Genetic Polymorphisms Influence Cognition in Patients Undergoing Carotid Interventions

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Abstract

While carotid interventions help decrease the risk of stroke, nearly 40% of patients experience cognitive deterioration. Genetic polymorphism in apolipoprotein E (*ApoE*) and brain-derived neurotrophic factor (*BDNF*) have been implicated in cognitive impairment; however, it is unclear whether they may influence cognitive changes in patients undergoing carotid intervention.

In this study, we seek to assess the role of genetic polymorphisms in carotid intervention-related cognitive change. Polymorphisms related to cognitive function were chosen for this preliminary analysis.

Over 2 years, patients undergoing carotid interventions were prospectively recruited. Patients underwent neuropsychological testing 2 weeks prior to and at 1 month following their procedure. Saliva samples were collected for genetic analysis. Logistic regressions were used to identify associations between polymorphisms and cognitive measures.

A total of 91 patients were included; all were male with an average age of 70 years. The majority of patients exhibited hypertension (95%) and a history of smoking (81%). Presence of *ApoE* 4 allele was associated with depression ($p = 0.047$). After correcting for age and genetic polymorphisms in *BDNF* and serotonin transporter (*5-HTT*), *ApoE* 4 allele was associated with depression ($p = 0.044$) and showed a trend with baseline cognitive impairment ($p = 0.10$). Age ≥ 70 years was associated with baseline cognitive impairment after adjusting for the three genetic polymorphisms ($p = 0.03$). Patients with *ApoE* 4 and *BDNF* A polymorphisms performed less well on the visual and verbal memory measures, respectively.

Polymorphisms in *ApoE* and *BDNF* may provide insight on cognition in patients undergoing carotid interventions; however, the mechanism of this relationship remains unclear.

Keywords

- internal carotid artery
- carotid stenosis
- genetic
- carotid endarterectomy
- carotid stent
- cognition
- polymorphism

Cognitive impairment is common among the elderly population. It was estimated that 35.6 million people had a diagnosis of dementia in 2010 and that this number would increase to 115.4 million by 2050.¹ With an increasing life expectancy and growing aging population, cognitive impairment has a

significant economic impact on our health care systems as individuals with cognitive deficiencies are admitted to the hospital three times more often than individuals with other conditions. Alzheimer disease (AD) and related conditions are estimated to be the third most costly condition to treat in the

United States.² Thus, there is a great need to better understand these diseases and to find solutions to decrease this public health burden.

Cognitive deficit has been observed across a broad range of patient populations, including those undergoing surgical interventions. Cognitive decline has been observed in patients undergoing coronary artery bypass graft surgery, as well as in patients undergoing noncardiac surgeries such as knee replacements.^{3,4} Age has been identified as an important risk factor for cognitive decline following surgery; therefore, decline is a relevant concern in patients undergoing vascular interventions as these patients tend to be in their later years of life. We and others have identified that nearly 40% of patients experienced cognitive decline following carotid intervention.⁵⁻⁷ While factors such as microembolization and other comorbidities have been identified as potential risk factors for cognitive change, genetic markers have not been well explored. In this study, we seek to determine whether genetic polymorphisms might provide additional insight on risk for cognitive decline following carotid intervention.

We focused our study on polymorphism in three genes: apolipoprotein E (*ApoE*), brain-derived neurotrophic factor (*BDNF*), and serotonin transporter (*5-HTT*). These genes were chosen for initial exploration due to their relatedness to cognition based on prior studies. *ApoE* has been widely associated with lipoprotein metabolism and cardiovascular disease. The *E4* allele has a frequency of approximately 14% and has been related to susceptibility of developing AD.⁸ A polymorphism in *5-HTT* results in a shortened version of the *5-HTT* protein. This “short” variation has been linked to depression and other psychiatric issues.⁹ *BDNF* is translated to a protein involved in neuronal growth and survival. The “A” allele of *BDNF* indicates a single-nucleotide polymorphism where methionine was substituted for valine and has been linked to brain dysfunction; however, its precise effect remains unclear.¹⁰ We herein contribute to the understanding of how these polymorphisms manifest themselves in an elderly population susceptible to cognitive decline.

Methods

Patient Selection

Patients with high-grade carotid artery stenosis, based on NACET duplex ultrasound criteria, and scheduled to undergo carotid artery interventions at the VA Palo Alto Health Care System were recruited to participate. Patients were offered carotid artery stenting (CAS) as an alternative to carotid endarterectomy (CEA) if they exhibited a high lesion on preoperative imaging, showed reversibility on persantine-thallium stress test, or had received prior radiation to the neck. Both symptomatic patients with >60% stenosis and asymptomatic patients with >80% stenosis were treated with carotid revascularization procedures in addition to aggressive medical therapy. Additional inclusion criteria required that the patient be of 40 years of age or older without significant psychiatric disorder, voluntarily participate in the study and sign an informed consent document approved by the Stanford

IRB, and be available for follow-up neuropsychological testing. Demographics and clinical risk factors were collected and entered into an electronic spreadsheet for each patient.

Neuropsychological Testing

Patients underwent neuropsychological testing prior to and at 1 month following carotid intervention. Preoperative sessions were conducted within 2 weeks prior to carotid intervention. Postoperative sessions were conducted at 1 month postintervention for all patients to avoid the potential influence of general anesthesia in the CEA cohort. Sessions included a battery of measures to evaluate executive function, motor speed and strength, visual and verbal memory, and language. The primary outcome measures analyzed were the Mini Mental Status Examination (MMSE) as a measure of general cognitive function, the Rey Auditory Learning Test (RAVLT) to evaluate verbal memory, the Rey–Osterrieth Complex Figure Test to evaluate visual memory, and the Geriatric Depression Scale (GDS) to determine mood and depression. Parallel forms of RAVLT were used to minimize practice effect. The Rey–Osterrieth measure consisted of three tasks: patients were asked to copy a complex figure, and then to recall it at immediate (5 min) and delayed (20 min) recall time points.

Genetic Analysis

Saliva samples were collected from all patients using Oragene 500 saliva collection kits (DNA Genotek Inc., Ontario, Canada). DNA was extracted and analyzed to determine allelic variation in each patient for three different genes: *ApoE*, *BDNF*, and *5-HTT*. Alleles for *ApoE* included the 2, 3, or 4 isoforms. For *BDNF*, either A or G alleles were reported. *5-HTT* alleles were reported as either short or long.

Statistical Analysis

Data were collected in an electronic spreadsheet and analyzed by a statistician using the R statistical computing environment, version 3.0.3.¹¹ Univariate logistic regression was used to search for associations between genetic polymorphisms, cognitive results, and clinical risk factors. Multivariate logistic regression models were chosen based on a causal path diagram¹² and used to evaluate relationships between genetic polymorphisms and cognitive data (→ Fig. 1). We calculated *p* values for two-sided Wald tests of the model coefficients, which are adjusted for the other variables in the model. We also estimated 95% confidence intervals for the adjusted odds ratios using the Wald method.

Results

A total of 91 patients undergoing carotid interventions were recruited and included in the study. All patients were male and had an average age of 70 years, ranging from 55 to 88 years. Of the 91 patients, 54 of the patients underwent CAS and 37 underwent CEA. Typical vascular disease risk factors were present, including diabetes in 41 (45%) of the patients, hypertension in 86 (95%), and CAD in 48 (53%). Forty (44%) of the patients were symptomatic: 18 patients had prior strokes and 22 had preoperative TIA symptoms. The majority of patients

Fig. 1 A causal pathway diagram was used to build the multivariate models. Models were designed based on variables (black outline) that were predicted to have an impact on outcomes (gray outline).

had a history of smoking (81%) and more than one-third (41%) had a history of severe alcohol abuse (► **Table 1**).

Carotid revascularization was successful for all patients, without incidence of major stroke, myocardial infarction, or cranial nerve injury. Average baseline MMSE was 27.7, and 21% of the patients scored <27, indicating cognitive impairment. Thirty-two percent of the patients exhibited baseline depression by scoring >9 on the GDS despite an absence of diagnosis of clinical depression. Forty-four percent of the patients (38) experienced a decline in RAVLT scores and were considered to have a decline in verbal memory. Similarly, 42% of the patients showed a decline in score on the Rey–Osterrieth Copy task, a measure of visual memory; however, only 24 and 18% showed decline on the immediate and delayed recall tasks, respectively.

Genetically, 25 (28%) patients carried one or two *ApoE* 4 alleles. Two patients had a 2/2 *ApoE* genotype that has been

associated with hyperlipoproteinemia type III, a rare lipid processing dysfunction. For the *5-HTT* gene, 62 (68%) of the patients carried the short allele of the gene that results in lower *5-HTT* transcription. Twenty-eight (31%) of the patients had one or more of the methionine single-nucleotide polymorphism for the *BDNF* gene.

Univariate analysis revealed significant associations between clinical risk factors and cognitive outcomes (► **Table 2**). Evidence of baseline depression was found to be associated with *ApoE* 4 allele ($p = 0.047$), current smoking ($p = 0.03$), and prior symptoms ($p = 0.01$). Additionally, current smoking had a significant negative univariate association with age ($p \leq 0.001$) and a positive association with prior symptoms ($p = 0.02$).

Using a multivariate model, depression was found to be associated with *ApoE* 4 allele after adjusting for age, procedure type, and genetic polymorphisms in *BDNF* and *5-HTT*

Table 1 Risk factors

Risk factor	N = 91
Diabetes	41 (45%)
Hypertension	86 (95%)
Obesity	38 (42%)
Coronary artery disease	48 (53%)
Smoking history	74 (81%)
Severe alcohol abuse history	37 (41%)
Symptomatic (prior stroke or TIA)	40 (44%)

Abbreviation: TIA, transient ischemic attack.

Table 2 Univariate analysis: depression is associated with *ApoE* 4, smoking, and prior symptoms

Variable	p Value
<i>ApoE</i> 4 allele	0.047
Current smoking	0.033
Symptomatic (prior stroke or TIA)	0.01
CAS	0.076
Contralateral occlusion	0.095

Abbreviation: CAS, carotid artery stenting; TIA, transient ischemic attack.

Table 3 Multivariate analysis: depression is associated with *ApoE* 4 after adjusting for age, procedure, and polymorphisms in *BDNF* and *5-HTT*

	GDS ≤ 9: N (%)	GDS > 9: N (%)	Adjusted OR estimate	OR CI 2.5%	OR CI 97.5%	p Value
(Intercept)	59 (64.84%)	32 (35.16%)	0.3556	0.1042	1.0857	0.0800
Age < 70	32 (58.18%)	23 (41.82%)	–	–	–	–
Age ≥ 70	27 (75.00%)	9 (25.00%)	0.3777	0.1313	1.0044	0.0586
CAS	31 (57.41%)	23 (42.59%)	2.5777	0.9854	7.2506	0.0608
≥1 <i>ApoE</i> 4 alleles	12 (48.00%)	13 (52.00%)	2.8074	1.0394	7.8533	0.0436
≥1 <i>BDNF</i> A alleles	18 (64.29%)	10 (35.71%)	0.9030	0.3134	2.5056	0.8462
≥1 <i>5-HTT</i> short alleles	40 (65.57%)	21 (34.43%)	0.9097	0.3362	2.5129	0.8524

Abbreviations: CAS, carotid artery stenting; CI, confidence interval; GDS, Geriatric Depression Scale; OR, odds ratio.

Table 4 Multivariate analysis: baseline cognitive dysfunction is associated with age ≥ 70 y after adjusting for polymorphisms in *ApoE*, *BDNF*, and *5-HTT*

	Baseline MMSE ≥ 27: N (%)	Baseline MMSE < 27: N (%)	Adjusted OR estimate	OR CI 2.5%	OR CI 97.5%	p Value
(Intercept)	72 (79.12%)	19 (20.88%)	0.0486	0.0087	0.1891	0.0001
Age < 70	47 (85.45%)	8 (14.55%)	–	–	–	–
Age ≥ 70	25 (69.44%)	11 (30.56%)	3.3949	1.1284	11.0853	0.0337
1+ <i>ApoE</i> 4 alleles	17 (68.00%)	8 (32.00%)	2.5706	0.8262	8.0373	0.0996
1+ <i>BDNF</i> A alleles	20 (71.43%)	8 (28.57%)	2.3834	0.7496	7.7811	0.1402
1+ <i>5-HTT</i> short alleles	46 (75.41%)	15 (24.59%)	2.0810	0.6213	8.4295	0.2604

Abbreviations: CI, confidence interval; MMSE, Mini Mental Status Examination; OR, odds ratio.

(► **Table 3**). Also, an association between baseline cognitive deficit (MMSE < 27) and age ≥ 70 years was identified after adjusting for all three polymorphisms (► **Table 4**). No significant associations were found between genetic polymorphisms and decline in verbal or visual memory using similar models.

When comparing average scores of patients with and without polymorphism in each gene, differences in performance were found for patients with *ApoE* 4 alleles and for patients with *BDNF* A alleles (► **Figs. 2** and **3**). Patients with the *ApoE* 4 allele had lower scores on the visual memory measure, compared with those who did not carry the 4 allele. Similarly, patients with the *BDNF* A risk allele did not score well on the verbal memory measure compared with those not carrying the A allele. No significant difference in memory performance was found for those carrying the *5-HTT* risk allele.

Discussion

In this prospective study, we demonstrate that genetic polymorphisms may provide additional insight on the cognition of patients undergoing carotid interventions. Personal genetics are currently being developed to become more applicable to clinical decision-making; this study contributes knowledge to this domain for an elderly population with vascular disease. Our findings suggest that there are differ-

ences between patients with and without genetic polymorphisms. Such knowledge could be used to help appropriately counsel patients on risks for cognitive change following surgery.

ApoE has been of interest to many groups due to its association with late-onset AD.⁸ Several studies have suggested that the *ApoE* 4 polymorphism is associated with impaired recovery following brain injury.¹³ Heyer et al looked specifically at *ApoE* genotype in patients undergoing CEA.¹⁴ They found that those with the 4 allele exhibited cognitive decline in multiple cognitive domains at 1 day and 1 month postoperatively. In our cohort, we did not find any association between *ApoE* 4 and cognitive decline following intervention; however, our data demonstrate that on an average patients carrying the 4 allele scored lower on visual memory tasks at 1 month postintervention (► **Fig. 2**). While the cohort as a whole appears to improve in performance on this measure, we believe that a learning effect may have falsely elevated the postinterventional scores. A slight trend ($p = 0.12$) with *ApoE* 4 and low baseline cognition, as measured by MMSE, was also observed. Additionally, we found an association between the 4 alleles and depression. These results suggest that while *ApoE* 4 may not have a direct impact on cognitive change following intervention, it may have an impact on cognitive function in general for this cohort. It is difficult to directly compare our results with those of Heyer et al as we included

Fig. 2 Patients carrying the *ApoE* 4 allele performed less well compared with those not carrying the 4 allele for the copy (A), immediate recall (B), and delayed recall (C) tasks of the visual memory measure.

Fig. 3 Patients carrying the *BDNF* A allele performed less well on the verbal memory task (Rey Auditory Learning Test) compared with those not carrying the A allele.

CAS patients in our study; however, we did correct the procedure type in our multivariate analysis.

While polymorphism in *ApoE* has been studied extensively, *5-HTT* and *BDNF* are less well characterized. Polymorphisms in these genes have been studied across several animal models and disease states in humans; however, there are limited data within the cardiovascular disease population. *BDNF* has been associated with depression and schizophrenia¹⁵; however, in

our present study, we found no association between the risk allele and depression. This may be because there are many other variables that influence depression that were not included in the scope of this study. While we also did not find any association between *BDNF* polymorphism and cognitive decline following intervention, patients carrying the risk allele for this gene performed less well on the verbal memory task compared with those not carrying the allele (—Fig. 3). While these differences did not quite reach significance, average scores were consistently lower at each of the testing time points (preoperative, postoperative, and 6 months postoperative) for patients with the polymorphism. Given this, we predict that *BDNF* polymorphism might have a negative impact on cognition in general, similar to our observations for those carrying the *ApoE* 4 allele.

The *5-HTT* short allele polymorphism has been linked with depression and anxiety in larger populations.¹⁶ Similar to our other analyses, we found no associations between *5-HTT* polymorphism and cognitive changes. Additionally, we did not find any difference in cognitive performance between patients carrying the *5-HTT* risk allele and those not carrying the allele. This may suggest that for these patients, *5-HTT* is not a modulator of cognition. It is also possible that we do not see cognitive associations replicated in our analysis due to the smaller size and specificity of our cohort.

There are several limitations to this study. Primarily, our sample size is relatively small and we were therefore not able to include a wide variety of variables in our multivariate models. Nevertheless, we included the most relevant variables that may impact cognitive performance in our multivariate analysis. Even when limiting our variables to procedure type, age, and the polymorphisms, our confidence intervals were still relatively wide, indicating that there is some uncertainty in the associations. We chose procedure type and age because we believed that these were the two most likely factors influencing cognitive differences between patients. Another limitation was the inclusion of patients who were either heterozygous or homozygous for each risk allele in the same analysis group. Due to the small number of patients who were homozygous for each polymorphism in our cohort, we identified patients as carriers if they had at least one allele exhibiting the polymorphism. This may limit the influence of polymorphism as individuals who are heterozygous for a given polymorphism may not experience the same effects as those who are homozygous. Since we did not perform a genomewide analysis of these patients, we also do not know what other genetic variations exist in these individuals that could be impacting cognitive function. Given the exploratory nature of this project, we focused our analysis on these three genes. An important next step to support and expand upon these findings would be to increase our cohort and to perform a more comprehensive genetic analysis.

Conclusion

Our findings suggest that genetic polymorphisms may be able to serve as risk factors for cognitive function in elderly patients undergoing carotid artery interventions. While the

specific relationships between polymorphism and cognition remain unclear, we demonstrate that patients carrying risk alleles for these genes perform less well on cognitive measures. Discerning these relationships is important to clarify the expectations for cognitive health following surgery to patients and their caregivers. Further investigation of these polymorphisms in patients with high-grade carotid artery stenosis is warranted to validate these findings.

Note

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