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Effects of the BDNF Val66Met polymorphism on functional status and disability in young stroke patients

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Data Availability Statement: The genetic data utilized in this study are available via request from the database of Genotypes and Phenotypes (dbGaP) at https://www.ncbi.nlm.nih.gov/gap/ Search Study Term: phs000292.v1.p1 The data is available upon request due to the the informed consent under which the data or samples were collected. Because of the sensitive nature of the data collected for this study, that could potentially be used to identify specific individuals, the phenotype and stroke outcomes data utilized in this study are available upon reasonable request.

Abstract

Background and purpose

The preponderance of evidence from recent studies in human subjects supports a negative effect of the BDNF Val66Met polymorphism on motor outcomes and motor recovery. However prior studies have generally reported the effect of the Met allele in older stroke patients, while potential effects in younger stroke patients have remained essentially unexamined. The lack of research in younger patients is significant since aging effects on CNS repair and functional recovery after stroke are known to interact with the effects of genetic polymorphisms. Here we present a study of first-ever ischemic stroke patients aged 15–49 years that examines the effect of Met carrier status on functional disability.

Methods

829 patients with a first ischemic stroke (Average age = 41.4 years, SD = 6.9) were recruited from the Baltimore-Washington region. Genotyping was performed at the Johns Hopkins University Center for Inherited Disease Research (CIDR). Data cleaning and harmonization were done at the GEI-funded GENEVA Coordinating Center at the University of Washington. Our sample contained 165 Met carriers and 664 non-Met carriers. Modified Rankin scores as recorded at discharge were obtained from the hospital records by study personnel blinded to genotype, and binarized into "Good" versus "Poor" outcomes (mRS 0–2 vs. 3+), with mRS scores 3+ reflecting a degree of disability that causes loss of independence.

Results

Our analysis showed that the Met allele conveyed a proportionally greater risk for poor outcomes and disability-related loss of independence with mRS scores 3+ (adjusted OR 1.73, 95% CI 1.13–2.64, $p = 0.01$).

Conclusions

The *BDNF* Val66Met polymorphism was negatively associated with functional outcomes at discharge in our sample of 829 young stroke patients. This finding stands in contrast to what

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would be predicted under the tenets of the resource modulation hypothesis (i.e. that younger patients would be spared from the negative effect of the Met allele on recovery since it is posited to arise as a manifestation of age-related decline in physiologic resources).

Introduction

It is a common observation that the effects of genetic variations become more prominent as the brain's physiologic resources decline with age. This interaction between aging and genetic polymorphisms has been referred to as the "resource modulation hypothesis", first articulated by Lindenberger et al in 2008 [1]. Several recent studies [2–4] have exemplified interactions of this sort for a common single nucleotide polymorphism (SNP) referred to as Val66Met, a missense mutation in the pro-domain of *BDNF* that leads to a methionine (Met) substitution for valine (Val) at codon 66. Multiple prior studies have examined the effects of the Val66Met polymorphism in older stroke patients, however, the effect of this polymorphism in younger stroke patients has been less well-studied. To address this knowledge gap, here we examined the effects of the Val66Met polymorphism on post-stroke functional outcomes in early-onset ischemic stroke patients aged 15–49 years.

Methods

Full details of the experimental protocol are reported elsewhere [5]. In brief, 829 participants with first ischemic stroke aged 15–49 years (average = 41.4, SD = 6.9) were recruited from the Baltimore-Washington region. Genotyping was performed at the Johns Hopkins University Center for Inherited Disease Research (CIDR). Data cleaning and harmonization were done at the GEI-funded GENEVA Coordinating Center at the University of Washington. These data had been collected previously at our clinical site as part of the "GENEVA" initiative (Gene Environment Association Studies) and "GEOS" study (Genetics of Early Onset Stroke). Modified Rankin scores at the time of discharge from the acute hospital were obtained from hospital records by raters blinded to the results of the genetic data. Scores were binarized for analysis into "Good" versus "Poor" outcomes (mRS 0–2 vs. 3+, respectively). We performed a case-only analysis, fitting a logistic regression model that included age, gender, and principle components to the data. This was constructed as an additive model to account for potential gene "dosage" effects (i.e. Val-Met vs. Met-Met). We did not model treatment effects (e.g. for thrombolysis), since the allocation of a given allele is random in the study population and therefore is not influenced by treatment factors. This study was conducted with the consent of all study subjects and was approved by the University of Maryland at Baltimore Institutional Review Board.

Results

Our sample contained 165 Met carriers and 664 non-Met carriers. **Table 1** details patient characteristics and risk factors. **Table 2** shows the association of Met carrier status with outcome, in the entire population and as stratified by ethnicity and gender. Analysis of the full sample comprising 829 cases revealed a significant association between the Met allele and mRS outcomes, where the Met allele conveyed a proportionally greater risk for poor mRS outcomes (adjusted OR 1.46, 95% CI 1.003–2.137, $p = 0.05$). When length of stay was added to the model (for n = 805 cases with available length-of-stay data) both the magnitude and significance of the association increased (adjusted OR 1.73, 95% CI 1.13–2.64, $p = 0.01$), likely because some

Table 1. Age, sex, race and risk factor characteristics.

	Cases (n = 829)
Age	
Mean age (years)	41.5 ± 6.9
Sex	
Female (%)	41.8
Male (%)	58.2
Self-Reported Race (%)	
European Ancestry (EA)	52.8
African Ancestry (AA)	42.2
Other	5.1
Stroke Risk Factors	
Hypertension (%)	43.1
Diabetes mellitus (%)	16.9
Current smokers (%)	42.1
Angina/MI (%)	5.5
≥ 1 Vascular Risk Factor* (%)	69.2

*Vascular risk factors: hypertension, diabetes mellitus, current smoking, angina/MI.

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of the random, non-genetic influences on outcome were removed. The effect was in the same direction across both ethnicities, and for males and females, but only reached significance in the male group. There was no significant difference in the proportion of stroke subtypes (i.e. lacunar, cardioembolic, atherosclerotic or indeterminate) within each genotype.

Discussion

BDNF plays a critical role in nervous system development and function, and perturbations in BDNF trafficking can lead to impairments in CNS function. The Val66Met polymorphism affects a region of the BDNF prodomain that interacts with secretory machinery, leading to reduced activity-dependent secretion of BDNF [6] and system-level motor function effects including reduced brain activation in the primary sensorimotor cortex contralateral to the moving limb [7].

Our analysis showed that the *BDNF* Val66Met polymorphism was negatively associated with functional outcomes at discharge in our cadre of young stroke patients. In general, the preponderance of evidence from recent studies in human subjects has supported a *negative effect* of the Met allele on motor outcomes and motor recovery. For example Kim et al. showed

Table 2. Results of case-only analysis using an additive model that accounted for sex, age, and principal components.

	n (Met/Non-Met)	Odds Ratio (mRS 0–2 vs. 3+)	*p*	*CI*
All cases	829	0.68	0.05	0.47–0.997
Ethnicity				
European	448	0.73	0.14	0.48–1.11
African-American	381	0.50	0.11	0.21–1.16
Gender				
Male	482	0.57	0.03	0.34–0.95
Female	347	0.80	0.43	0.49–1.41

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poorer motor outcomes in Met carriers on the Fugl-Meyer at 30 and 90 days [8]. Helm et al. [9] and Charalambous et al. [10] found a slower rate of motor adaptation in Met carriers. Shiner et al. reported reduced post-therapy motor improvement in Met carriers with moderate to high motor function [11]. Van der Vliet et al. showed diminished motor learning for Met carriers [12]. Apart from these behavioral studies, negative effects are also reported in neuroimaging and neurostimulation studies. For example, Kim et al. showed decreased brain activation in Met carriers with hemiparesis 12 months post-stroke [7], and Fridriksson et al. showed that Met carriers were less likely to benefit from A-tDCS for aphasia [13]. Some studies, however, have reported *no effect* of the polymorphism on motor outcomes or treatment response. For example, French et al. demonstrated that the *BDNF* genotype did not account for differences in functional mobility at 6 months [14]. Others have even reported the *opposite effect*, including Quin et al. [15] who reported an adaptive role for the Met allele in their rodent model of stroke, and Mirowska-Guzel et al. who showed less favorable stroke rehabilitation outcomes in *non*-Met carriers in the first 90 days post stroke [16].

This inconsistency in the direction of findings across studies could be attributed to many factors, including ethnicity, gene-gene interactions, stroke severity, or—germane to the present study—variations in age. Age effects can furthermore have sex-specific interactions due to changes in hormone expression across the lifespan. Our analysis showed a significant effect of the Met allele in males but not females, and while it is possible that sample size alone accounts for this finding (n = 482 males vs. 347 females), the potential for a protective effect of estrogen in this sample of young females also warrants comment. Sex differences in brain BDNF expression have previously been shown in animal studies to vary with estradiol levels [17]. Furthermore in human studies, plasma BDNF levels are higher during the luteal phase of the menstrual cycle [18]. The effects of estrogen on stroke risk and stroke recovery are complex, with known protective effects of 17β-estradiol on stroke risk for women compared to men [19], but also increased stroke risk with high estrogen levels during pregnancy or with the use of high-estrogen oral contraceptives. Estrogen effects on stroke severity and stroke recovery are less well-studied. The possibility that the BDNF Val66Met polymorphism may have a sexually dimorphic effect on stroke recovery is thus an important consideration in study design.

A limitation to the interpretation of our findings is that baseline NIHSS and mRS data were not available for all patients in our sample, hence the possibility that initial stroke severity was greater for Met carriers, or that pre-hospitalization functional deficits were present, cannot be entirely ruled out. The lack of appropriate baseline and longitudinal measures in large data sets is a key methodological concern for stroke outcomes research in general, with such data frequently missing from clinical stroke registries and administrative databases. For example, despite its well-established clinical significance, NIHSS documentation in the first 5 years of the Get With The Guidelines-Stroke Program (GWTG) was missing in 60.3% of cases [20]. The significance of this problem is now becoming increasingly clear as the field of stroke genetics continues to expand beyond its initial focus on factors affecting stroke *risk* to those factors affecting stroke *recovery*. While stroke *outcomes* can be measured at a discrete time point without consideration of baseline status, *recovery per se* is a relative measure and necessitates reference to a defined baseline. Recognizing the critical significance of this issue for stroke recovery and rehabilitation trials, our research group is actively engaged with the International Stroke Genetics Consortium Global Alliance to generate a consensus statement outlining common data elements and data collection timepoints for appropriate baseline and longitudinal measures in stroke genetics studies (manuscript in review).

In closing, to our knowledge this is the first study to analyze the effects of the *BDNF* Val66Met polymorphism in early-onset stroke patients. The average age across a sample of recent studies was 60.3 years (SD 9.4), while the average age in our sample was 41.9 years (SD 6.8).

Our finding that the Met allele carried a proportionally greater risk for poor mRS outcomes among young stroke survivors stands in contrast to what would be predicted under the tenets of the resource modulation hypothesis (i.e. that younger patients would be spared from the negative effect of the Met allele on recovery since it is posited to arise as a manifestation of age-related decline in physiologic resources). (1–4) This novel finding warrants replication in other cohorts of early-onset stroke subjects.

Author Contributions

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