SHORT COMMUNICATION

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Apolipoprotein E genotype and Cognition in Bipolar Disorder

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Apolipoprotein E (APOE) has been extensively studied as a risk factor for sporadic and late onset Alzheimer's Disease (AD). APOE *allele* *3, the most frequent variant, is not associated to cognitive dysfunction (CD) or to increased AD risk. Differently, the *4 *allele* is a well-established risk factor for CD, while the *2 *allele* is associated with survival and longevity. CD is an important feature of Bipolar Disorder (BD) and recent data suggest that CD may be one of its endophenotypes, although controversial results exist. The aim of this research is to study the association of APOE genotype (*APOE*) and neurocognitive function in a sample of drug free young BD-type I patients. Sample consisted of 25 symptomatic BD (type I) patients (age 18–35 years old). They were submitted to an extensive neuropsychological evaluation and genotyped for *APOE*. Subjects with *allele* *2 presented better cognitive performance. The presence of *allele* *4 was associated with worse performance in a few executive tasks. *APOE* *3*3 was associated with overall severe dysfunction on cognitive performance. In young individuals with nontreated BD-type I, *APOE* may predict cognitive performance. Further and larger studies on *APOE* and cognition in BD are required to clarify whether *APOE* is a BD cognitive endophenotype.

Introduction

Genetic studies identified DNA polymorphisms related to severe cognitive deficits in several neurological disorders [1]. Apolipoprotein E (APOE) is a risk factor for both sporadic and late onset familial Alzheimer's Disease (AD) [2–7], and may influence cognition even in healthy individuals [8]. Recent *in vitro* studies suggested that all forms of APOE influence synaptic formation [9]. In healthy individuals, APOE allelic distribution differs broadly across various ethnic backgrounds, resulting in imprecise prevalence estimates for the entire population [10]. The APOE *allele* variant *3 is more prevalent (48–89%) and does not seem to be related to cognitive impairment in normal aging. Differently, the *4 *allele* is a well-established risk factor for AD, while the *2 *allele* is associated with survival and longevity [11–13].

The relevance of APOE genotype (*APOE)* in Bipolar Disorder (BD) is unclear [14], with controversial findings regarding associations and frequencies [15]. The most studied *allele* is *4 and, to the best of our knowledge, studies on the importance of *alleles* *2 and *3 in mood disorders are not available. Similarly, increased frequency of *allele* *4 in affective disorders has not been described, although different groups of patients with increased frequency of *4 have been identified [16]. For example, increased frequency of *allele* *4 in late-onset depression has been reported, as compared to early-onset major depression [17], but findings have never been replicated [18]. Similarly, *allele* *4 seems to be more frequent among patients with depression and psychotic features [19]. The relevance of *allele* *4 and cognitive function in affective disorders is yet controversial; while one study reported decreased scores on the Mini-Mental State Examination

test [20], a second study failed to replicate the findings [18].

Endophenotypes are quantitative and inherited traits that are characteristic of a disorder. They are typically assessed by laboratory methods rather than by clinical observation [21]. Cognitive dysfunction (CD) is a common feature of BD, and data suggest that it may be a BD endophenotype, persisting during euthymia. Acutely ill BD patients often have dysfunctions in several cognitive domains, such as attention, executive function, learning, memory, and psychomotor speed [22,23]. Accordingly, in this study we investigated the association between *APOE* genotype and CD in a sample of young individuals with BD-type I, while in an acute untreated episode (drug free for at least 2 weeks).

Methods and Materials

Sample

Our sample consisted of 25 individuals with BD-type I (18–35 years old). Of them, 10 were in the depressive phase, while 15 were in the manic phase. BD was diagnosed using the DSM-IV criteria [24] and the Structured Clinical Interview (SCDI-I/P) [25,26]. Interviews were conducted during an acute episode (mania or depression) [respectively defined as a Young Mania Rating Scale [27] score $\geq$7 or a Hamilton Depression Rating Scale [28] $\geq$7]. Only drug free patients (at least 2 weeks without oral antidepressants and oral antipsychotics, or four weeks without depot medication) were included. We did it so to exclude the effects of medication on cognition. Since most euthymic patients are on medication, we excluded both, treated and euthymic patients. Patients with neurological disorders, previous head trauma, physical illnesses requiring medical intervention, substance abuse and those who had an electroconvulsive therapy (ECT) course in the preceding six months were excluded.

Neurocognitive Assessments

The neurocognitive test battery assessed memory with the logical memory subtest [29]; attention with the digit span [30] and trail making test part A – TMT-A [31]; perceptual motor skill with the Rey–Osterrieth complex figure – ROCF [32]; executive functioning with the Wisconsin card sorting test – WCST [33]; controlled oral word association test – FAS [32]; stroop color-word test – SCWT [32]; trail making test part B – TMT-B [31]; letter number sequencing – LNS [30]; Raven's progressive matrices test – RPMT; intelligence quotient – IQ with the Wechsler abbreviated scale of intelligence – WASI [34].

APOE Genotyping

Genomic DNA was extracted from the peripheral blood using salting-out protocol [35] and then genotyped for the *alleles APOE**2, *3, and *4. Genotyping was performed by real-time PCR allelic discrimination. PCR amplification for all SNPs (rs429358 and rs7412) was performed in 5 μl reactions with 5 ng of template DNA, 1× TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), 1× each primer and probe assay, and H_2O. Thermal cycling initiated with a first denaturation step of 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 seconds and annealing at 60 °C for 1 min. The allele-detection process was performed for 1 min at 60 °C on a 7500 Real-Time System (Applied Biosystems, Foster City, CA) to determine the allelic discrimination.

Statistical Analyses

A nonparametric test was used to compare the neuropsychological performance, socio-demographic variables and genetic variables (factors: alleles *2, *3, and *4 and genotypes *3*4, *3*3, *2*3, and *4*4). The sample was stratified by gender and ethnicity and neurocognitive tests were correlated with *APOE* alleles using *Spearman's* correlation, with a significance level of $P < 0.05$. Statistical analysis was carried out using SPSS 15.0 (SPSS Inc., Chicago, IL).

Results

As displayed in Table 1, no significant differences were seen for age or educational level after stratification by gender and ethnicity. The relative frequency of *APOE* *4 *allele* was 10% (n = 5/50), with 4% (n = 1) of homozygosis in the overall sample. The prevalence of *APOE* *3 *allele* was 82% (n = 41/50), with 68% (n = 17) in homozygosis. Finally, the prevalence of *APOE**2 *allele* was 8% (n = 4/50), with no homozygosis. The YMRS mean score was 20.56 (SD 8.06), while the HDRS mean score was 21.6 (SD 7.59). Figure 1 displays the YMRS and HDRS individual scores. No differences between symptoms scales scores were observed as a function of genotype ($P = 0.091$).

Cognitive Performance by Gender/Ethnicity

Caucasian patients were less likely to have perseverative behavior in the WCST ($P = 0.013$). African Americans showed better verbal fluency in the FAS total ($P = 0.041$)

Table 1 Demographic characteristics of enrolled sample, overall and as a function of genotype

	n	Age		Educational level		Genotype				
		Mean (SD)	p	Mean (SD)	p	*2*3	*3*3	*3*4	*4*4	p
Gender										
Male	10	27.7 (5.9)	0.721	12.9 (1.5)	0.557	2	11	2	1	0.822
Female	18	28.44 (4.8)		12.17 (3.7)		2	6	1	0	
Race										
Caucasian	20	28.52 (5.2)	0.903	12.71 (3.1)	0.494	4	14	1	1	0.145
African American	5	28.2 (5.6)		11.6 (3.6)		0	3	2	0	

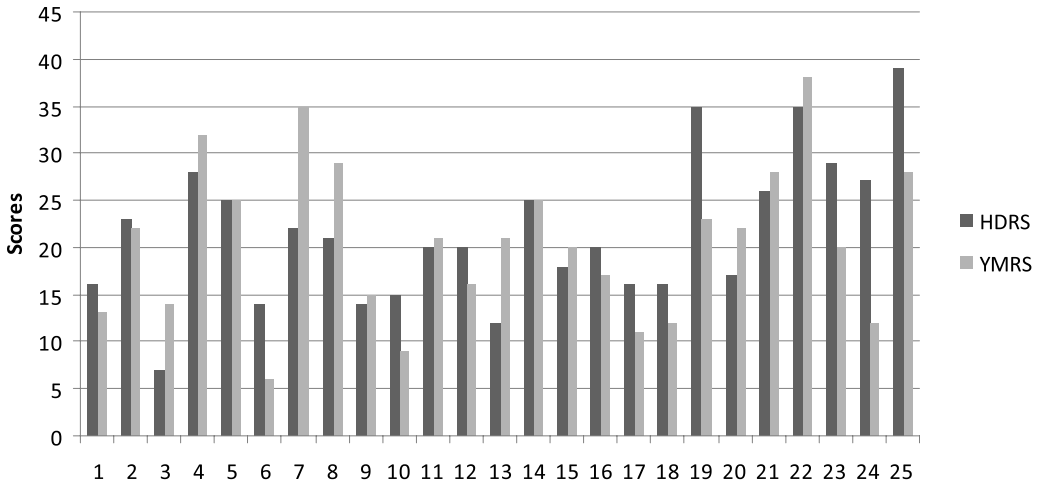


Figure 1 Individual YMRS and HDRS scores.

and FAS letter F ($P = 0.029$). African American females were less likely to have perseverative behavior in the WCST ($P = 0.033$), and more likely to have better verbal fluency in the FAS total ($P = 0.049$) and FAS letter F ($P = 0.042$), but with increased number of mistakes in the WCST ($P = 0.009$).

Cognitive Performance by Alleles

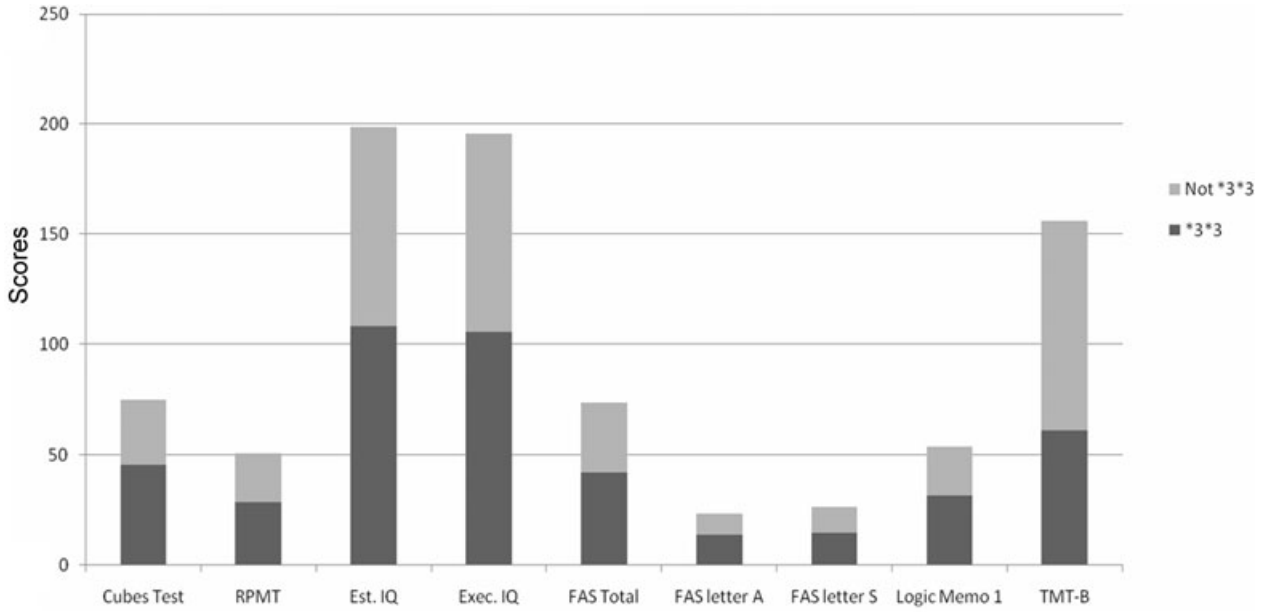
We found significant differences between groups regarding *APOE allele* *4 (homozygous or heterozygous) and performance in SCWT part 2 ($P = 0.029$), FAS letter F ($P = 0.026$) and FAS letter S ($P = 0.019$); we observed a nonsignificant trend in FAS total ($P = 0.057$) and logic memory I ($P = 0.088$). The presence of *allele*3 (homozygous or heterozygous) in males was associate with worse performance on the perseveration sections of SCWT ($P = 0.008$). The presence of *allele*2 was associated with the best performance among all genotypes on the cubes test ($P = 0.018$), RPMT ($P = 0.030$), estimated IQ ($P = 0.036$), execution IQ ($P = 0.025$) and TMT-B ($P = 0.030$).

Cognitive Performance x Genotype

As contrasted to other genotypes, those with genotype *3*3 (Fig. 2) had worse performance on the Cubes Test ($P = 0.011$), RPMT ($P = 0.02$), Estimated IQ ($P = 0.021$), Execution IQ ($P = 0.04$), FAS Total ($P = 0.043$), FAS Letter F ($P = 0.056$), FAS Letter A ($P = 0.034$), FAS Letter S ($P = 0.049$), Logic Memory I ($P = 0.008$) and TMT-B ($P = 0.01$). Total IQ ($P = 0.086$) and Logic Memory II ($P = 0.075$) approached significance.

Discussion

To the best of our knowledge, this is the first study to report the cognitive effects of *APOE* genotype on BD. Main effects were seen with *allele* *3 and genotype *3*3, in which global deficits in cognition were very similar to AD with allele *4. Indeed, allele *4 was associated with CD, although effect was seen in fewer tests than for allele *3. We speculate that results differ from AD, where *4 is associated with more severe CD, due to important differences in the pathophysiology of AD and BD. The finding that



Footnote: Graphic displays significant neurocognitive dysfunction in individuals with the *3*3 genotype, as compared to other genotypes. Cubes Test (P=0.011), RPMT (P=0.02), Estimated IQ (P=0.021), Execution IQ (P=0.04), FAS Total (P=0.043), FAS Letter F (P=0.056), FAS Letter A (P=0.034), FAS Letter S (P=0.049), Logic Memory I (P=0.008) and TMT-B (P=0.01).

Figure 2 Scores on neurocognitive tests as a function of genotype.

allele *2 was associated with better cognitive function is supported by analyses from healthy subjects [11–13].

Despite the small size of our sample, we observed that women were more severely impaired in several domains, as contrasted to men. It may be that gender influences APOE distribution of plasma lipids and of apolipoproteins [36], and may affect APOE efficacy in redistributing myelin cholesterol for neurotrophic processes. Estrogen has a protective effect against age-related cognitive decline [37–39], and its replacement enhances memory in post-menopausal women [40,41].

Our data indicate that patients with *3*3 genotype have important memory and executive deficits regarding logical memory, working memory, planning, impulse inhibition, sequencing, and global measures of execution tasks, suggesting a pattern of fronto-temporal dysfunction. A recent study [42] demonstrated that BD patients have a disruption in fronto-temporal neural circuitry that may underlie memory and executive difficulties, often observed in patients with BD. However, genotype *3*3 was the most prevalent and our sample was small, this may have acted as a confounding factor. This limitation will be addressed when we have the final sample analyzed.

Studies on the *APOE* effect in nondemented individuals yielded inconsistent findings [43]. A recent meta-analysis [14] showed that allele *4 increased the risk of CD in cognitively healthy adults. We found statistically significant differences between groups with and without allele *4 across multiple cognitive domains, with carriers showing significantly poorer performance on measures of episodic memory, global cognitive ability, executive functioning and perceptual motor skills. We argue that allele *4 when associated with variables related to inflammatory processes may induce apoptosis, therefore being a risk factor for CD in younger individuals [44,14].

The *APOE* *2 allele has been associated with neuronal survival and longevity, being a protective factor against AD [12,45]. Very few studies analyzed the impact of *APOE* genotype in BD, and conclusive findings regarding the relationship between BD and neurocognition are lacking. Associations between *allele* *4 frequency and CD in BD have not been reported, even when cognition was assessed at different stages of the disorder (e.g., during depressive and euthymic periods).

The frequency of *allele* *4 is known to be the same for unipolar and bipolar patients [16]. Previous studies reported a significantly greater frequency of *allele* *4 among BD patients with psychotic features as compared to those without [16]. Although there is some evidence of an association between the *APOE**4 allele and a sub-sample of early onset BD with psychotic symptoms, no association with intermediate or late onset BD patients has been described [46]. Accordingly,

describing the association between *APOE* gene and specific mood disorders is of importance, since it may flag individuals with BD at risk of cognitive deterioration [46].

Conclusion

To our knowledge, this is the first study describing an association of *APOE* genotype and cognitive performance in BD. Even being a preliminary report, we consider it relevant to share our findings with the scientific community. Further and larger studies on *APOE* and cognition in BD are required to clarify the existence of the hypothesis of *APOE* being a cognitive endophenotype in BD.

Limitations of the present study include the small sample size and the absence of a control group.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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