1.2	0.25	4.1 ± 1.0	4.6 ± 1.1	0.35
HDL-cholesterol (mmol/l)	1.0 ± 0.2	1.0 ± 0.3	0.82	0.9 ± 0.2	1.0 ± 0.3	0.45
LDL-cholesterol (mmol/l)	3.5 ± 0.5	3.1 ± 1.1	0.44	2.5 ± 1.1	3.0 ± 0.9	0.25
Glucose (mmol/l)	9.1 ± 2.6	8.7 ± 4.2	1.00	5.4 ± 1.7	7.8 ± 3.6	0.21
Triglycerides (mmol/l)	2.0 (2.0 - 2.6)	1.3 (1.0 - 2.1)	0.040	1.8 (1.2 - 2.2)	1.4 (1.0 - 2.0)	0.59
Clusterin (ug/ml)	319.6 ± 109.9	252.2 ± 66.8	0.21	296.8 ± 34.1	347.6 ± 77.6	0.18
Aβ1-42 (ng/ml) #	0.6 (0.5 - 0.9)	0.7 (0.4 - 1.8)	0.40	1.5 (1.0 - 1.5)	0.8 (0.4 - 1.7)	0.31
Aβ1-40 (ng/ml) #	50.1 (36.2 - 57.7)	75.4 (52.1 - 144.7)	0.23	202.5 (165.0 - 239.9)	50.2 (41.0 - 70.7)	0.16
TNF-α (pg/ml)	7.2 (6.2 - 8.3)	9.3 (6.3 - 12.5)	0.32	10.7 (8.6 - 15.0)	7.7 (6.4 - 8.8)	0.06
IL-6 (pg/ml)	7.8 (7.7 - 7.8)	9.6 (6.8 - 16.7)	0.51	7.9 (2.6 - 10.3)	7.2 (3.3 - 9.6)	0.91
CRP (ng/ml)	11.0 (10.3 - 33.9)	42.9 (16.1 - 73.6)	0.18	70.2 (17.0 - 109.2)	35.2 (18.0 - 103.2)	0.86

Note: Data presented as Mean ± SD for normal variables whereas Median (1st Quartile – 3rd Quartile) for non-normal variables. P<0.05 considered as significant. P-values are obtained from Mann Whitney-U Test.

For IL-6 gene SNP rs1800795 and TNF-α gene SNP rs1799724, no significant differences were observed for any of the measured biochemical parameters in relation to different observed genotypes (Table 6 & 7 respectively).

Table 6. Descriptive statistics according to IL-6 gene SNP rs1800795

Parameter	Control				Patients			
	GG	GC	CC	P-values	GG	GC	CC	P-values
N	3	5	15		3	11	25	
Age (Years)	64.0 ± 6.6	67.6 ± 8.7	68.7 ± 6.7	0.63	87.5 ± 6.4	71.2 ± 9.7	74.9 ± 7.8	0.07
BMI (Kg/m2)	28.5 ±	32.4 ± 2.8	28.9 ± 7.4	0.59		24.8 ± 5.6	28.8 ± 4.9	0.15
Systolic BP (mmHg)	109.0 ±	134.5 ± 0.7	131.5 ± 14.6	0.51		125.0 ± 29.0	135.9 ± 14.0	0.25
Diastolic BP (mmHg)	67.0 ±	75.5 ± 0.7	67.0 ± 8.2	0.31		71.3 ± 16.4	71.0 ± 12.0	0.82
Total cholesterol (mmol/l)	5.8 ± 0.7	4.8 ± 0.9	4.6 ± 1.3	0.23		4.7 ± 1.0	4.5 ± 1.1	0.61
HDL-cholesterol (mmol/l)	1.2 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	0.11		1.1 ± 0.4	0.9 ± 0.2	0.35
LDL-cholesterol (mmol/l)	4.0 ± 0.4	3.2 ± 0.7	2.8 ± 1.1	0.17		3.4 ± 0.9	2.7 ± 0.9	0.18
Glucose (mmol/l)	9.5 ± 6.6	6.2 ±	8.9 ± 3.7	0.95		7.8 ± 3.9	7.3 ± 3.4	0.49
Triglycerides (mmol/l)	1.4 (0.7 - 1.9)	1.3 (1.3 - 1.7)	1.7 (1.1 - 2.5)	0.70		1.1 (0.9 - 2.1)	1.6 (1.3 - 2.1)	0.38
Clusterin (ug/ml)	257.4 ± 125.5	231.3 ± 67.5	278.6 ± 78.1	0.61		347.0 ± 76.4	337.0 ± 76.3	1.00
Aβ14-2 (ng/ml) #	0.8 (0.3 - 1.5)	0.6 (0.4 - 0.6)	0.7 (0.4 - 1.8)	0.58		0.9 (0.5 ± 1.6)	0.7 (0.4 - 1.5)	0.66
Aβ140 (ng/ml) #	64.3 (30.8 - 861.7)	55.1 (32.6 - 91.0)	83.6 (52.1 - 144.7)	0.60		45.0 (31.9 ± 56.1)	59.3 (50.2 - 200.9)	0.07
TNF-α (pg/ml)	9.4 (8.9 - 11.0)	11.3 (9.3 - 13.5)	8.3 (6.1 - 11.1)	0.24		9.7 (7.7 - 14.6)	7.3 (5.9 - 8.4)	0.07
IL-6 (pg/ml)	11.7 (9.8 - 18.5)	13.3 (7.2 - 21.8)	7.8 (6.1 - 9.5)	0.19		7.3 (2.6 - 8.7)	6.8 (4.6 - 9.6)	1.00
CRP (ng/ml)	75.4 (14.8 - 105.3)	43.5 (14.7 - 60.7)	26.2 (13.9 - 66.1)	0.49		32.4 (27.9 - 70.2)	37.9 (18.0 - 103.2)	0.92

Note: Data presented as Mean ± SD for normal variables whereas Median (1^{st} Quartile – 3^{rd} Quartile) for non-normal variables. P<0.05 considered as significant. P-values are obtained from Mann-Whitney U test when two groups are compared and Kruskal-Wallis Test when three groups are compared.

Table 7. Descriptive statistics according to TNF -α gene SNP rs1799724

Parameters	Control			Patients		
	CC	CT	P-values	CC	CT	P-values
N	16	6		36	5	
Age (Years)	69.5 ± 6.8	63.0 ± 6.5	0.18	73.3 ± 8.7	79.8 ± 10.4	0.18
BMI (Kg/m2)	28.4 ± 7.1	32.6 ± 6.3	0.37	27.4 ± 5.7	28.5 ± 0.1	0.79
Systolic Blood Pressure (mmHg)	129.2 ± 13.7	133.3 ± 21.4	0.46	134.6 ± 18.0	127.3 ± 19.1	0.49
Diastolic Blood Pressure (mmHg)	67.0 ± 8.8	70.0 ± 3.0	0.66	72.0 ± 12.3	67.8 ± 14.1	0.53
Total cholesterol (mmol/l)	4.8 ± 1.2	4.8 ± 1.4	0.91	4.8 ± 0.9	3.7 ± 1.3	0.09
HDL-cholesterol (mmol/l)	1.0 ± 0.3	1.0 ± 0.2	1.00	1.0 ± 0.2	0.9 ± 0.6	0.30
LDL-cholesterol (mmol/l)	3.1 ± 1.1	3.0 ± 1.1	0.91	3.1 ± 0.8	2.3 ± 1.3	0.19
Glucose (mmol/l)	9.6 ± 4.1	7.3 ± 3.2	0.48	7.3 ± 3.4	8.1 ± 4.1	0.73
Triglycerides (mmol/l)	1.5 (1.0 - 2.2)	1.7 (1.3 - 2.0)	0.62	1.5 (1.2 - 2.2)	1.0 (0.9 - 1.9)	0.37
Clusterin (ug/ml)	256.9 ± 73.4	298.2 ± 98.2	0.42	339.9 ± 81.2	341.9 ± 55.2	0.91
Aβ142 (ng/ml) #	0.8 (0.5 - 1.8)	0.4 (0.3 - 0.8)	0.07	0.8 (0.4 - 1.1)	1.5 (1.0 ± 1.7)	0.19
Aβ140 (ng/ml) #	75.1 (34.7 - 144.7)	64.3 (50.1 - 98.2)	1.00	53.2 (41.1 - 125.5)	82.0 (44.9 ± 239.9)	0.63
TNF-α (pg/ml)	8.7 (5.9 - 11.2)	8.5 (7.2 - 9.4)	0.86	7.7 (6.0 - 9.7)	8.4 (7.7 - 8.6)	0.70
IL-6 (pg/ml)	7.8 (6.8 - 9.8)	11.7 (7.8 - 24.7)	0.19	7.2 (5.9 - 9.6)	5.0 (2.6 - 7.6)	0.26
CRP (ng/ml)	18.4 (13.9 - 75.4)	35.4 (14.8 - 60.7)	1.00	37.9 (27.9 - 103.2)	17.0 (2.1 - 109.2)	0.36

Note: Data presented as Mean ± SD for normal variables whereas Median (1st Quartile – 3rd Quartile) for non-normal variables. P<0.05 considered as significant. P-values are obtained from Mann-Whitney U test.

AD patients who carried genotype GG for TNF-α SNP rs1800629 (Table 8) showed significantly higher levels of serum IL-6 at $p = 0.04$).

Table 8 Descriptive statistics according to TNF-α SNP rs1800629

Parameters	Normal			Patients			
	AG	GG	P-values	AA	AG	GG	P-values
N	11	12		1	10	26	
Age (Years)	66.3 ± 5.5	69.1 ± 8.1	0.28	78.0	77.1 ± 9.3	72.8 ± 8.3	0.38
BMI (Kg/m2)	28.7 ± 8.2	30.1 ± 5.0	0.40	31.6	26.9 ± 4.7	27.9 ± 5.6	0.62
Systolic Blood Pressure (mmHg)	128.1 ± 15.5	133.0 ± 13.3	0.54	153.0	137.9 ± 18.2	130.3 ± 19.2	0.35

Diastolic Blood Pressure (mmHg)	70.0 ± 7.2	66.0 ± 8.6	0.34	67.0	68.7 ± 14.3	72.7 ± 12.9	0.71
Total cholesterol (mmol/l)	4.5 ± 1.3	5.0 ± 1.2	0.29		4.4 ± 1.5	4.7 ± 0.9	0.70
HDL-cholesterol (mmol/l)	1.0 ± 0.2	1.0 ± 0.3	0.93		0.9 ± 0.2	1.0 ± 0.3	0.17
LDL-cholesterol (mmol/l)	2.9 ± 1.0	3.3 ± 1.1	0.31		2.9 ± 1.3	3.0 ± 0.8	0.71
Glucose (mmol/l)	9.8 ± 4.1	7.3 ± 3.1	0.22		7.4 ± 4.5	7.7 ± 3.0	0.30
Triglycerides (mmol/l)	1.4 (1.1 - 1.9)	1.6 (1.1 - 2.4)	0.63		1.3 (0.9 - 2.1)	1.5 (1.0 - 2.1)	0.71
Clusterin (ug/ml)	250.8 ± 67.3	279.1 ± 93.6	0.52		335.0 ± 95.6	339.5 ± 68.1	0.92
Aβ142 (ng/ml) #	0.8 (0.5 - 1.8)	0.6 (0.3 - 1.0)	0.15		0.6 (0.4 ± 0.8)	1.0 (0.8 - 1.5)	0.39
Aβ140 (ng/ml) #	58.2 (34.7 - 92.1)	98.2 (56.1 - 144.7)	0.28		50.1 (45.1 ± 57.7)	112.2 (50.2 - 239.9)	0.16
TNF-α (pg/ml)	8.7 (7.2 - 11.3)	8.8 (4.9 - 11.8)	0.57		8.4 (8.0 - 9.7)	7.7 (6.9 - 8.6)	0.39
IL-6 (pg/ml)	9.5 (7.7 - 11.7)	7.8 (6.9 - 18.7)	0.89		2.9 (2.6 - 3.3)	8.3 (6.3 - 10.3)	0.04
CRP (ng/ml)	35.4 (17.3 - 75.9)	30.7 (12.5 - 68.9)	0.50		27.9 (18.0 - 37.9)	70.2 (17.0 - 109.2)	0.51

Note: Data presented as Mean ± SD for normal variables whereas Median (1st Quartile – 3rd Quartile) for non-normal variables. P<0.05 considered as significant. P-values are obtained from Mann-Whitney U test.

Genotyping analysis

The genotype analysis for the CLU gene SNP rs11136000 revealed no significant differences in distribution between the two groups (Figure 1 A & B; Table 9), while for the other CLU gene SNP, rs1532278, the GA genotype was significantly higher in patients (57.1%) than in the controls (26.1%), [p = 0.036, OR = 3.67, 95% CI (1.10 – 12.32)] (Table 9; Figure 1 C & D). For the two selected SNPs in the IL-6 gene and two in the TNF-α gene, the genotype analysis found no significant difference in the distribution of various genotypes compared to that of the two studied groups (Table 9).

Figure 1. Genotype analysis of CLU gene SNP rs 11136000 and rs15322278 shows no significant difference in patient (A) and control groups (B) with reference to rs11136000 while GA was significantly higher in patients (D) than in control (C) in case of rs15322278.

Table 9. Odds of Alzheimer according to genotypes

	Normal (N = 23)	Patients (N = 42)	OR (95%CI)	P-value
CLU SNP rs11136000				
GG	9 (39.1)	8 (19.5)	0.34 (0.08 - 1.39)	0.134
GA	9 (39.1)	20 (48.8)	0.96 (0.26 – 3.59)	0.953
AA	5 (21.7)	13 (31.7)	Reference	
CLU SNP rs1532278				
GG	12 (52.2)	12 (29.3)	Reference	
GA	6 (26.1)	24 (58.5)	3.67 (1.10 – 12.32)	0.036
AA	5 (21.7)	5 (12.2)	0.92 (0.21 – 4.05)	0.909
IL-6 SNP_rs1800796				
CC	5 (22.7)	4 (10.3)	0.39 (0.09 – 1.64)	0.20
CG	--	--	--	--
GG	17 (77.3)	35 (89.7)	Reference	
IL-6 SNP_rs1800795				
GG	3 (13.0)	3 (7.7)	0.60 (0.11 – 3.36)	0.56
GC	5 (21.7)	11 (28.2)	1.65 (0.44 - 6.12)	0.45
CC	15 (65.2)	25 (64.1)	Reference	
TNF-α SNP_rs1799724				
CC	16 (72.7)	36 (87.8)	Reference	
CT	6 (27.3)	5 (12.2)	0.37 (0.10 – 1.39)	0.14
TT	--	--	--	--
TNF-α SNP_rs1800629				
AA	--	1 (2.7)	--	--
AG	11 (47.8)	10 (27.0)	0.38 (0.13 - 1.17)	0.09
GG	12 (52.2)	26 (70.3)	Reference	

Note: Data presented as N (%); OR (95%CI) indicates and Odds ratio and its 95% confidence interval; P-value < 0.05 considered significant. P-values are obtained from logistic regression analysis.

Discussion

The present study aimed to evaluate the associations of selected polymorphisms in the CLU, IL-6, and TNF-α genes with the risk of AD in the Saudi population. The CLU gene is one of the ten non-*APOE* genome-wide significant risk loci that carries SNPs with functional evidence. Serum CLU levels are implicated in the onset, prevalence, progression, and severity of AD, as well as brain atrophy, through several pathways such as lipid metabolism, beta-amyloid aggregation and clearance, apoptosis, neuroinflammation, and neuronal cell cycle control [20]. A significant association between the CLU gene SNP rs11136000 and a reduced risk of LOAD has been reported in the European population [21-22]. The present study found that the heterozygote GA for *CLU* rs1532278 was significantly higher in patients compared to that of the controls ($p = 0.036$). Our result finds support in a study that reported a strong and significant association between rs1532278 and an increased risk of AD through its involvement in enhancing beta-amyloid deposition in AD brains [23]. A previous study on white Europeans recruited from six sites in the UK, Italy, France, Finland, Greece, and Poland, found a significant increase in CLU gene expression in AD patients compared to that of mild cognitive impairment and control groups [24]. Similarly, a recent study on Australian AD patients reported higher CLU levels in the case group compared to the control group at baseline and after 18 months [25].In line with these studies, the present study also found the serum CLU to be significantly higher in AD patients compared to that of the control group ($p = 0.004$).

Lipid metabolism has been traced to be one of the possible routes leading to AD progression affected by increased CLU levels. However, in this study lipids, including the total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride levels were similar for both the control and AD patient groups, indicating that there was no significant relationship between lipids and an increased risk of AD. Despite our sample size, our findings are not inconsistent

with the existing literature. Reitz et al. confirmed a weak association between the lipid level and the risk of AD [26]. Similarly, a recent study evaluated the concentration of blood lipids, such as total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol, in a random subset of individuals and observed no differences in the blood lipid concentrations between the controls and AD patients [27]. Serum concentrations of Aβ1-40 and Aβ1-42 were not significantly different in AD patients and control groups (Tables 4 & 5) in relation to different genotypes of CLU. This observation suggests that increased CLU serum levels may not be the outcome of Aβ aggregates. A previous meta-analysis noted the importance of using the cytokine (IL-6, TNF-α) levels as biomarkers of AD [28]. Serum IL-6 and TNF-α levels have also been reported to be increased in some studies [29] (Shibata et al., 2004), while in others, no significant differences were observed in comparison to the healthy controls [30]. The peripheral levels of CRP, IL-6, or TNF-α in AD patients and those of the control were not significantly different between the two groups in this study.

The current study evaluated genotypic distribution of IL-6 gene rs1800796 G/C (-572 G/C) and rs1800795 G/C (-174 G/C) in the IL-6 promoter among healthy and AD patients in a Saudi population. Previous studies have been inconsistent in linking IL-6 rs1800796 polymorphisms to AD risk. This may be attributed to different ethnic groups or sample sizes used in different studies. In 2008, Baune and his colleague found that there was a negative association between rs1800796 polymorphism and AD in the general elderly population. Similarly, Hui-Ping Qi et al. found that *IL-6 C/G* can be a neuroprotective factor against AD with specific genotypes (GG + GC). However, a study conducted on a Brazilian population found no association between rs1800796 polymorphism and increased susceptibility for AD occurrence [31]. The genotypic distributions for polymorphism at position-572 of the IL-6 gene promoter showed no significant difference between AD patients and the healthy control group

in the present study. However, it was interesting to note that in our study, the levels of TNF-α in AD patients with the CC genotype was higher than in AD patients with GG genotypes for SNPs rs1800796 of the *IL-6* gene. This finding showed a trend towards significance (p=0.06; Table 5). These findings also support the hypothesis, which suggests that interactions between cytokines can affect cytokine production through T-cell activation, which in turn produces more mediators. Several studies in the past have attempted to find an association between the *IL-6* rs1800795 polymorphism with AD, but the results have been contradictory. Studies conducted across diverse geographical areas have yielded different results; some have shown a positive association while others have found none [32-33]. Hongmei Yue and Wei Han studied the correlation between *IL-6 C>G (-174)* in a Chinese population and found no association with AD. Similarly, the result of the present study indicates that there was no association between *IL-6 rs1800795* polymorphism and the occurrence of AD in Saudi subjects [34].

TNF-α is another important pro inflammatory cytokine that is unregulated in Alzheimer's patients. Pro inflammatory cytokines have been known to activate the nuclear-factor kappa B (NF-KB), which in turn activates the secretion of APOE [35]. A meta-analysis investigated the associations between 5 TNF-α polymorphisms (−850, −308, −863, −238, and −1031) and AD. The study reported no significant difference in the genotype distribution of −308 polymorphism in AD among Caucasian Australians and Northern Europeans [36]. Another meta-analysis performed on a multi-ethnic population reported that TNF-α −308 A/G polymorphism may be significantly associated with AD in East Asians but not in European or Middle Eastern populations [37]. This supports our finding of various genotypes of rs 1800629 among the subjects of the present study. We found that TNF-α rs1800629 polymorphism lacks an association with susceptibility for increased or decreased risk of AD in Saudi subjects. Similarly, other studies also found that TNF-α *SNPs_rs1800629* does not play a role in AD

progression [38]. The level of IL-6 was found to be significantly low among AD subjects carrying AG genotypes compared to those carrying GG genotypes for SNP_rs1800629 of *TNF-α* gene (p=0.040). This finding suggests that TNF-α -308 A/G polymorphism (SNP_rs1800629) may affect the level of IL-6 in AD patients with specific genotypes. This result can be explained by the fact that TNF-*α* is known to not only strongly induce the secretion of IL-6 but it may also modify IL-6 signaling and vice versa via a crosslink [39].

Conclusion: Striking finding of this study is the observation that genetic variants in rs1532278 SNP of CLU gene, GA heterozygous allele variant, and the serum levels of the CLU protein may be associated with an increased risk of Alzheimer's disease among Saudi Arabian subjects. Secondly, AD patients who were carriers of GG genotype for TNF-α SNP rs1800629 had significantly higher serum levels of IL-6, highlighting the possible association of this genotype with AD. Further studies using a larger sample size are needed to confirm the present finding.

Ethics approval and consent to participate:

The study was approved by the Ethical Committee, at the College of Science and the internal review board at the college of medicine, King Saud University (# KSU-SE-18-24) and all experimental methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from all participants and from a parent and/or legal guardian as vulnerable population (Alzheimer diseased participants) involved in the study.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

Author Contributions

SA and AAl were involved in conception of the study, selection of the appropriate methodology for the research and drafting the manuscript, suggested the research topic, revised the manuscript and provided support as deemed appropriate during the course of the study. TM was involved in conception, patient recruitment, and screening. TM, and R Al were involved in patient clinical assessment, the procurement of blood samples and revision of manuscript, NA analysed data, SS revised the manuscript and assisted in data analysis, SHH and A Alamro co-drafted the manuscript.

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