Apolipoprotein E polymorphism is associated with susceptibility to schizophrenia among Saudis

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Abstract

Introduction: Apolipoprotein E (APOE) genotypes influence the phenotype of several neurodegenerative disorders including Alzheimer's and Parkinson disease and may affect schizophrenia pathogenesis. This study was undertaken to determine the association between APOE gene polymorphisms and schizophrenia in the Saudi population.
Material and methods: APOE allele and genotype frequencies were studied in 380 Saudi subjects including schizophrenia patients and matched controls using polymerase chain reaction (PCR) and reverse-hybridization techniques.
Results: The frequencies of the APOE allele ε2 and genotypes ε2/ε3 and ε2/ε4 were significantly higher in the schizophrenia patients as compared to controls, suggesting that the ε2 allele and its heterozygous genotypes may increase the susceptibility to schizophrenia. In contrast, the frequencies of the ε3 allele and ε3/ε3 genotype were lower in patients as compared to controls, suggesting a protective effect of APOE ε3 for schizophrenia. This study indicated that APOE ε4 was differentially associated with schizophrenia depending on the symptoms as the frequency of the ε4 allele was significantly higher in schizophrenia patients with positive symptoms. By contrast, no significant association between APOE ε4 and schizophrenia patients with negative symptoms was observed. Genotypes ε2/ε2 and ε4/ε4 were absent in patients and controls. Moreover, the age of onset was significantly lower in patients with the APOE ε2/ε3 genotype. There was no significant difference in the frequencies of APOE alleles and genotypes between male and female schizophrenia patients.
Conclusions: The results of this study clearly show that APOE alleles and genotypes are associated with risk of developing schizophrenia and early age of onset in Saudis.

Key words: schizophrenia, apolipoprotein E (APOE), polymorphism, Saudis.

Introduction

Schizophrenia is a clinical syndrome of severe mental illness characterized by a breakdown of thought processes, hallucinations, delusions and functional deterioration. According to the World Health Organization estimate, schizophrenia affects around 24 million people worldwide, with a prevalence ranging from 0.7% to 1.1% and with a lifetime morbidity risk of 0.5–2.7% [1, 2]. Due to its early age of onset and the lifelong disabili-

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ty, which invariably brings emotional and financial devastation to its victims and their families, schizophrenia is considered one of the most catastrophic mental illnesses [3, 4]. In spite of extensive studies, schizophrenia still remains a diagnosis of exclusion, since none of its clinical features are pathognomonic, and, by and large, biochemical imaging, physiologic and psychological tests lack the sensitivity and specificity required for a firm diagnosis [5]. The causes and pathogenesis of schizophrenia remain vexingly obscure. Recent studies clearly suggest that schizophrenia is a multifactorial disease involving several genes with each susceptibility gene having only a modest individual effect [6]. A combination of complex genetic and environmental processes that may cause the disease have been proposed [7]. Genetic studies have implicated several susceptibility loci, but the clinical impact of these loci in the pathogenesis of schizophrenia is yet to be established.

There is ample evidence to suggest that cognitive dysfunction in schizophrenia is closely associated with alterations in synapses, dendritic spines and myelin [8–10]. Lipoprotein cholesterol being an indispensable component of synapses and myelin, its availability in oligodendrocytes is a rate-limiting factor in maturation and repair of neurons [11, 12]. Apolipoprotein E (APOE), being closely associated with uptake and distribution of cholesterol in the brain and other parts of body, is an excellent candidate for a pathologic role in cardiovascular and neurological disorders including schizophrenia [13, 14]. Apolipoprotein E synthesized in astrocytes and oligodendrocytes plays a critical role in growth development and repair of neurons [15, 16]. Significantly altered APOE levels in brains of people with schizophrenia have been reported [17–19].

Apolipoprotein E, a 34-kDa glycosylated protein, is composed of 299 amino acids encoded by a four exon polymorphic gene on chromosome 19q13.2. The gene encoding APOE has three polymorphic variants in humans designated as ε2, ε3 and ε4. These variants differ from one another by the presence of either a C or a T nucleotide at codons 112 and 158. These three alleles encode different APOE isoforms which vary significantly in structure and function including receptor binding capacity and lipid metabolism [20]. As each individual human being carries two allelic copies in a gene, six possible genotypes (ε2/ε2, ε3/ε3, ε2/ε3, ε3/ε4, ε2/ε4, and ε4/ε4) are formed by different combinations of these three alleles [21, 22]. The frequency of these genotypes differs significantly among different ethnic groups; however, APOE ε3/ε3 is the most predominant genotype and ε3 the most common allele in the majority of the population [23–25].

Evidence suggests that the APOE genotype may influence the phenotype of several neuropsychiatric illnesses [26, 27]. The association between APOE genotype and schizophrenia was first reported by Harrington et al. in 1995 [28]. However, subsequent studies on APOE genotype distribution in schizophrenia have produced conflicting findings [29–38].

The prevalence of psychiatric disorder is quite high in the Kingdom of Saudi Arabia (KSA) and other Arab countries [39]. The Saudi population, being a closed and isolated society with a high rate of consanguinity (inbreeding), may represent a valuable resource for studying genetic etiology of schizophrenia as compared to other heterogeneous populations. In this study we examined the APOE allele/genotype frequencies in Saudi schizophrenic patients.

Material and methods

Subjects

A total of 380 subjects including 180 schizophrenia patients visiting a psychiatric clinic and 200 age- and sex-matched healthy controls visiting a community health clinic of Prince Sultan Military Medical City, Riyadh were recruited to this study. All the subjects were biologically unrelated Saudis. The diagnosis of schizophrenia was based on the criteria mentioned in the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, DSM-IV-TR Version. These criteria use the self-reported experience of the patient and reported abnormalities in behavior and a comprehensive clinical assessment by a neuro-psychiatrist. After initial selection, to ensure the diagnostic reliability a systemic search into the case notes of the patients was made. Patients with questionable diagnosis, known organic disorder, or mental retardation were excluded. The remainder of the patients were interviewed by the psychiatrist to ascertain whether patients met *DSM-1V-TR* criteria for schizophrenia. After confirmation of schizophrenia diagnoses, patients were further assessed for positive and negative symptoms using the Positive and Negative Syndrome Scale (PANSS) involving further clinical interview, cognitive testing, motor assessment, and careful review of medical and historical records as described by Kay et al. [40]. This assessment procedure applied to the PANSS includes 30-item and 7-point rating instruments. Each item on the PANSS is accompanied by a complete definition as well as detailed anchoring criteria for all seven rating points, which represent increasing levels of psychopathology: 1 = absent, 2 = minimal, 3 = mild, 4 = moderate, 5 = moderate-severe, 6 = severe, and 7 = extreme. Of the 30 psychiatric

parameters assessed on the PANSS, 7 parameters (delusions, conceptual disorganization, hallucinatory behavior, excitement, grandiosity, suspiciousness, hostility) were chosen a priori to constitute a Positive Scale, seven others (blunted affect, emotional withdrawal, poor rapport, passive-apathetic social withdrawal, difficulty in abstract thinking, lack of spontaneity and flow of conversation, stereotyped thinking) a Negative Scale, and the remaining 16 a general psychopathology scale. The PANSS was scored by summation of ratings across items, such that the potential ranges were 7–49 for the Positive and Negative Scales. The assessment was conducted by two trained psychiatrists independently and almost the same rating was obtained for each patient by both the psychiatrists.

Based on the above criteria, only 180 patients scoring 7–49 either for positive or negative symptoms were included in this study. Among the confirmed 180 cases there were 52 females and 128 males with a mean age of 39 ±12.5 years and mean disease duration of 9 ±4.5 years. Age of onset of disease ranged from 19 to 64 years. The female to male ratio of schizophrenia patients in our study was 1 : 2.5.

The matched control group consisted of 200 healthy unrelated voluntary blood donors (aged 26–55 years) from the same Saudi population. Each control subject was interviewed and examined by a psychiatrist to rule out subjects with a family history of any psychiatric illness. A number of baseline parameters to rule out any psychotic illness were adopted as described by Johnstone *et al.* [41]. Controls subjects less than 26 years old and having first- or second-degree relatives with schizophrenia or any psychiatric disorder or with past or present involvement in substance abuse were excluded. The control group consisted of 50 females and 150 males with mean age of 36 ±10 years. This study was approved by the ethical committee of the hospital and informed consent was obtained from each subject.

Genotyping

Genomic DNA was extracted from the blood of schizophrenia patients and controls using the QIAamp DNA mini kit (Qiagen CA, USA). The genotypes of the APOE polymorphisms were determined using the ApoE StripAssay kit based on polymerase chain reaction (PCR) and reverse-hybridization technique (ViennaLab Labordiagnostika GmbH, Vienna, Austria). The procedure included three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification product to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and color substrates. To cross-check the results the genotypes of the APOE polymorphisms were also determined by PCR and restriction fragment length polymorphism (RFLP) technique. Primers were designed on the basis of the sequence data for APOE available in GenBank to amplify the coding sequence of APOE. The PCR was performed using PuRe Taq Ready-To-Go PCR Beads (GE Healthcare, UK) with primers as described earlier [25].

200–300 ng of genomic DNA was used as a template in 25 µl reaction volume. Genomic DNA was amplified for 40 cycles. Each cycle consisted of: 94°C for 30 s, 68°C for 10 s, 72°C for 1 min; PCR products obtained were separated by electrophoresis on 1.5% agarose gel in TAE buffer, visualized by ethidium bromide fluorescence. Fragments with the expected size were cut from the gel, purified using the GFX PCR DNA Gel band purification kit (GE Healthcare, UK). Purified DNA was digested with *Cfo* I (Hha I) enzyme, separated by agarose gel electrophoresis to identify the genotype. On the basis of size and number of various fragments generated, APOE genotypes were determined as ε2/ε2 with 144 bp and 96 bp, ε3/ε3 with 144 bp and 48 bp, ε4/ε4 with 72 bp and 48 bp, ε2/ε3 with 144 bp, 96 bp and 48 bp, ε3/ε4 with 144 bp, 72 bp and 48 bp, and ε2/ε4 with 144 bp, 96 bp, 72 bp and 48 bp fragments. The prevalence of various genotypes in patients and controls was determined. Complete matching of results was obtained following both of the above-mentioned procedures.

Statistical analysis

Frequencies of various alleles and genotypes for each polymorphism were compared between patients and controls and analyzed by Fisher's exact test, and *p*-values < 0.05 were considered as significant. The strength of the association of disease with respect to a particular allele/genotype is expressed by the odd ratio interpreted as relative risk (RR) according to the method of Woolf as outlined by Schallreuter *et al.* [42]. The RR was calculated only for those alleles and genotype which were increased or decreased in schizophrenia patients as compared to normal Saudis.

Etiologic fraction (EF) indicates the hypothetical genetic component of the disease. Values > 0.0 are of significance. It was calculated for a positive association (RR > 1) [43].

Preventive fraction (PF) indicates the hypothetical protective effect of one specific allele/genotype for the disease. It was calculated for a negative association (RR < 1) [43]. Values < 1.0 indicated the protective effect of the genotype/allele against manifestation of the disease.

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Results

The results of frequency of APOE alleles and genotypes in the schizophrenia patients and the control subjects are summarized in Tables I and II. In both schizophrenia patient and control groups the genotype distributions were in Hardy-Weinberg equilibrium. The ε2 allele was present in 9.17% of schizophrenia patients and altogether absent in controls ($p = 0.0001$). The frequency of ε3 alleles was significantly lower in schizophrenia patients (83.33%) compared with that in controls (95.75%, $p = 0.0001$, RR = 0.222, PF = 0.606). On the other hand, the frequency of allele ε4 was higher in patients compared with that in controls ($p = 0.06$, RR = 1.826, EF = 0.277) (Table I). Our study on various genotypes of APOE also showed variations in patient and control groups. The prevalence of ε3/ε3, ε3/ε4, ε2/ε3, and ε2/ε4 was 70.0, 11.67, 15, and 3.33% in patients and 91.5, 8.5, 0 and 0% in the control group, respectively. Though the frequency of ε3/ε3 genotype was higher in both the

test and control Saudi population, the statistical analysis of data showed a significant difference in ε3/ε3 genotype frequency between patients and controls, being higher in controls than in patients ($p = 0.0001$, RR = 0.217, PF = 0.598). The difference in the frequencies of the genotype ε3/ε4 was not statistically significant between the two groups ($p = 0.31$, RR = 1.449, EF = 0.171). The genotypes ε2/ε3 and ε2/ε4 were present in 15% and 3.33% of patients, respectively, but totally absent in the controls ($p = 0.0001$, $p = 0.01$, respectively). The genotypes ε2/ε2 and ε4/ε4 were absent in both patient and control groups. These results indicated that allele ε2 and genotypes ε2/ε3 and ε2/ε4 are associated with schizophrenia and may be a risk factor while allele ε3 and genotype ε3/ε3 may be protective for schizophrenia in Saudis. The frequency distribution of genotypes and alleles of APOE polymorphism were not significantly different in male and female patients, indicating that gender plays no role in genotype/allele distributions among Sau-

Table I. Apolipoprotein E genotype/allele frequencies in schizophrenia patients and matched controls

Genotype/ allele	Schizophrenia (N = 180)		Control (N = 200)		Value of p	RR	EF*/PF
	N	Frequency (%)	N	Frequency (%)			
ε3/ε3	126	70.00	183	91.50	0.0001♣	0.217	0.598
ε3/ε4	21	11.67	17	8.50	0.3100	1.449	0.171*
ε2/ε3	27	15.00	0	0	0.0001♣	–	–
ε2/ε4	6	3.33	0	0	0.0100♣	–	–
ε2/ε2	0	0	0	0	–	–	–
ε4/ε4	0	0	0	0	–	–	–
ε3	300	83.33	383	95.75	0.0001♣	0.222	0.606
ε4	27	7.50	17	4.25	0.0600	1.826	0.277*
ε2	33	9.17	0	0	0.0001♣	–	–

N – Number of subjects, ♣statistically significant, EF – etiological fraction, PF – preventive fraction.

Table II. Comparison of apolipoprotein E genotypes and alleles with negative or positive symptoms in schizophrenia patients and controls

Genotype/ allele	With negative symptoms (N = 94)		With positive symptoms (N = 86)		Control (N = 200)	
	N	Frequency (%)	N	Frequency (%)	N	Frequency (%)
ε3/ε3	71	75.53♣**	55	63.95*	183	91.50
ε3/ε4	8	8.51	13	15.12	17	8.50
ε2/ε3	11	11.70*	16	18.60*	0	0
ε2/ε4	4	4.26*	2	2.33	0	0
ε3	161	85.64*	139	80.81*	383	95.75
ε4	12	6.38	15	8.72*	17	4.25
ε2	15	7.98*	18	10.47*	0	0

*♣P-value < 0.05 as compared to the frequency in patients with positive symptoms. *P-value < 0.05 as compared to the frequency in controls, N – number of subjects.*

di patients with schizophrenia. The APOE ε2 allele was associated with age of onset of schizophrenia ($p = 0.001$). The groups of patients with genotypes ε2/ε3 and ε2/ε4 had a lower age of onset (21 ±4 years) than the patients with genotypes ε3/ε3 and ε3/ε4 (31 ±10 years).

The difference in the frequency distribution of APOE genotypes and alleles between the two types of schizophrenia was not significant except for the frequency of genotype ε3/ε3, which was significantly higher in patients with negative symptoms compared with patients with positive symptoms (Table II). However, when compared with controls separately, a similar pattern was observed for both types of schizophrenia except that the frequency of allele ε4 was significantly higher in patients with positive symptoms ($p = 0.045$) but not in patients with negative symptoms.

Discussion

In this study we found that frequencies of various isoforms of APOE were significantly different between the Saudi schizophrenia patients and healthy controls (Table I). One interesting finding of this study is that significantly higher frequencies of allele ε2 ($p = 0.0001$) and heterozygous genotypes ε2/ε3 ($p = 0.0001$) and ε2/ε4 ($p = 0.010$) were observed in Saudi schizophrenic patients, whereas allele ε2 was completely absent in Saudi healthy controls. Moreover, patients with genotypes ε2/ε3 and ε2/ε4 had a lower age of onset than the patients with genotypes ε3/ε3 and ε3/ε4, indicating that the APOE ε2 allele was associated with age of onset in schizophrenia ($p = 0.001$). Earlier studies reported low frequencies of allele ε2 in control populations [44–51], while it was completely absent in healthy Saudis [25, 52] and South American Amerindians [53]. This study clearly suggests that carriers of APOE ε2/ε3 and APOE ε2/ε4 are at higher risk of developing schizophrenia. Our finding is in agreement with Schurhoff *et al.* [54], who reported that the APOE ε2/ε3 genotype may confer higher susceptibility to schizophrenia in the French male population. Halford *et al.* [55] observed that American Caucasians with schizophrenia-associated tardive dyskinesia had significantly higher frequency of the APOE ε2 allele. However, no such association was found in African-Americans, suggesting a definite role of race/ethnicity. Contrary to our findings, studies carried out on Korean [56] and Kuwaiti populations [37] showed a significant protective effect of APOE ε2 against the development of schizophrenia. Kimura *et al.* [33] also observed a protective role of APOE ε2 against early onset schizophrenia in the Japanese population.

The mechanism by which APOE ε2 affects susceptibility to schizophrenia is far from clear. Although schizophrenia is primarily considered to be a neurodevelopmental disease, there is a growing consensus that the disorder involves neurodegeneration [57]. The hypothesis of neurodegenerative change in schizophrenic patients has been supported by observation of increased vulnerability to apoptosis, increase in cell loss and reduced neuronal size as well as decreased expression of synaptic proteins in schizophrenia [58–60]. A meta-analysis of published work showed a clear association between APOE and neurodegenerative disorders [61]. Some studies reported higher frequency of APOE ε2 in Parkinson's disease as compared to controls [62]. On the other hand, the possession of APOE ε2 has been associated with late-onset Alzheimer's disease, longevity in Down's syndrome, protection against the development of dementia, and protection against early-onset schizophrenia [33, 37, 56, 63, 64]. The mechanism by which APOE participates in the different pathogenic processes remains to be elucidated. A definite role of APOE ε2 in regulation of neuronal sprouting, regeneration and apoptosis has been demonstrated [64]. The APOE ε2 isoform differs from the APOE ε3 isoform by one amino acid, at position 158, with ε2 containing cysteine and ε3 containing arginine. This single amino acid difference results in markedly reduced/defective binding of APOE ε2 to the low density lipoprotein family of receptors, which in turn results in profound metabolic consequences, particularly type III hyperlipidemia. Additionally, the two cysteines in APOE ε2 (positions 112 and 158) permit APOE ε2 to form disulfide-linked multimeric protein complexes. These unique properties of APOE ε2 may contribute to its role in the etiology of neurological as well as coronary artery diseases.

The results of this study also showed significantly lower frequencies of allele ε3 and genotype ε3/ε3 among Saudi schizophrenic patients as compared to healthy controls (Table I), suggesting that the ε3 allele confers protection against schizophrenia in the Saudi population. Our findings are in agreement with several published studies on other populations including Chinese [65], Finnish [66] and Serbian [38], in which decreased frequencies of genotype ε3/ε3 and allele ε3 were observed in schizophrenia patients compared to controls, and the effect was independent of sex and age of onset of illness. Kampman *et al.* [66] also suggested that the APOE ε3 allele may function in a protective manner in later onset schizophrenia and its absence could be associated with higher risk for early-onset schizophrenia. The results of a recent study by Kecmanovič *et al.* [38] further strengthened the reports that the ε3 allele could serve as a protective factor against symptoms more abundant in undifferentiated types of schizophrenia. In contradiction to these, some re-

ports on schizophrenic patients from Taiwan [34], Korea [56] and Mexico [36] stated that the development of schizophrenia in these populations is associated with higher frequency of allele ε3. Yet some other investigators found no association between the frequency of APOE ε3 and schizophrenia [30–32, 67–70]. This inconsistency in published data may be attributed to small sample size, problems of sample stratification, mismatch between cases and controls, diagnostic criteria of the disease, clinical status of patients/severity of disease and method of detection of APOE (protein isoelectric focusing or PCR-based genotyping).

The frequency of the ε4 allele was also higher in Saudi schizophrenia patients as compared to controls, a difference which was just short of significance ($p = 0.06$). However, upon stratification of patients with positive symptoms and with negative symptoms, the frequency of the ε4 allele was significantly higher in schizophrenia patients with positive symptoms as compared with that in controls. These results point to a higher risk of the disease among APOE ε4 carriers. A significant role of APOE ε4 in the pathogenesis of schizophrenia was first reported by Harrington *et al*. [28]. However subsequent studies revealed conflicting results [31, 34, 65, 66, 70–74]. A recent meta-analysis of published data clearly showed that APOE ε4 is significantly associated with schizophrenia [75]. Moreover, an association between early age of onset in schizophrenia and higher frequencies of the ε4 allele has also been reported [31, 66].

On the other hand, population-based studies from other geographical locations/ethnic groups failed to find any significant association of APOE ε4 and schizophrenia [29, 30, 32–36, 69, 73, 76, 77].

Overall, APOE ε4 has been shown to be a major risk factor for a variety of neurodevelopmental and neurodegenerative disorders including schizophrenia and Alzheimer's disease (AD). The mechanism by which carrying the APOE ε4 allele translates to neuropathology remains elusive. The APOE plays a critical role in redistributing lipids among CNS cells for normal lipid homeostasis, repairing injured neurons, maintaining synapto-dendritic connections, and scavenging toxins [19]. In multiple pathways affecting neuropathology of neuropsychological disorders, APOE may act by itself or in concert with age, oxidative stress, ischemia and inflammation to cause neurological disorders, accelerating progression, altering prognosis, or lowering age of onset. The role of APOE ε4 in diabetic peripheral neuropathy has been suggested by some investigators though it is still debated and needs further studies [78]. It has also been suggested that unique structural features of APOE ε4 are responsible for ε4-associated neuropathology

[79]. Although the structures of APOE ε2, ε3, and ε4 are in dynamic equilibrium, APOE ε4, which is detrimental in a variety of neurological disorders, is more likely to assume a pathological conformation. Importantly, ε4 displays domain interaction and molten globule formation (the formation of stable, reactive intermediates with potentially pathological activities). APOE ε4 uniquely undergoes neuron-specific proteolysis, resulting in bioactive toxic fragments that enter the cytosol, alter the cytoskeleton, disrupt mitochondrial energy balance, and cause cell death [80]. In recent years potential therapeutic strategies, including the use of "structure correctors" to convert APOE ε4 to an "APOE ε3-like" molecule, protease inhibitors to prevent the generation of toxic APOE ε4 fragments, and "mitochondrial protectors", have been attempted to prevent cellular energy disruption.

Overall, the results of this study clearly suggest that all three isoforms of APOE play a role in the pathogenesis of schizophrenia, but the way these isoforms modify the onset and course of the disease warrants further studies.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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