Genetic polymorphisms of ACE2 G8790A (rs2285666) and Apo E (rs 121918398) in Alzheimer's disease among cohort of Egyptian patients: Relation to disease susceptibility and severity

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Abstract

Background: Multiple family members have been reported to have Alzheimer's disease (AD), according to family and twin studies. Several ApoE genetic variations have been linked to AD because of their involvement in lipid dysregulation. It has been observed that the Angiotensin converting enzyme 2 (ACE2) gene influences the accumulation of β-amyloid peptide (Aβ).

Objectives: To assess the ACE2 G8790A (rs2285666) and Apo E (rs 121918398) SNPs among cohort of Egyptian AD patients and to explore their probable connection to the occurrence and severity of AD.

Patients and methods: This is a case-control research included 50 Egyptian patients with AD and 50 healthy participants. AD patients were assessed using mini-mental state examination. Genetic analysis for ACE2 (rs2285666) SNPs and Apo E (rs 121918398) SNPs was done using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR).

Results: The age, and gender distributions of the study and control groups were matched. Patients with AD were more prevalent in rural areas. In terms of ACE2 genotyping, a higher frequency of AA genotype and A allele in the AD group, indicating their significant association with increased AD risk. They were linked to the severity of the illness as well. Regarding Apo E genotyping, there were statistically frequent (**P**<0.001) E4 allele and the E3/E4 genotype among the AD group, and were considerably linked to more severe illness.

Conclusion: An ACE2 homozygous mutation with an A allele is thought to be a genetic risk factor and major predictor of the severity of AD. The incidence and severity of AD are significantly predicted by the APO E4 allele.

Keywords: Genetic polymorphisms; Alzheimer's disease; Angiotensin converting enzyme; Apolipoprotein E; Egypt.

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Introduction

Dementia is a significant decline in cognitive function that impairs an individual's capacity to perform daily activities. The most common type of dementia, Alzheimer's disease (AD), affects at least two thirds of those 65 years of age and older. The most significant factor is age, and growing older is the primary reason. Beginning at age 65, the prevalence of AD about doubles for every five years of age increase (**Kumar et al., 2024**). 6.9 million Americans aged 65 years and older are expected to develop Alzheimer's by 2024. This number could rise to 13.8 million by 2060. Of them, 73% are 75 years of age or older. In the US, women make up more than two thirds of Alzheimer's cases (**2024 Alzheimer's disease facts and figures, 2024**). In Upper Egypt, 1% of those 50 years of age and older had AD (**EL Tallawy et al., 2019**).

Alzheimer's disease is a neurodegenerative disorder that gradually impairs behavioral and cognitive abilities. Memory, comprehension, language, attention to detail, logical thinking, and judgment are some of these abilities. The characteristic of AD is neurodegeneration, which is caused by the progressive and slow death of neurons. The neurodegenerative process typically begins in the entorhinal cortex of the hippocampus region. It has been established that both early-onset and late-onset AD are influenced by genetic factors. AD is a complicated disease with several known risk factors (**Kumar et al., 2024**).

Extracellular accumulation of the β-amyloid peptide (Aβ) and intra-neuronal collection of tau protein (tau) characterize AD. Aβ is normally produced in the brain with a rapid turnover. It is the product of the splitting of amyloid precursor protein (APP) which is a large transmembrane precursor protein coded by the APP gene on chromosome 21 (**Roda et al., 2022**).

Aβ is not generated when APP is catabolized by other enzymatic activity (α- and Z-secretase complexes). The majority of Aβ's function is unknown. The MAPT gene, which codes for the phosphoprotein tau, is found on chromosome 17q21. Tau attaches to neurotubules through a domain consisting of three or four sequence repeats, and it controls the polymerization of these structures. There is an option to splice the second repetition (exon 10) differently. Thus, tau isoforms with three or four repetitions (3R or 4R) exist. Both isoforms build up and become hyperphosphorylated in AD (**Calderon-Garcidueñas and Duyckaerts, 2018**).

Studying specific genetic markers is crucial in understanding AD susceptibility, as well as delving into the neural substrates affected by these genetic variations, to better describe novel pathways contributing to AD susceptibility and severity (**Battaglia et al., 2024**; **Jászberényi et al., 2024**; **Tanaka et al., 2023**; **Battaglia et al., 2024**). Three genetically determined forms of the lipid transporter ApoE exist: APOE2, APOE3, and APOE4, which are coded by the corresponding alleles (ε2, ε3, ε4). A very important risk factor is APOE ε4. One APOE E4 allele increases the chance of AD development threefold, while both alleles increase the risk tenfold. In addition to ApoE4, genome wide association studies have shown genetic variants linked to late onset AD (LOAD) at over 20 loci (**Shih et al., 2018**). By generating and increasing Aβ accumulation, aggregation, and deposition in the brain, APOE4 probably raises the risk of AD (**Zhao et al., 2018**). It is well recognized that APOE serves as a lipid transport protein, and that dysregulation of lipids is a major characteristic of a number of neurological disorders, including AD. The APOE4 genotype has been associated with a higher risk of disease due to molecular disturbances in lipid metabolism. It has been reported that APOE4 disturbed the cellular lipidome, leading to an increase in unsaturation of fatty acids and an

accumulation of intracellular lipid droplets in human astrocytes (**Sienski et al., 2021**).

Angiotensin converting enzyme (ACE) is well known for its two functions, which are crucial for blood pressure regulation: it breaks down active bradykinin (BK) and converts inactive Ang I to active Ang II (**Van De Beek et al., 2019**). In humans, the ACE and ACE2 genes are found on chromosomes 17q23 and Xp22, respectively. Alzheimer's disease patients did not receive enough research on ACE2, and one study found that AD patients had downregulated ACE2 in their basal nucleus, hippocampus, entorhinal cortex, middle frontal gyrus, visual cortex, and amygdala (**Cui et al., 2021**). When combined with evidence from both in vitro and in vivo experiments showing that ACE cleaves and clears Aβ in an activity-dependent manner, these results imply that people with normal functioning ACE & ACE2 genes increasing ACE protein, and potentially ACE activity, may be better able to clear accumulating Aβ aggregates and less likely to develop AD (**Kauwe et al., 2014**; **Bekiet et al., 2023**).

Therefore, in the current research, we evaluated the ACE2 G8790A (rs2285666) and Apo E (rs 121918398) SNPs among cohort of Egyptian AD patients to explore the genetic profile of these two SNPs and their probable connection to the occurrence and severity of AD.

Patients and methods

Study design: In this case-control study, patients were either followed up in Outpatient Clinics or were recruited from the Neurology and Psychiatry Department of Qena University Hospital in Egypt, during the period between January 1st, 2020, and January 1st, 2021.

Ethical consideration: The approval was provided by institutional review board (IRB) of Faculty of Medicine, Qena, South Valley University, (Ethical approval code: SVU-MED-NAP020-4-24-1-802). All the participants provided written consent or their caregivers before participation in the study.

Participants: The study included 50 Alzheimer's patients of 60 years old or older of both genders and accepted to sign the written informed consent as study group. Fifty healthy age, sex participants irrelevant to the included patients and have no family history of AD, were included as control category. Patients with altered conscious level, mental retardation, or dementia rather than Alzheimer's disease were excluded. Diagnosis of Alzheimer's disease was according to Diagnostic and Statistical Manual, fifth edition (DSM-V) (**Nina-Estrella, 2017**). Advanced medical imaging, such as brain magnetic resonance imaging (MRI) or computed tomography (CT), is utilized to rule out other cerebral pathologies or dementia subtypes. Additionally, it might forecast the transition from moderate cognitive impairment in the prodromal stages to Alzheimer's disease (**Zaki et al., 2022**).

Methodology: Patients registry was reviewed for demographics (age, sex, BMI), associated medical disorders (hypertension, diabetes, chronic liver disease, others) and the findings in the neurologic examination. All patients were assessed using the following scores:

Mini-Mental state examination (**Folstein et al., 1975**): This is essentially intended for regular and serial use and has proven validity and reliability. It is a cognitive screening tool that offers a rapid, objective assessment of cognitive function. It can be used to demonstrate cognitive impairment, assess the level of severity of the condition, and monitor how cognitive decline develops over time. The MMSE assesses a variety of cognitive domains, such as verbal memory, calculation, visual construction, orientation, repetition, and language. The test taker's score is as simple as counting the correct answers. Per interpretations, a score of 24 or higher (out of 30) indicates normal cognition. A score outside of this range may indicate mild

(19–23 points), moderate (10–18 points), or severe (9 points) cognitive impairment.

Genetic analysis: Genetic analysis for ACE2 (rs2285666) SNP and Apo E (rs 121918398) SNP was done using restricted fragment length polymorphism polymerase chain reaction (RFLD PCR). The primer sequences, the PCR condition which used, and the amplification products size and alleles sizes, all were described in the (**Table.1**) (**Nahid et al., 2022**; **Qian et al., 2015**; **Ahmed et al., 2022**). The restriction enzyme (Alu I) (purchased from New England Biolabs in USA – Catalog no R01395) was used for digestion of the PCR products of ACE2 G8790A (rs2285666) SNP (**Fig.1**). The restriction enzyme (Hhal I) (purchased from the same company with catalog no R01375) was used for digestion of the PCR products of Apo E (rs 121918398) SNP (**Fig.2 & 3**).

Table 1. Polymerase chain reaction data for genotyping of ACE2 (rs2285666) and Apo E (rs 121918398) single nucleotide polymorphisms

Single nucleotide Polymorphism	PCR Primers (5′ - -3′)	PCR amplifications products Size (bp)	Restriction enzyme	Alleles size (bp)	PCR Conditions
ACE2 (G/A) (rs2285666)	**F** AAACCACTGA AATGACTTACT TACTG **R** GCCTCACTGTC CTATGACTTTA T	817	Alu I restriction enzyme	G = 817 bp A = 589 and 228 bp GA = 817, 589, and 228 bp	Heating the mixture at 94°C for 5 min and underwent 35 cycles of 50 seconds at 94°C, 50 seconds at 52°C, and 50 seconds at 72°C in a thermal cycler. At 72°C for 10 minutes, the reaction was terminated.
Apo E gene (E /E) (rs121918398) three alleles and six genotypes	**F** ACAGAA TTC GCCCCG GCC TGGTAC AC **R** TAAGCT TGG CACGGC TGT CCA AGG A-	244	Hha I restriction endonuclease.	(E3/4 (91, 72 and 48 bp),E3/3 (91 and 48 bp) and E4/4 (72 and 48 bp) genotypes).	The mixture was heated 94°C for 5 min and underwent 35 cycles of denaturation at 95°C for 45 sec; annealing at 65°C for 45 sec and extension at 72°C for 1 min; and a final extension at 72°C for 10 min.

Statistical analysis

The analysis was performed using SPSS (version 21, Chicago, IL, USA). Number and percentages were used to represent qualitative variables; while mean (±SD) represented the normally distributed quantitative parametric variables and median (min-max) described quantitative non-parametric data. Chi-square test: compared qualitative variables. Hardy-Weinberg equilibrium: to test prevalence of studied genes among AD in comparison to control group. Student t-test: compared normally distributed quantitative variables between 2 categories. Analysis of variance: compared normally distributed quantitative variables between >2 categories. P-value < 0.05 was deemed significant.

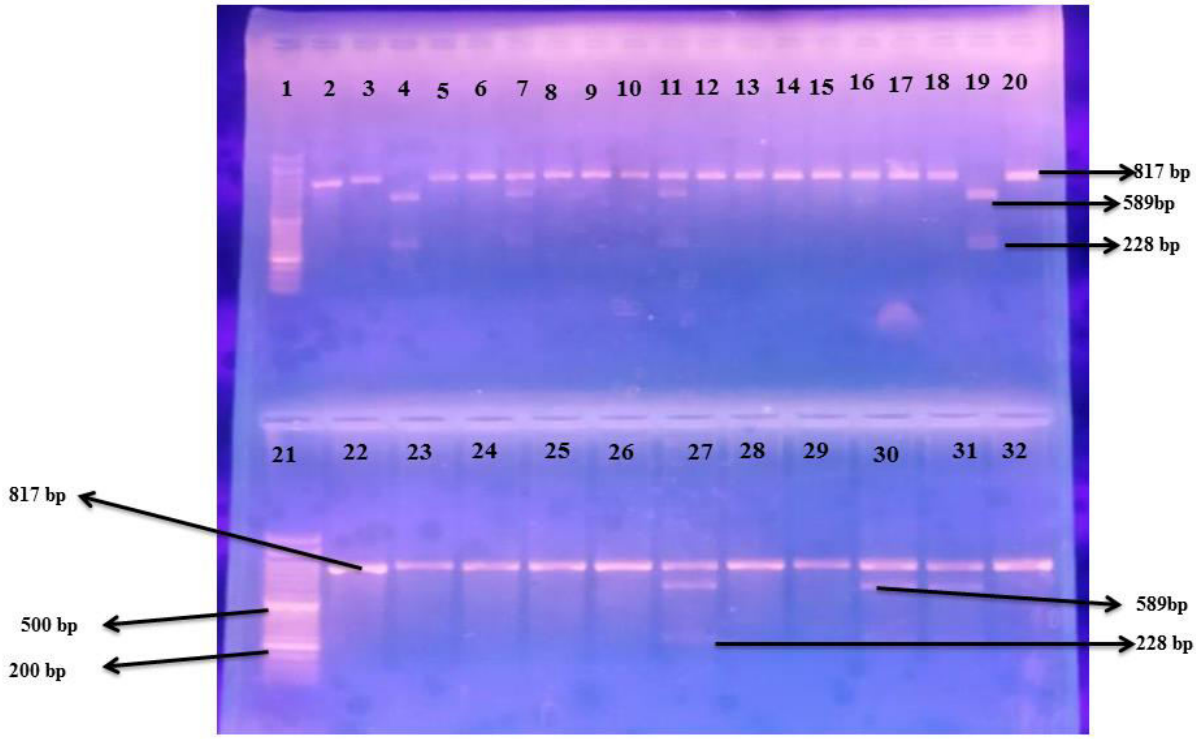

Fig.1. Gel electrophoresis of ACE2 (rs2285666) SNP using PCR-RFLP method. Numbers refer to lanes. Lane 1& 21 show 50 bp DNA ladder; Lanes (2,3,5,6,8-10,12-18,20,22-26,28,29,& 32) represents represent wild genotypes (GG) with 817 bp band; Lanes 7, 11, 27, 30, 31 are heterozygous mutant (GA) genotypes with 817, 589, 228 bp bands; Lanes 4 & 19 are homozygous mutant (AA) genotypes with 589& 228 bp bands.

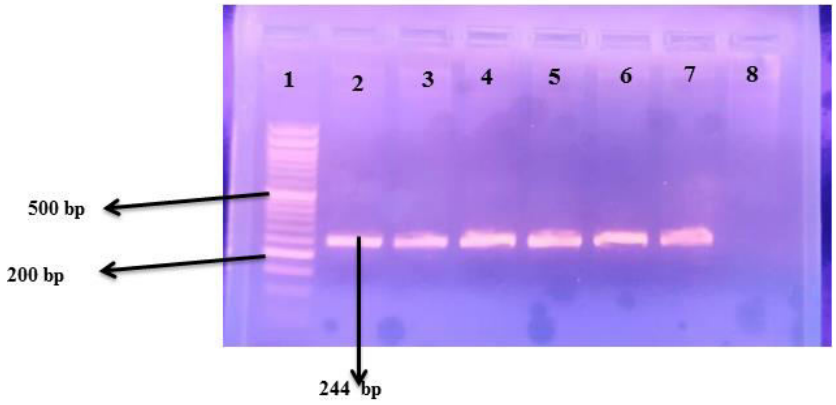

Fig.2. Gel electrophoresis of the PCR products of APOE gene using PCR-RFLP method. Numbers refer to lanes. Lane 1 shows 50 bp DNA ladder; Lanes (2-7) showed amplified DNA segments (undigested PCR product) of length 244 bp; Lanes 8 represents no template control (NTC).

Results

The study included 50 Alzheimer's disease with mean age 73.2 ± 6.4 years and female predominance with mean disease duration 7.2 ± 1.2 years. Age and sex in the study and control groups were matched. The majority of cases were from rural areas compared to the control group (80% vs. 60%; p= 0.029), (**Table. 2**).

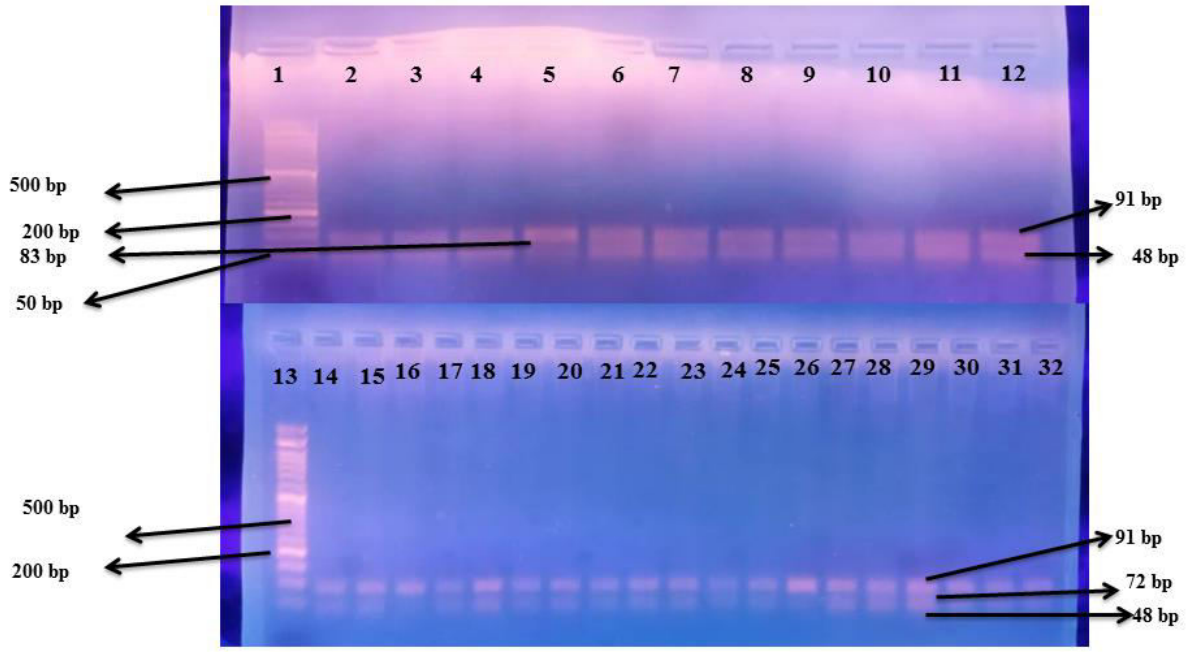

Fig.3. Gel electrophoresis of APOE genotypes using PCR-RFLP method. Numbers refer to lanes. Lane 1& 13 show 50 bp DNA ladder; Lanes (2,3,4,6-12, 14,15, 17-25, 30-32) represents represent ε 3/ ε 3 genotype with 91, 48 bp bands; Lanes (5, 16, 26) are ε2/2 genotypes with 91, 83 bp bands; Lanes (27-29) are ε 3/ ε 4 genotypes with 91, 72, 48 bp bands.

Table 2. Demographic and baseline characteristics of the included participants

Variables	Study category (n= 50)	Control category (n= 50)	P value
Age (years) mean ± SD	73.2 ± 6.4	70.2 ± 4.3	0.2
Disease duration (years) mean ± SD	7.2 ± 1.2		
Sex No. (%) - Male - Female	 17 (34%) 33 (66%)	 15 (30%) 35 (70%)	0.09
Residency No. (%) - Urban - Rural	 12 (24%) 38 (76%)	 20 (40%) 30 (60%)	**0.04**

Individuals were categorized into three groups based on their MMSE score: mild (23 patients), moderate (21 patients), and severe (n = 6) disease. The three groups did not significantly differ in terms of age, age at diagnosis, or dominant hand. On the other hand, there were notable differences in the duration of the disease among the three groups, with severe disease being linked to a longer illness duration (**P=** 0.03). There was a statistically significant higher difference in the percentage of male patients with mild disease compared to the higher number of female patients in the moderate and severe category (**P=** 0.03). A higher percentage of patients with moderate or severe diseases came from rural areas, whereas a higher percentage of patients with mild diseases came from urban areas, with a significant difference (**P=** 0.001). Poor concentrations as well as memory problems were seen in all of the patients. Patients with moderate or severe disease, however, reported difficulty accomplishing everyday duties, disorientation regarding time and location,

and resignation from job. Patients with serious illnesses also complained of fluctuations in mood, poor decision-making skills, and difficulties with language (**P**< 0.001), (**Table.3**).

Table 3. Comparison between different Alzheimer's disease severity groups according to MMSE as regards demographics and complaints

Variables	Mild (n= 23)	Moderate (n= 21)	Severe (n= 6)	P value
Age (years) mean ± SD	73.78 ± 6.7	75.7 ± 10.5	78.33 ± 8	0.54
Disease duration (years) mean ± SD	7.1 ± 2.2	8.2 ± 2.3	11.1 ± 1.2	***0.03***
Age No. (%) - >65 - <65	 20 (87%) 3 (13%)	 17 (81%) 4 (19%)	 5 (83.3%) 1 (16.7%)	0.86
Sex No. (%) - Male - Female	 13 (56.5%) 10 (43.5%)	 4 (19.1%) 17 (80.9%)	 0 (0%) 6 (100%)	**0.03**
Residency No. (%) - Urban - Rural	 10 (43.5%) 13 (56.5%)	 2 (9.5%) 19 (90.5%)	 0 (0%) 6 (100%)	**0.001**
Complaints				
Difficulty in remembering	23 (100%)	21 (100%)	6 (100%)	0.85
Poor concentration	23 (100%)	21 (100%)	6 (100%)	0.85
Problems in finishing daily tasks	0 (0%)	21 (100%)	6 (100%)	**<0.001**
Confusion with time and place	0 (0%)	21 (100%)	6 (100%)	**<0.001**
Language problems	0 (0%)	0 (0%)	6 (100%)	**<0.001**
Poor judgment in decision	0 (0%)	0 (0%)	6 (100%)	**<0.001**
Mood changes	0 (0%)	0 (0%)	6 (100%)	**<0.001**
Withdrawal from the work	0 (0%)	21 (100%)	6 (100%)	**<0.001**

Level of significance < 0.05.

Genetic profiling for ACE2 (rs2285666) SNPs showed statistically significant variations in the genotype and allele distribution, with the AA genotype showing a higher frequency in the AD group compared to the controls (30% vs. 6%) and A alleles seemed to be associated with an increased incidence of AD (OR: 2.8; **P=** 0.003). Although the GG genotype seemed to provide protection (60% vs. 74%) with the G allele occurring more frequently in the control group (65% vs. 84%; **P=** 0.002) (**Table. 4, Fig.4**).

Genetic profiling for Apolipoprotein E (rs 121918398) SNP showed statistically significant variations in the genotype and allele distributions showing a higher frequency of the E3/E4 genotype in the AD group (44% vs. 10%) but among the AD group, E2/E2 was more common (6% vs. 20%). Additionally, the control group's E3/E3 genotype frequency was noticeably greater (50% vs. 70%), **P<** 0.001. E4 allele appeared to carry risk for AD (Alzheimer's disease vs. control: 22% vs. 5%; OR: 5.3; **P=** 0.001) while E2 and E3 appeared to be protective with statistically significant differences (**P<** 0.001) (**Table. 4, Fig.5**).

Table 4. Genetic profile of ACE2 (rs2285666) and Apo E (rs 121918398) single nucleotide polymorphisms among the studied categories

Variables	Study category (n= 50) No. (%)	Control category (n= 50) No. (%)	P value
ACE2 genotypes - GG - GA - AA	 30 (60%) 5 (10%) 15 (30%)	 37 (74%) 10 (20%) 3 (6%)	**0.006**
ACE2 alleles - G - A	 65 (65%) 35 (35%)	 84 (84%) 16 (16%)	**0.002**
Apo E genotypes - E2/E2 - E3/E3 - E3/E4	 3 (6%) 25 (50%) 22 (44%)	 10 (20%) 35 (70%) 5 (10%)	**<0.001**
Apo E alleles - E2 - E3 - E4	 6 (6%) 72 (72%) 22 (22%)	 20 (20%) 75 (75%) 5 (5%)	**<0.001**
	95% confidence interval **Lower: Upper**	**Odds ratio**	**P value**
ACE 2 genotype: - AA vs. GG - GA vs GG - AA vs. GA	 0.89: 0.3 0.29: 22.58 8.5: 22.23	 4.48 1.7 2.5	 **0.04** 0.48 0.4
ACE 2 alleles (A)	1.4: 5.5	2.8	**0.003**
Apo E genotypes - E3/E4 vs. E3/E3 - E3/E4 vs. E2/E2 - E3/E3 vs. E2/E2	 1.5: 5.13 0.9: 111.15 86.2: 27.3	 13.1 16.6 5.07	 **0.019** **0.001** 0.06
Apo E alleles (E4)	1.9: 14.8	5.3	**0.001**

Chi square test for categorical data; Level of significance < 0.05

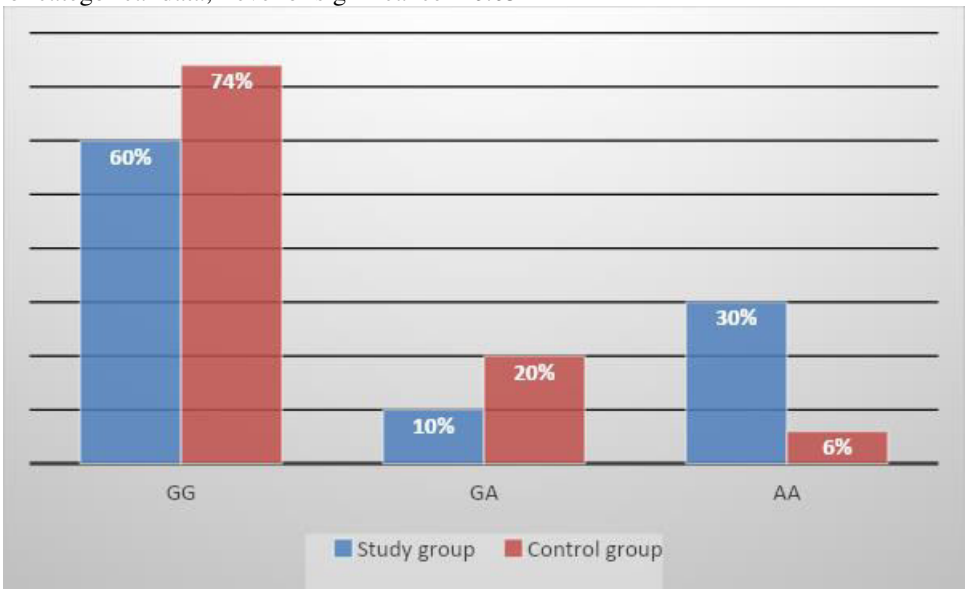

Fig.4. Frequencies of ACE2 (rs2285666) genotypes between both groups

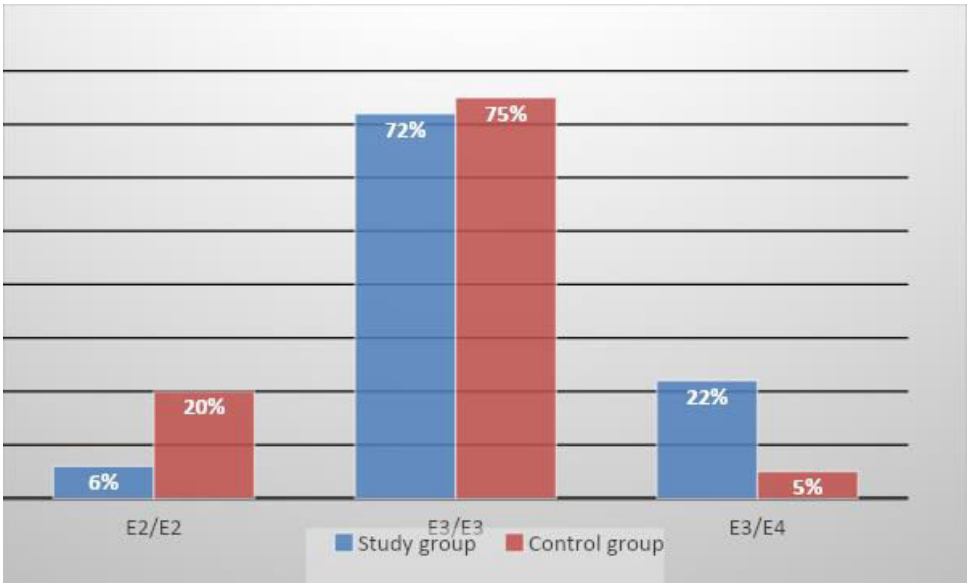

Fig.5. Frequencies of different Apo E (rs 121918398) genotypes between both groups

With relation to ACE2 genotypes, there were statistically significant higher frequency of the AA genotype in the group with severe disease and the lowest frequency in the group with mild disease, and higher frequency of the GG mutation in the group with mild disease and the lowest frequency in the group with severe disease (**P=** 0.004). A allele had the highest frequency among severe disease group and G allele was the commonest among mild disease group with significant variance (**P=** 0.009). When it came to Apo E genotypes, the groups with severe and mild diseases showed the highest and lowest frequencies of E3/E4, respectively, while the groups with mild diseases showed greater frequencies of E3/E3 and E2/E2 genotypes with statistically significant differences (**P<** 0.001). The most prevalent allele among those with severe disease was E4, and there was a statistically significant difference (**P=** 0.01) (**Table. 5**).

Table 5. Comparison between different Alzheimer's disease degrees in terms of ACE2 (rs2285666) and Apo E (rs 121918398) genes and alleles

Variables	**Mild (n= 23) No. (%)**	**Moderate (n= 21) No. (%)**	**Severe (n= 6) No. (%)**	**P value**
ACE2 genotypes				
- GG	14 (60.9%)	15 (71.4%)	1 (16.7%)	**0.004**
- AG	5 (21.7%)	0 (0%)	0 (0%)	
- AA	4 (17.4%)	6 (28.6%)	5 (83.3%)	
ACE2 alleles				
- G	33 (71.7%)	30 (71.4%)	2 (16.7%)	**0.009**
- A	13 (28.3%)	12 (28.6%)	10 (83.3%)	
Apo E genotypes				
- E2/E2	2 (8.7%)	1 (4.8%)	0 (0%)	**<0.001**
- E3/E3	18 (78.3%)	7 (33.3%)	0 (0%)	
- E3/E4	3 (13%)	13 (61.9%)	6 (100%)	
Apo E alleles				
- E2	4 (8.7%)	2 (4.8%)	0 (0%)	**0.01**

- E3	39 (84.8%)	27 (64.3%)	6 (50%)	
- E4	3 (6.5%)	13 (30.9%)	6 (50%)	

Chi square test for categorical data; Fisher exact test for categorical data with frequencies less than 5; Level of significance < 0.05

All patients and healthy participants were divided according to E4 carrier state. As regard ACE2 genotype, in E4 non-carriers, AA genotype had higher incidence among AD patients than normal volunteers with statistically significant difference. Also, A allele expression was higher among AD patients than normal volunteers with statistically significant difference in E4 carrier state. While in E4 carrier state, there were no statistically significant differences as regard ACE 2 (**Table. 6**).

Table 6. Effect of E4 of Apo E (rs 121918398) carrier status on the ACE2 (rs2285666) genotypes and alelles in AD versus control category:

Variables	E4 non- carriers			E4 carriers		
	Study group (n= 28)	Control group (n= 45)	P value	Study group (n= 22)	Control group (n= 5)	P value
ACE2 genotypes						
- GG	17 (60.7%)	35 (77.8%)		13 (59.1%)	2 (40%)	
- GA	4 (14.3%)	10 (22.2%)	**0.002**	1 (4.5%)	0 (0%)	0.59
- AA	7 (25%)	0 (0%)		8 (36.4%)	3 (60%)	
ACE2 alleles						
- G	38 (67.9%)	80 (88.9%)	**0.002**	27 (61.4%)	4 (40%)	0.22
- A	18 (32.1%)	10 (11.1%)		17 (38.6%)	6 (60%)	

Discussion

The aim of this study was to examine the relationship between the genetic polymorphisms of ACE 2 and Apo E and the risk of AD, as well as the severity of the disease. To achieve this goal, we conducted this study on 50 Alzheimer's disease patients who were compared to 50 controls. We observed significant associations of APOE E4 allele, E3/E4 genotype, and the AA genotype of ACE2 (rs2285666) SNPs with raised risk and severity of AD among cohort of Egyptian patients.

The majority of patients in the AD group, according to the current study, were from rural areas (76%), with a statistically significant difference. The majority of AD patients were reported to dwell in rural areas by Ramadan et al. (**Ramadan et al., 2019**) & Abner et al. (**Abner et al., 2016**), which is consistent with the findings of the current study. However, Deng et al. (**Deng et al., 2015**) could not find any appreciable differences in residency between the AD and control groups.

Regarding the mean age of the patients with regard to the severity of dementia according to MMSE, there were no notable differences. Wattmo et al. (**Wattmo et al., 2016**) & Henneges et al. (**Henneges et al., 2016**) did not find remarkable differences between the AD stages as regard age and age at presentation. In contrary, Ferretti et al. (**Ferretti et al., 2020**) found that age is significant predictor for AD severity.

In the present study, disease duration was longer among patients with severe dementia than moderate and mild disease groups. Riecher-Rossler (**Riecher-Rossler, 2017**) demonstrated statistically significant longer disease duration among severe disease group with inverse correlation between disease duration and disease severity scores (MMSE or DSRS). Ferrrati et al. (**Ferretti et al., 2020**) proposed that disease duration is a highly significant predictor for disease severity.

On the other hand, Henneges et al. (**Henneges et al., 2016**) could not find significant correlation between disease duration and disease severity.

The majority of patients in the current study were male and had milddementia, whereas the majority of female patients had moderate dementia. Luy et al. (**Luy et al., 2015**) proposed that longer life expectancy of female patients exposes them to severer forms of AD.

In the current research; ADL, PSQA, and DSRS showed statistically significant differences with poor scores among patients with moderate and severe disease group. Wattmo et al. (**Wattmo et al., 2016**) showed lower DAD, ADL, and higher DSRS and PSQA scores among moderate to severe disease groups than mild disease groups. Also, Kato et al. (**Kato-Narita et al., 2011**) reported statistically significant lower MMSE values among patients with more severe diseases (**P<** 0.001). He reported significantly lower DAD score among moderate disease group.

To the best of our knowledge, this is the first research to include the genetic polymorphisms of ACE2 G8790A (rs2285666) as a predictor for AD. The findings of this research displayed that G allele is protective against AD with lower frequencies of homozygous GG and GA and higher frequency of AA genotype among AD groups. Additionally, the AD group have greater frequency of the A allele compared to the control group. Cui et al. (**Cui et al., 2021**) compared ACE2 expression in 12 regions of the brain between healthy and AD patients. Machine-learning Optimal-filtering Detection-procedure (MOD) analysis revealed that AD patients experienced down regulation of ACE2 in the hippocampus, basal nucleus, visual cortex, entorhinal cortex, middle frontal gyrus, and amygdala in AD patients. Genetic mutation of ACE2 (G8790A) was reported to be associated with some other diseases. A considerable correlation between the occurrence of cardiovascular disease and the ACE2 genetic mutation was identified by Yousif et al. (**Yousif et al., 2022**). Strong associations were shown between ACE2 (G8790A) and type 2 diabetes, dyslipidemia, and the frequency of cardiovascular events by Patel et al. (**Patel et al., 2012**) and Liu et al. (**Liu et al., 2018**). ACE2 (G8790A) was linked to a moderate risk of diastolic blood pressure (DBP ≥ 80 mmHg) and a high risk of systolic blood pressure (SBP ≥ 130 mmHg), according to Wang et al.'s findings (**Wang et al., 2014**). Instead of focusing on G8790A, Deng et al. (**Deng et al., 2015**) examined other ACE2 gene sequences and found no statistically significant variations in the rs710446 or rs4343 sequences between the AD and control groups. According to Xin et al. (**Xin et al., 2021**), there is a minor but substantial correlation between North Europeans' risk of AD and the ACE2 sequence rs1799752 polymorphism. Although, polymorphisms in the ACE2 sequence at positions 4343, 4291, and 4309 are not expected to be major contributors to the development of AD. According to Kehoe et al. (**Kehoe et al., 2016**), ACE-2 activity was significantly lower in AD patients compared to age-matched controls, and had a negative correlation with Aβ levels and phosphorylated tau (p-tau) pathology.

Based on Apo E genotyping, the current study found statistically significant differences in Apo E genetic polymorphisms between the AD and control groups. Specifically, the AD group had a higher frequency of the E3/E4 genotype, while the control group had a higher frequency of the E2/E2 and E3/E3 genotypes, which demonstrated a protective role. E4 allele frequency was also greater in the AD group compared to the control group. Ramadan et al. (**Ramadan et al., 2019**) & Durmaz et al. (**Durmaz et al., 2019**) demonstrated that genotyping analysis of Apo-E displayed that the risk of AD was strongly correlated

with Apo E3/E4 with adjusted odds ratio of 4.35 (CI=1.62–11.66) and **P=** 0.003. Xu et al. (**Xu et al., 2021**) found that Apo E4 allele had higher frequency among AD patients than control group. Similar results were obtained in previous studies (**Lucatelli et al., 2011**; **Yildiz et al., 2015**; **Saddiki et al., 2020**).

The relatively small sample size, single ethnicity, limited genetic variants examined, the lack of analysis of some demographic data of the participants such as assessments of education and socioeconomic status, and lack of functional validation were the main study's limitations. Additionally lack to account for potential confounding variables, such as cardiovascular risk factors, which could independently influence the risk and severity of Alzheimer's disease.

Conclusion

This preliminary study identified associations suggesting the ACE2 AA genotype and APOE E4 allele/E3/E4 genotype may increase Alzheimer's susceptibility and severity in this Egyptian population. However, larger longitudinal studies controlling for confounders and incorporating functional data are needed to validate these variants as robust predictive biomarkers before making definitive claims. These findings could be helpful in screening members of AD families and also can expect the AD course and severity among patients diagnosed with AD.

List of abbreviations

- β-amyloid peptide (Aβ)
- Alzheimer's disease (AD)
- Angiotensin converting enzyme (ACE)
- Diagnostic and Statistical Manual, fifth edition (DSM-V)
- Mini-mental state examination (MMSE)
- Activity of daily life (ADL)
- Patient-specific quality assurance (PSQA)
- Machine-learning Optimal-filtering Detection-procedure (MOD)

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