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Association of *APOE* Polymorphisms and Stressful Life Events with Dementia in a Pakistani Population

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Abstract

Dementia is a major public health problem worldwide. Alzheimer's disease (AD) is a major form of dementia and the *APOE*4* allele is an established genetic risk factor for AD. Similarly, stressful life events are also associated with dementia. The objective of this study was to examine the association of *APOE*4* and stressful life events with dementia in a Pakistani sample, which to our knowledge has not been reported previously. We also tested for an interaction between stressful life events and *APOE*4* on dementia risk. A total of 176 subjects (61 cases and 115 controls) were recruited. All cases and healthy controls were interviewed to assess cognition, co-morbidities, history of stressful life events and demographics. Blood genotyping for the *APOE* polymorphism (E2/E3/E4) was performed. *APOE*4* and stressful life events were each independently and significantly associated with the risk of dementia (*APOE*4: P*=0.00697; stressful life events: *P*=5.29E-09). However, we did not find a significant interaction between *APOE*4* carrier status and stressful life events on risk of dementia (*P*=0.677). Although the sample size of this study was small, the established association of *APOE*4* with dementia was confirmed the first time in a Pakistani sample. Furthermore, stressful life events were also found to be significantly associated with dementia in this population.

Keywords

dementia; Pakistan; stressful life events; *APOE*

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1. Introduction

Dementia is a syndrome associated with aging, involving decline in multiple cognitive domains, of sufficient severity to interfere significantly with daily living. The most common form of dementia is Alzheimer's disease (AD). AD is a neurodegenerative condition that results in deterioration of memory and other cognitive functions and ultimately leads to death [1]. Approximately 35 million people worldwide are affected with dementia and this number is expected to reach 65 million in 2030 and 113 million by 2050 [2]. Dementia rates are expected to increase in the least developed and developing countries, as estimates suggest that 71% of the expected 81.1 million dementia cases will be in the developing world by 2040 [3].

The most established genetic risk factor for dementia is the *E*4* allele of apolipoprotein E (apoE, protein; *APOE*, gene). The association of the *APOE* polymorphism with AD has been well established, where the *APOE*4* allele is associated with increased risk of AD and inheritance of *APOE*2* allele confers protection [4, 5]. The risk attributed to *APOE*4* is gene-dosage related and shows variation in different ethnic groups. Among European-derived populations, the odds ratios (ORs) for one and two copies of *APOE*4* alleles are about 3 and 15, respectively [6]. However, the strength of the association is weaker in African-Americans and Hispanics [7]. In order to lower disease risk or delay onset of dementia, more emphasis is being placed on psychosocial risk factors that make disease onset and progression more likely [8]. A number of studies suggest stressful life events may have adverse effect on cognitive function, especially in old age [9]. The hypothalamic-pituitary adrenal (HPA) axis is activated in response to stress and secretes the stress hormone cortisol. Given its ability to cross blood brain barrier, it binds to receptors in various regions of the brain known to be involved in memory and learning such as the hippocampus, amygdala, and frontal lobes and consequently influences cognitive function [10].

There are no published reports that show association of *APOE* with the risk of developing dementia in Pakistani population, or the impact of negative life events on developing dementia. We sought to confirm the expected *APOE* association for the first time in a Pakistani sample. We also hypothesized that there is an association between previous stressful events and risk of dementia. The present case-control study aims to show the (1) association of *APOE*4* allele with risk of dementia in Pakistani population, (2) exposure to stressful life events and risk of dementia, and (3) combined interaction effect on dementia of stressful life events and *APOE*4* status.

2. Materials and methods

2.1. Study sample and diagnostic procedures

This case-control study was approved by Board of Advanced Studies and Ethical Committee of the University of the Punjab, and by the Institutional Review Board of the University of Pittsburgh. All controls gave written informed consent to participate in this study. All patients provided either written or verbal informed consent (if unable to write due to educational background) or assent with consent provided by a family member.

A total of 176 subjects were included in this study (61 dementia patients and 115 controls). Dementia patients were recruited from various clinical settings of Lahore (Alzheimer's Pakistan Day Care Center for Alzheimer's and related dementias; Psychiatry department of the Services Institute of Medical Sciences; out-patient service of the Punjab Institute of Mental Health) and Rawalpindi (out-patient service of the Armed Force Institute of Mental Health) from February 2011 to September 2012. As there is no specialized center/facility in Pakistan for the diagnosis of dementia based upon rigorous research diagnostic criteria, primary dementia diagnosis was formulated by psychiatrists/psychologists/neurophysicians based upon clinical presentation and information from patients and families/caregivers. However, physicians adhere to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM- IV) criteria for dementia screening. Depending on cultural, educational and socioeconomic demographics, only few participants were able to be evaluated by MRI/CT scans. Cases that had been diagnosed and evaluated by clinicians were again interviewed at the same time by study staff for personal history and clinical history. Accompanying family members, available for all cases, were also asked for verification or correction of information provided by patients.

Control subjects were recruited from the same communities as cases and fulfilled inclusion criteria (described below). They consisted of hospital employees, friends and colleagues of study staff, attendants of other patients, manual laborers. An attempt was made to match controls to cases according to literacy and educational background. Control subjects were evaluated by the study investigator.

2.2. Inclusion criteria

Inclusion criteria for the case group consisted of a dementia diagnosis documented by the patient's physician. Control group inclusion criteria included subjects with no clinical history of dementia and cognitively normal status based on Mini Mental State Examination (MMSE) (see below).

2.3. Cognitive assessment

An Urdu translation of the standard MMSE was administered, with some modifications, to assess severity of cognitive impairment, but was not used for dementia diagnosis. Considering socio-cultural differences and the low education status of the population under study, the following MMSE items were omitted: serial seven subtractions, spelling backwards, sentence writing and copy of drawing. This gave a total possible score of 23 instead of 30, where higher score indicated better cognition.

2.4. Stressful life events assessment

Subjects were assessed for stressful life events with a set of standardized questionnaires. All interviews were conducted in person and the information was verified by a family member (siblings/children) for all patients. The stressful life events items were used as described and validated in Tsolaki *et al*. [11]. A subset of items was selected for our study with cultural relevancy in mind. In Pakistan, there is a very strong family system and families generally live together. Adult children share the parents' home and generally know detailed information about their parent's life and vice versa. Grandparents also tend to live in the

same house. All the life events selected for our study were considered “major” and siblings and/or adult children were able to verify the information. All subjects (cases and controls) were asked whether they underwent any of the stressful events (death of spouse; death of son, daughter or grandchild; death of sibling or other beloved ones; stroke; traumatic brain injury; other surgical intervention-serious health problems; illness of spouse, parents, son, daughter or grandparents; problems in family-stressful situation; financial difficulties-professional problems) in their lifetime and the answers were coded in “yes” or “no” format.

2.5. Co-variates

Co-variates of interest included socio-demographic variables: age, sex, marital status, years of formal education, and income groups. Clinical variables were used to characterize the sample, including presence of heart disease, stroke, diabetes, hypertension, obesity, depression, and head injury from medical record review (cases) or self-report (some cases and all controls).. Subjects were also assessed for smoking, and alcohol/drug abuse.

2.6. Blood sample collection and screening

Six ml of blood was drawn from study participants by venipuncture, out of which 3ml was taken in plain tube for screening purposes and 3ml was stored in EDTA vial for later genetic analysis. Isolated serum from the 3 ml blood stored in plain tube was screened for Human Immunodeficiency virus (HIV), Hepatitis C virus (HCV), Hepatitis B virus surface antigen (HbsAg) using one step device (Acu-®check) and following the manufacturer’s instructions. Syphilis screening was done macroscopically by Randox reagin test (non treponemal) that detects reagin antibodies. Out of 203 participants 12.31% tested positive for HCV and 0.98% for HbsAg. The HCV^+ and $HbsAg^+$ samples were excluded from subsequent analysis. However none of the participants tested positive for HIV and Syphilis.

2.7. *APOE* genotyping

DNA was isolated from blood using the iPrep™ PureLink® gDNA Blood kit protocol (Invitrogen). After the extraction, DNA was quantified using the Quant-iT™ PicoGreen® dsDNA assay kit (Life Technologies, NY, USA). *APOE* genotypes were determined using TaqMan SNP Genotyping Assays (Applied Bio Systems, Foster City, CA). The assay identification numbers for SNPs rs7412 and rs429358 were C__904973_10 and C__3084793_20, respectively. The TaqMan analysis was performed after all samples were placed on 384 well plates. Every plate had a mixture of cases and controls, and ten percent of the samples were repeated in order to assess error rate. Assays were run following the Applied BioSystems TaqMan’s protocol. Plates were read and analyzed on ABI Prism 7900HT Sequence Detection Systems.

2.8. Statistical analysis

Sex and age adjusted logistic regression analysis was performed to test for associations of *APOE* polymorphisms with dementia risk using R-statistical software. The effect of stressful life events on dementia status was analyzed using linear logistic regression model after adjusting for three demographic variables (i.e. age, gender and income levels). The ORs for these events were calculated to assess the difference between groups in exposure. In order to

determine whether dementia patients experienced more total stressful events than non-demented subjects, generalized linear regression with log link function appropriate for count data, adjusted for age, gender and income level was used. Given multiple comparisons a more conservative *P*-value less than 0.01 was considered statistically significant.

3. Results

The demographic and clinical variables of dementia cases and controls are given in Table 1. Cases were older and there were more women than controls. Cases were not different than controls on marital status and educational background. The majority of the cases (72%) and controls (83%) had less than 8 years of formal education and only 28% of cases and 17% of controls had more than 8 years of formal education. However, they differed on income where a greater percent of controls fell into the lower income category. Cases and controls were also different on a number of comorbidities, with cases having higher proportions of heart diseases, hypertension, stroke, diabetes and depression, and controls showing a higher rate of smoking than cases.

Comparison of *APOE* genotype and allele frequencies between cases and controls is presented in Table 2. The genotype distribution was in Hardy-Weinberg equilibrium (*P*=0.63). The frequency of the *APOE*4*allele was more than double in cases compared to controls (20% vs. 9%). The sex- and age- adjusted OR for *APOE*4* carriers was 2.81 (95% CI: 1.33–6.03; *P*=0.00697). Only one of 56 cases was homozygous for the E4/E4 genotype and that subject was 70 years of age with disease duration of three years. Therefore, the observed significant association of *APOE*4* with dementia is mainly attributed to the E3*/E4* subgroup, and thus a dose dependent increase in risk of dementia could not be explored. No member of the control group was E4/E4 homozygote. In contrast, no appearance of the *APOE*2* allele was observed in cases compared to its 5% frequency in controls, which is only suggestive of its protective effect against dementia. Given our small sample size no subject with the relatively rare E2/E2 genotype was observed. We also investigated the distribution of *APOE* genotypes among different age categories, but found no considerable difference (data not shown), likely due to our small sample size.

Table 3 shows the frequency of each stressful life event in demented patients and controls. The most frequently reported event in cases was family problems (33%), followed by health problems and spouse death (28%), financial difficulties (27%), and death of siblings and other close relatives (18%). The most pronounced stress event in controls was financial difficulties (16%), followed by brain injury and spouse death (15%), death of son, daughter and grandchild (10%). The most significantly associated stressful life event with dementia was health problems (OR=35.0; p=1.5E-05) followed by financial difficulties/professional problems (OR=3.62; p=0.004), and problems within family/stressful situations (OR=3.46; p=0.003). The mean number of events experienced by dementia patients was 1.75±1.44 and the mean number of events experienced by non-demented subjects was 0.79±0.96. The rate of experiencing more total stressful events was significantly higher among dementia patients (*P*=5.29E-09) than non-demented subjects. However, we did not find a significant interaction between *APOE*4* carrier status and total number of stressful life events on dementia risk (P=0.677).

4. Discussion

The aging population in Pakistan has increased dramatically due to decline in mortality. It is projected that in 2058 the mean life expectancy in Pakistan will be 70.7 years as compared to its current value of 66.7 years [12]. According to Alzheimer's disease International (ADI) 2006 estimates, in Pakistan the prevalence of dementia was 330,100 people with annual incidence of 107,300 cases. This number is projected to be 566,600 by 2020 and 1,916, 200 by 2050 [13]. This alarming projected increase in dementia prevalence in Pakistan heralds the need for effective preventive and therapeutic treatments.

To our knowledge, this is the first study in a Pakistani sample that has examined the association of two established risk factors for dementia, including stressful life events and *APOE*4* allele. The majority of our cases were obtained from the Punjab Institute of Mental Health, Lahore, Pakistan, originating from different regions of Punjab. The control group was matched to cases and shared a similar profile in terms of education. The majority of the cases (72%) and controls (83%) had less than 8 years of formal education and only 28% of cases and 17% of controls had more than 8 years of formal education. This is consistent with previous findings that low education is a risk factor for dementia [14, 15].

Although our sample size was relatively small, we have confirmed the established association of the *APOE*4* allele as risk factor for dementia. In our sample, the frequency of the *APOE*4* allele was more than double in cases than in controls (20% vs. 9%) with an adjusted OR of 2.81. Lack of the *APOE**2 allele in cases compared to its 5% frequency in controls is only suggestive of its protective effect as our case sample size was small. Future large case-control studies may help to establish its protective effect in this population. These observations are in line with previously reported associations of these alleles with dementia in diverse ethnic groups [16]. Likewise, a large population-based dementia study carried out in India [17], which is genetically similar to the Pakistani population, has also shown a similar association of *APOE*4* with AD (OR=2.26; 95%CI: 1.29–3.95).

Our findings show that dementia patients experienced more stressful events than controls. Since we did not systematically assess timing of events relative to dementia onset, it cannot be assumed from the present results that stressful events were causative for dementia. However, that stress may be causative is in line with a previous study with more detailed retrospective assessments of timing of life events [18], and a prospective study of distress-proneness [19]. In the present study, events like surgical intervention/serious health problems, family-related stress, and financial difficulties/professional problems were significantly associated with risk of dementia. Results of this study replicate previous finding that psychosocial stress is associated with increased risk of dementia and AD [20, 21].

Previous studies have reported that older APO*E*4* allele carriers who were exposed to high stress were more likely to have worse cognitive status, compared to older persons with stress but without the E*4 allele [8, 9, 22]. In contrast, we did not find a significant interaction between *APOE*4* carrier status and stressful life events for risk of dementia (P=0.677). Our finding is consistent with another study where no modifying effect of *APOE* genotype on

stressful life events was identified in predicting cognitive decline [23]. However, other studies where an *APOE*4* and stress interaction was reported differed in a number of important ways from ours. They included non-demented older adults and detailed cognitive test performance either cross-sectionally [8, 22] or longitudinally [9] as the outcome. Stressful life events were assessed retrospectively within a more specific and recent time-frame [8, 9] or characterized in more detail regarding duration and level of severity [8]. One of the studies examined cortisol level as a biologic index of stress [22].

This study has many limitations. First, the sample group is not random and not representative of the general population. Our control group sampling was based upon convenience. We have more males in the control group than females as men generally work outside the home, and thus were more available for sampling. Elderly women usually reside at home, and due to cultural factors, we were limited in reaching out to women for recruitment. Second, the sample size is limited, partly a reflection of the unawareness of early indications of dementia. In Pakistan, forgetfulness is considered as normal part of aging and family members generally take care of elderly at home until symptoms become severe. As a result many early to moderate cases do not seek professional health services. Another challenge is an absence of specialized memory clinics and diagnostic facilities, and a general lack of adherence to standard diagnostic criteria for mental health in Pakistan [24]. Moreover, there is no registry for dementia patients, and often inconsistent maintenance of medical records. Most of the patients in lower or middle income groups cannot afford any specialized testing like imaging (MRI/CT), making dementia difficult to diagnose. In general, there are formidable challenges to dementia research in this population.

Since cases had higher proportions of a number of illnesses than controls, it should be noted that these illnesses (e.g., stroke, depression) may have contributed to cognitive changes not attributable to AD-related neuropathology, adding further to diagnostic heterogeneity among cases. While these illnesses may be considered as stressful events, as well, their presence may indicate limitations with respect to the diagnostic procedure. A further limitation is the language barrier and low literacy rate in Pakistan, especially in the aged population. As patients belong to families where a number of different dialects are spoken (Urdu, Punjabi, Siraiki, etc.), it is difficult for investigators to interview potential subjects and utilize different cognitive batteries for assessment. There is a need for valid assessment tools translated and validated in Pakistani languages and sub-cultures.

Finally, as noted above, precise timing of the occurrence of stressful life events was not assessed. This may lead to overestimation of the association because many of the cases were diagnosed at very late stages of the disease, and the corresponding stressful events could be outcomes rather than predictors of dementia. Self-reported events could be subject to recollection bias. We did not assess severity of stressful events, though some informants reported events experienced by patient as being highly stressful and triggering the onset of disease. Some of the participants experienced multiple events, like spouse death and death of sibling, but this was not systematically recorded. We believe, therefore, our stress measure likely lacked sensitivity to individual differences of interest (perhaps in particular limiting power to detect the stress x genotype interaction). Despite these limitations and relatively small samples size, our findings of the associations of *APOE* and stressful life events with

dementia are consistent with multiple previous reports and thus providing some validation to our study design.

5. Conclusion

In conclusion, presence of stressful life events and *APOE*4* are independently associated with dementia. We did not find an interaction between stressful life events and *APOE*4* status. Our findings will likely stimulate additional well-designed studies in this population on larger sample size, longer follow up period, and at multiple study sites. It also emphasizes the need to improve mental health research with older people in Pakistan.

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Highlights

- *APOE*4* allele is associated with increased risk of dementia in Pakistani population.
- Stressful life events are also associated with dementia risk in Pakistani population.
- No significant interaction was observed between *APOE*4* and stress in Pakistani population.

Table 1

Sample description of cases and controls

	n (%)		p-values
	cases	controls	
	61	115	
SEX			
Male	30 (49.2)	80 (69.6)	0.008
AGE	Mean ± (SD)		
Mean age	71.32 ± (9.9)	66.37 ± (7.6)	0.003
MARITAL STATUS			
Married	44 (72.1)	93 (80.9)	0.415
Never Married	2 (3.3)	2 (1.7)	
Widowed	14 (23.0)	16 (13.9)	
Divorced	1 (1.6)	2 (1.7)	
Separated	0	2 (1.7)	
EDUCATION			
Illiterate	39 (65.0)	84 (74.3)	0.309
<8 years	4 (6.7)	10 (8.8)	
8–12 years	11 (18.3)	14 (12.4)	
13 or more years	6 (10.0)	5 (4.4)	
INCOME GROUPS			
lower	21 (35.0)	74 (64.3)	0.003
lower middle	19 (31.7)	21 (18.3)	
upper middle	15 (25.0)	16 (21.7)	
high	5 (8.3)	4 (3.5)	
CO-MORBIDITIES			
Heart Disease	8 (13.3)	7 (6.1)	0.104
Stroke	6 (10.0)	1 (0.9)	0.007
Diabetes	14 (23.3)	8 (7.0)	0.002
Hypertension	22 (36.7)	19 (16.5)	0.003
Obesity	1 (1.7)	11 (9.6)	0.06
Depression	12 (20.0)	6 (5.2)	0.002
Head Injury	9 (15.0)	17 (14.8)	0.969
Smoking	17 (28.3)	56 (48.7)	0.01
Alcohol/drug abuse	1 (1.7)	7 (6.1)	0.267
FAMILY HISTORY OF DEMENTIA			
Present	15 (25.0)	6 (5.2)	0.0002
CLINICAL MEASURES	Mean ± (SD)		
Mean modified MMSE (total possible score 23)	12.95 ± (5.0)	22.75 ± (0.8)	<0.001

Table 2

APOE genotype count and allele frequencies

	Cases n (%)	Controls n (%)
***APOE* genotype**		
E2/E3	0 (0.0)	9 (8.33)
E3/E3	35 (62.5)	79 (73.1)
E2/E4	0 (0)	1 (0.9)
E3/E4	20 (35.7)	19 (17.6)
E4/E4	1 (1.88)	0 (0.0)
Total	56*	108*
Allele frequency		
*APOE*2*	0	0.05
*APOE*3*	0.80	0.86
*APOE*4*	0.20	0.09
Sex and Age adjusted *APOE*4* OR and *P* value		
OR	95 % CI	*P*-value
2.81	1.33–6.03	0.00697

*Results are included for only 164 samples that passed stringent quality control criteria for genotyping, remaining 12 samples were failed on genotyping

Table 3

Results of testing the effects of life stressful events on dementia, with adjustment for demographic variables (i.e. age, gender and income level)

stressful event	Cases		controls		OR (95% CI)	P
	N	%	N	%		
spouse death	17	28.33	17	14.8	1.84 (0.79–4.27)	0.154
death of son, daughter, grandchild	10	16.66	12	10.4	2.10 (0.76–5.72)	0.143
death of sibling and other beloved persons	11	18.33	6	5.21	3.85 (1.25–12.84)	0.021
surgical intervention-serious health problems	17	28.33	2	1.73	35.20 (8.52–246.19*)	1.5E-05
illness of spouse, parents, son, daughter, grand parents	1	1.66	3	2.6	0.33 (0.01–3.38)	0.382
problems within the family, stressful situations	20	33.33	15	13	3.46 (1.51–8.12)	0.003
financial difficulties, professional problems	16	26.66	18	15.7	3.62 (1.50–8.99)	0.004
Stroke	6	10	1	0.86	9.96 (1.32–213.77*)	0.054
traumatic brain injury	9	15	17	14.8	0.66 (0.25–1.77)	0.400

* as sample size is relatively small, the variance of the log-odds is large such that the CI of odds ratio is wide