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The Met Allele of the BDNF Val66Met Polymorphism Predicts Poor Outcome Among Survivors of Aneurysmal Subarachnoid Hemorrhage

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Background and Purpose—Brain-derived neurotrophic factor (*BDNF*) plays a role in neuronal survival, plasticity and neurogenesis. The *BDNF* gene contains a common Val66Met polymorphism; the Met allele is associated with lower depolarization-induced *BDNF* release and differences in memory functions and brain morphology. We hypothesized that the Met allele is associated with poor recovery from subarachnoid hemorrhage.

Methods—A sample of 105 survivors was assessed at 3 months after subarachnoid hemorrhage using Glasgow Outcome Scale. Poor outcome was defined as severe disability or worse. DNA samples were genotyped for the Val66Met polymorphism.

Results—Higher percentage of the Met carriers had a poor outcome (29%) as compared with the Val/Val group (10%; $P=0.011$). In multiple logistic regression, this association between the Met allele and poor outcome was independent of several other prognostic factors such as patient age, clinical condition, and radiological severity of the bleeding (odds ratio 8.40; 95% CI, 1.60 to 44.00; $P=0.012$).

Conclusions—Genetically influenced variation in *BDNF* function plays a role in recovery from subarachnoid hemorrhage. These data indicate that augmentation of *BDNF* signaling may be beneficial to recovery from brain injury. (*Stroke*. 2007;38:2858-2860.)

Key Words: BDNF ■ polymorphism ■ outcome ■ SAH

Aneurysmal subarachnoid hemorrhage (SAH) is a severe medical illness, which among survivors is associated with disability, cognitive deficits, psychosocial impairments and lower quality of life.¹ Age, neurological condition on admission, and factors related to the severity and complications of SAH are predictive of mortality and recovery.^{1,2} However, a significant amount of variation in the long-term recovery from SAH remains unexplained.

Brain-derived neurotrophic factor (*BDNF*) plays a major role in neuronal survival, neurogenesis and synaptic plasticity.³ For example, *BDNF* attenuates glutamate toxicity and rescues cerebellar neurons from cell death. Animal studies show that *BDNF* reduces ischemic injury and improves functional recovery and postinjury regeneration.^{4,5}

BDNF contains a Val66Met polymorphism in the 5' proregion of the protein. In vitro studies using transfected neurons suggest that the Met allele decreases *BDNF* trafficking into secretory granules. Furthermore, lower depolarization-induced *BDNF* secretion in the Met-*BDNF* transfected neurons was reported while constitutive secretion in these neurons

remained unchanged.⁶ In humans, increasing body of evidence supports that the Val66Met is associated with a complex neuronal phenotype involving memory and subtle differences in brain morphology.^{6,7,8,9} For example, subjects with the Met allele have lower performance in tests measuring episodic memory, and lower hippocampal and prefrontal cortical gray matter volumes.^{6,8}

Considering the role of *BDNF* in neuronal survival and neuroplasticity, we hypothesized that the Met allele is associated with poor outcome of recovery as compared with the Val allele among SAH survivors.

Materials and Methods

Subjects

Subjects of this study were 105 SAH survivors who had participated in a clinical trial testing the efficacy of enoxaparin on the outcome of SAH¹⁰ (Table 1). The results of the study, which were negative, were reported earlier.¹⁰ All patients were assessed using the Glasgow Outcome Scale^{1,2,10} at the outpatient department 3 months after the SAH. Poor outcome was defined by Glasgow Outcome Scale as severe disability or worse. All patients were rated by J.S. who was

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Table 1. Characteristics of 105 Patients With SAH

| | Met (n=34) | Val/Val (n=71) |
|---|--------------|----------------|
| Female | 14 (41%) | 38 (54%) |
| Age (mean±SD) | 46.1 (±10.0) | 48.4 (±11.8) |
| Plasma glucose mmol/L ±SD on admission | 8.42 (±2.16) | 8.06 (±2.20) |
| D-dimer, µg/L ±SD after surgery | 1095 (±1106) | 1270 (±1093) |
| Fisher grade 3* | 24 (71%) | 49 (69%) |
| IVH | | |
| no | 19 (56%) | 39 (55%) |
| slight | 12 (35%) | 27 (38%) |
| moderate to severe | 3 (9%) | 5 (7%) |
| ICH >10 mm in diameter | 3 (9%) | 4 (6%) |
| Symptoms of spasm | | |
| no | 26 (77%) | 55 (78%) |
| reversible | 3 (9%) | 6 (9%) |
| fixed | 5 (15%) | 10 (14%) |
| WFNS grade on admission | | |
| I | 23 (68%) | 39 (55%) |
| II–III | 6 (18%) | 17 (24%) |
| IV–V | 5 (15%) | 15 (21%) |

*Defined as >1 mm of blood in vertical layers in CT.

IVH indicates intraventricular hemorrhage; ICH, intracerebral hemorrhage; WFNS, World Federation of Neurosurgical Societies.^{1,2,10}

blind to the genotypes of the subjects. Neurological condition on admission was determined using the World Federation of Neurological Surgeons (WFNS) scale.^{1,2,10} All subjects were Finnish.

Genetic Analysis

Genomic DNA was extracted using the Gentra PureGene kits from buccal cells. *BDNF* Val66Met was genotyped using PCR-RFLP.¹¹ About 25% of the samples were analyzed as duplicate for quality control purposes with perfect concordance of the results. Met/Met homozygotes (n=3) were grouped together with Met/Val heterozygotes (n=31). The Met allele frequency in this population was 18%.

Statistical Analysis

For univariate statistics, conventional statistical tests were used. Univariate and multivariate odds ratios and 95% CI of risk factors, including their interactions, for poor outcome at 3 months were analyzed with unconditional logistic regression. The variables tested included established factors for poor outcome: age, clinical condition (WFNS grade), presence of intraventricular bleeding, intracerebral hemorrhage >10 mm in diameter, Fisher grade 3 (defined as >1 mm of blood in vertical layers in CT), plasma glucose and D-dimer concentrations on admission.^{1,2}

Results

Of the subjects with the Met allele (n=34), 29% had a poor outcome (n=10) and 71% had a favorable outcome (n=24). Of the Val/Val subjects, 10% had a poor outcome (n=7) and 90% had a favorable outcome (n=64; $P=0.011$). Multiple logistic regression analysis showed that the Met allele is associated with poor outcome independent of other prognostic factors (Table 2).

Discussion

The major finding in this study is that the Met allele of the *BDNF* Val66Met polymorphism is associated with poor

Table 2. Risk Factors for Poor Outcome After Aneurysmal SAH

| | Univariate | | Multivariate | |
|---|------------|---------------|--------------|-------------|
| | OR | 95% CI | OR | 95% CI |
| Age (per year) | 1.05 | (1.00–1.11) | 1.08 | 0.98–1.20 |
| Plasma glucose at admission, per mmol/L | 1.33 | (1.05–1.68)* | 1.13 | 0.79–1.61 |
| D-dimer, per mg/L | 1.86 | (1.19–2.90)† | 1.82 | 0.98–3.40 |
| WFNS | | | | |
| 1 | 1 | | 1 | |
| 2–5 | 3.21 | (1.08–9.50)* | 3.63 | 0.74–17.97 |
| IVH | 5.16 | (1.56–17.14)† | 4.32 | 0.82–22.73 |
| Fisher 3** | 3.88 | (0.83–18.09) | 2.62 | 0.17–41.33 |
| ICH | 5.69 | (1.50–21.58)* | 7.60 | 1.12–51.48* |
| Met allele | | | | |
| No | 1 | | 1 | |
| Yes | 3.81 | (1.30–11.15)* | 8.40 | 1.60–44.00* |

OR indicates odds ratio; WFNS, World Federation of Neurosurgical Societies;^{1,2,10} IVH, intraventricular hemorrhage.

* $P<0.05$; † $P<0.01$.

**Defined as >1 mm of blood in vertical layers in CT.

ICH >10 mm in diameter.

outcome of recovery at 3 months after SAH. In our sample, 29% of the Met carriers had a poor outcome whereas in the Val/Val genotype group 10% of the subject had this outcome ($P=0.011$). The effect of the Met allele was independent of other known predictors of poor outcome (Table 2). These findings are in agreement with the data supporting the role of *BDNF* in neuronal survival and neuroplasticity.^{4,5} Furthermore, these data suggest that targeting *BDNF* signaling may promote recovery from SAH.

Although these data do implicate *BDNF* in recovery from SAH, they do not tell us about the mechanisms leading to better functioning. Glasgow Outcome Scale is a simple clinician-rated instrument used to assess subject's level of disability irrespective of whether the disability is due, for example, to cognitive or motor deficits.^{1,2,10} Future studies should address whether the effects of the Val66Met are mediated through cognitive mechanisms, such as memory, as suggested by previous studies.^{6,7,9}

One caveat of this study is that the sample is not representative of all SAH survivors. Because enrollment to this study was through a previous clinical trial, survivors with the most complicated cases of SAH were excluded.¹⁰ Thus, the population in this study is biased toward better outcome. On the other hand, reduction in clinical variation by exclusion of subjects with severe SAH, among whom genetic effects on recovery are unlikely to be seen, may have allowed the *BDNF* effect to be discovered. Regardless of this sampling bias, we feel that the findings are important and open new vistas toward understanding neurobiological mechanisms contributing to recovery from brain trauma. This bias should be borne in mind, however, if attempts are made to replicate these findings. Another caveat of the study is that the sample size was relatively small. Replication attempts in independent populations are thus warranted. Considering that all subjects

were Finns, we feel that likelihood of the findings resulting from population stratification is low.

In summary, these data support that genetically influenced variation in *BDNF* function is associated with recovery from SAH. These findings suggest that targeting BDNF signaling may enhance recovery from brain injury.

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Disclosures

None.

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