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Correlation between genetic polymorphisms and stroke recovery

Analysis of the GAIN Americas and GAIN International Studies

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Background and purpose: Recovery after stroke occurs on the basis of specific molecular events. Genetic polymorphisms associated with impaired neural repair or plasticity might reduce recovery from stroke and might also account for some of the intersubject variability in stroke recovery. This study hypothesized that the ApoE ϵ4 polymorphism and the val^{66}met polymorphism for brain-derived neurotrophic factor (BDNF) are each associated with poorer outcome after stroke. Associations with mitochondrial genotype were also explored.

Methods: Genotypes were determined in 255 stroke patients who also received behavioral evaluations in the Glycine Antagonist In Neuroprotection (GAIN) clinical trials. The primary outcome measure was recovery during the first month post-stroke, as this is the time when neural repair is at a maximum and so when genetic influences might have their largest impact. Two secondary outcome measures at 3 months post-stroke were also examined.

Results: Genotype groups were similar acutely post-stroke. Presence of the ApoE ϵ4 polymorphism was associated with significantly poorer recovery over the first month post-stroke ($P = 0.023$) and with a lower proportion of subjects with minimal or no disability (modified Rankin score 0–1, $P = 0.01$) at 3 months post-stroke. Indeed, those with this polymorphism were approximately half as likely to achieve minimal or no disability (18.2%) versus those with polymorphism absent (35.5%). Findings were confirmed in multivariate models. Results suggested possible effects from the val^{66}met BDNF polymorphism and from the R0 mitochondrial DNA haplotype.

Conclusions: Genetic factors, particularly the ApoE ϵ4 polymorphism, might contribute to variability in outcomes after stroke.

Spontaneous recovery occurs after a stroke but is highly variable and generally incomplete. Studies in animals have provided molecular insights into the basis of this recovery, indicating that the brain undergoes spontaneous repair and remodeling after a stroke. A better understanding of the molecular events underlying stroke recovery in humans would be useful to advance application of restorative therapeutics [3].

Genetic polymorphisms have provided useful insights at the molecular level in a number of domains of stroke research, such as pathogenesis [4] and therapy [5]. Although disease-related states can be influenced by numerous genes, a single gene can sometimes have substantial impact, through the consequences of specific gene polymorphisms. The current study examined the influence on stroke recovery of polymorphisms in two genes related to neural repair, in a phase III clinical stroke trial setting. The first polymorphism was the ApoE ϵ4 allele, which is associated with impairment of some neural repair processes [6,7] as well as increased neurologic risk in several settings [8–10]. The second was the val^{66}met brain-derived neurotrophic factor (BDNF) polymorphism, which is associated with reduced activity-dependent BDNF secretion, reductions in some forms of brain plasticity, and changes in brain structure and function [11,12].

The main hypothesis tested in the current study was that the ApoE ϵ4 polymorphism and the BDNF val^{66}met polymorphism are each associated with poorer recovery from stroke. The primary end-point was recovery over the first month post-stroke, when neural

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repair is maximal and thus genetic influences might be strongest. Two secondary end-points examined recovery and disability at 3 months after stroke.

An additional exploratory hypothesis was that mitochondrial DNA (mtDNA) haplotype also influences recovery after stroke. The rate of novel mutations in mtDNA is 10-fold higher compared to nuclear DNA, which has resulted in specific haplotype groups around the world. Studies suggest that mtDNA haplotype is associated with a number of clinical conditions such as degenerative diseases [13] and risk of stroke [14,15], and is important to the energy metabolism and so potentially to brain reformatting in the setting of stroke recovery.

Methods

Subjects

This report describes a subset of patients from the GAIN clinical trials. Sixty-seven of the 305 participating sites in the GAIN Americas [1] and GAIN International [2] trials, representing 17 of the 23 GAIN enrollment countries, added to the protocol the possibility to offer blood drawing to patients on a voluntary basis for later genotyping. Of the 3493 patients treated in the two GAIN studies, 255 (114 from GAIN Americas and 141 from GAIN International) also consented to have blood drawn and stored for later testing in this manner. Note that baseline features of the current cohort, as versus the overall GAIN trial enrollees, were very similar, for example, mean age 69 vs. 70 years, median NIHSS score 11 vs. 12, 18% vs. 15% with intracerebral hemorrhage, and 21% vs. 25% diabetic. As part of the GAIN studies, prior to any enrollment, personnel at each site were trained to score the NIH Stroke Scale (NIHSS) and modified Rankin Scale (mRS), including certification for NIHSS. The NIHSS was assessed acutely (immediately prior to treatment, that is, within 6 h of stroke onset) as well as 1 and 3 months post-stroke. Assessments also included mRS at 3 months post-stroke. Inclusion criteria for both GAIN trials included treatment started <6 h after stroke onset, age $\geq$18 years, limb weakness, and pre-stroke independence (pre-stroke mRS score $\leq$1). Exclusion criteria included unresponsiveness and rapidly improving symptoms. All research was approved by appropriate Institutional Review Boards.

Genotyping

Blood was aliquoted and stored at −80°C, and genomic DNA was extracted from white blood cells using the standard DNA isolation technique. The val^{66}met BDNF polymorphism and ApoE ϵ4 polymorphism, as well as mitochondrial DNA (mtDNA) haplotype, were then tested at the University of California, Irvine. A subject identifier linked stored blood samples with clinical data, and all samples were de-identified and anonymized prior to genotyping so that DNA analyses could be performed blinded to clinical data.

The presence of the BDNF val^{66}met polymorphism was determined as described previously [12]. ApoE genotype was assessed by PCR–RFLP (Restriction Fragment Length Polymorphism) analysis, as described elsewhere [16]. Determination of mtDNA haplotype was carried out by analysis of the hypervariable sequences of the non-coding region and by PCR–RFLP of the mtDNA coding region. Selected RFLP haplotype markers, defining the major mitochondrial haplogroups, were analyzed according to Torroni *et al.* [17].

Data analysis

The main study hypothesis was that the ApoE ϵ4 and the BDNF val^{66}met polymorphism, each of which has been linked with a reduction in certain neural repair or plasticity events, are each related to poorer outcome after stroke. Three outcome measures were examined. The primary outcome measure for the current analyses was behavioral recovery over the first month after stroke, the time when molecular reformatting of the brain is at a maximum after stroke [3] and so the timepoint when genetic factors might have their largest impact. Recovery was expressed as change in NIHSS score from acute stroke to 1 month post-stroke. The two secondary end-points were recovery over the 3 months after stroke, the time when spontaneous improvement reaches a final plateau for most patients, expressed as change in NIHSS score from acute stroke to 3 months post-stroke; and the probability of reaching minimal or no disability at 3 months post-stroke, expressed as the proportion of patients with mRS score 0–1.

Study hypotheses were first examined for bivariate relationships. Most outcome measures were not normally distributed and could not be transformed to a normal distribution, and so non-parametric statistical methods were used for bivariate analyses (Wilcoxon rank sums testing, Fisher's exact, or Chi-square tests, as appropriate). Because neither GAIN study found a difference between placebo and active treatment, these two arms were combined. Analyses used $\alpha < 0.05$ and were two-tailed. The two analyses of the NIHSS score were corrected for multiple comparisons and thus had threshold for significance of $P < 0.025$. Ten subjects died by 1 month, and 10 more by 3 months, during the study; for these subjects, missing data were imputed by assigning the poorest value for each scale. Amongst

subjects with missing scores not attributed to death, last available values were carried forward. Power calculations suggested a total of 84 patients needed to obtain 80% power, with 2-sided $\alpha < 0.05$, to detect a difference in proportion of subjects with favorable outcome of 0.28, which is the value reported for ApoE $\epsilon 4$ in relation to proportion of subjects with good outcome (Glasgow Outcome Scale score = 5) after traumatic brain injury [8]. The study was therefore not powered to detect a gene dose effect. Because the number of subjects with two copies of either polymorphism was small (Table 1), primary analysis therefore excluded any subjects with two polymorphism copies, although a secondary analysis was performed that included all subjects, with polymorphism status treated as 'present' versus 'absent'. An additional secondary analysis examined only those subjects with ischaemic stroke.

Study hypotheses were next examined using multivariate modeling, which adjusted for confounding variables and provided further insight into the independent relationships between the two genotypes of interest. A stepwise (P values of 0.25 to enter, 0.1 to leave) forward nominal logistic model was constructed for predicting the proportion of subjects with minimal or no disability at 3 months. For both of the genotypes of interest, the variable 'polymorphism present versus absent' was entered into the model, along with nine covariates: presence of hypertension, presence of diabetes mellitus, current smoking status, age, gender, treatment arm to which randomized, GAIN study into which subject was enrolled, stroke classification (intracerebral hemorrhage versus ischaemic), and baseline stroke severity (acute NIHSS score).

Additional secondary analyses explored the relationship between outcomes and mitochondrial haplotype. A range of haplogroups was considered, including from Caucasian (R0, including H and HV, U, and JT), African (L), and Asian haplogroups (M and N). Based on mitochondrial phylogeny, mtDNA haplotypes were clustered into one of six macrohaplogoups (R0, N, U, L, M, or JT) for statistical analysis. Outcomes were examined across the six haplotypes, and *post hoc* tests examined outcome in relation to each haplotype, the latter using corrected significance threshold of $P < 0.0083$.

Results

Genotype distributions

Of the 255 with stored blood, nine subjects had two copies of the val^{66}met polymorphism, two had two copies of the ApoE $\epsilon 4$ polymorphism, and one had two copies of both polymorphisms, leaving 241 subjects for primary analyses (subjects with 0–1 copies of either polymorphism) and 255 for secondary analyses (all subjects, that is, 0–2 copies of either polymorphism). The incidence of these two polymorphisms was not correlated ($P = 0.64$), and each was in Hardy–Weinberg equilibrium.

Table 1 Baseline characteristics according to BDNF and ApoE genotype

	No. of copies BDNF val^{66}met polymorphism			No. of copies ApoE $\epsilon 4$ polymorphism		
Measure	0	1	2	0	1	2
No. of subjects	158	85	12	195	57	3
Age (in years)	69.9 ± 12.5	67.8 ± 12.7	64.3 ± 13.3	69.0 ± 12.8	68.6 ± 12.1	68.5 ± 12.7
Gender	54%M/46%F	58%M/42%F	83%M/17%F	57%M/43% F	54%M/46% F	67%M/33%F
Ethnicity	89%C/5% A/6% O	73%C/26% A/1% O	58% C/42% A/0% O	83%C/14%A/3%O	80%C/14%A/6%O	100%C
Hypertension, %	57	68	67	61	61	67
Diabetes mellitus, %	22	20	25	21	31	33
Hypercholesterolemia, %	20	25	8	18	28	33
Index stroke was intracerebral hemorrhage, %	15	24	25	19	18	0
Died by 3 months post-stroke, %	8	9	0	7	12	0
NIH Stroke Scale score (acutely)	11.5	10.0	8.0	11.0	12.0	12.0

BDNF, brain-derived neurotrophic factor.

Values for behavioral measures are median; for age, mean ± SD; C = Caucasian, A = Asian, O = Other (Black, Hispanic, Other).

Primary analysis focused on the 241 subjects with 0–1 copies of either polymorphism, amongst whom ethnicity ($P < 0.0001$) varied according to BDNF val^{66}met polymorphism status. No baseline measured varied according to ApoE $\epsilon 4$ polymorphism status. The same was true in a secondary analysis that examined all 255 subjects for presence or absence of each polymorphism.

Baseline data

Genotype groups had overall similar characteristics at baseline (Table 1). Amongst the 241 subjects with 0–1 copies of either polymorphism, ethnicity ($P < 0.0001$) varied according to whether the val^{66}met BDNF polymorphism was present or absent (a higher proportion of Asian subjects had the val^{66}met BDNF polymorphism, as expected [18]), and no measure varied according to ApoE ϵ4 status. The same was true when baseline data were examined for the 197 of these patients with ischaemic stroke. This was also true when all 255 subjects were analyzed.

Genotype and recovery over the first month after stroke

Bivariate analysis of the primary end-point, change in NIHSS score from acute to 1 month after stroke amongst the 241 subjects with 0–1 copies of either polymorphism, found that presence of the ApoE ϵ4 polymorphism was associated with poorer recovery ($P = 0.023$, Table 2): subjects with this polymorphism ($n = 55$) had a median change in NIHSS score of only 4 points [interquartile range (IQR) 0–5], compared to 5 points [IQR 2–8] amongst those lacking the polymorphism ($n = 186$). Similar results were suggested for the presence of the BDNF val^{66}met polymorphism ($P = 0.036$), where those with the polymorphism ($n = 83$) had a median change in NIHSS score of only 4 points [IQR 1–6], compared to 5 points [IQR 2–8] amongst those lacking this polymorphism ($n = 158$). When the 197 patients with ischaemic stroke were analyzed for the presence or absence of each polymorphism (Table 3), the ApoE ϵ4 polymorphism remained associated with poorer recovery over the first month ($P = 0.047$). Results from a secondary analysis that included all 255 subjects did not reach significance for either genotype.

Genotype and outcomes at 3 months after stroke

Bivariate analysis was also performed on the two secondary end-points. The first of these was mRS score of 0–1 at 3 months after stroke, which indicates minimal or no disability. Amongst the 241 subjects with 0–1 copies of either polymorphism, presence of ApoE ϵ4 polymorphism was associated with a significantly ($P = 0.01$) lower proportion of patients achieving mRS score of 0–1 at 3 months after stroke [18.2% (10/55 patients) when polymorphism present; 35.5% (66/186 patients) when polymorphism absent; see Table 2 and Fig. 1a]. Presence of the ApoE ϵ4 polymorphism thus nearly doubled the risk of having significant disability at 3 months post-stroke. This too was confirmed in the 197 patients with ischaemic stroke ($P = 0.018$, Table 3), where a lower proportion of those with the ApoE ϵ4 polymorphism present had mRS score of 0–1 at 3 months compared to those with this polymorphism absent [19.6% (9/46 patients) when polymorphism present; 37.8% (57/151 patients) when

Table 2 Outcome measures according to BDNF and ApoE genotype for all patients

	No. of copies BDNF val^{66}met polymorphism			No. of copies ApoE ϵ4 polymorphism		
Measure	0	1	2	0	1	2
No. of subjects	158	85	12	195	57	3
Recovery over the first month (change in NIHSS score from acute to 1 month after stroke)	−5 (−1.75 to −8)	−4 (−1 to −6)	−6.5 (−0.5 to −8)	−5 (−1 to −8)	−4 (0 to −6)	−6 (−4 to −10)
Recovery over the first 3 months (change in NIHSS score from acute to 3 months after stroke)	−6 (−2 to −10)	−5 (−1.5 to −7.5)	−6.5 (−1.5 to −8.75)	−6 (−2 to −9)	−5 (0 to −8)	−9 (−8 to −10)
Minimal or no disability at 3 months (proportion of subjects with mRS score of 0–1 at 3 months)	51 (32%)	25 (29%)	8 (67%)	71 (36%)	12 (21%)	1 (33%)

BDNF, brain-derived neurotrophic factor.

Primary analysis focussed on the 241 subjects with 0–1 copies of either polymorphism and found that recovery over the first month was poorer when either the ApoE ϵ4 ($P = 0.023$) or BDNF val^{66}met ($P = 0.036$) polymorphism was present, and that the proportion of subjects with minimal or no disability at 3 months was lower ($P = 0.01$) amongst those in whom the ApoE ϵ4 polymorphism was present. A secondary analysis of all 255 subjects found that presence of ApoE ϵ4 was associated with a lower proportion of subjects with minimal or no disability at 3 months as compared to subjects in whom this polymorphism was absent ($P = 0.029$), but that presence or absence of the BDNF val^{66}met polymorphism was not related to any of the three outcomes. There was no interaction of any of these results with gender. Note that a negative value for NIHSS score change indicates clinical improvement. NIHSS values are median (IQR); mRS values, number of subjects (percent genotype).

Table 3 Outcome measures according to BDNF and ApoE genotype for patients with ischaemic stroke

	No. of copies BDNF val^{66}met polymorphism			No. of copies ApoE ϵ4 polymorphism		
Measure	0	1	2	0	1	2
No. of subjects	134	65	9	158	47	3
Recovery over the first month (change in NIHSS score from acute to 1 month after stroke)	−5 (−1 to −8)	−4 (−1 to −6)	−7 (−2 to −9)	−5 (−1 to −7)	−4 (0 to −6)	−6 (−4 to −10)
Recovery over the first 3 months (change in NIHSS score from acute to 3 months after stroke)	−6 (−2 to −9)	−5 (−1.5 to −7.5)	−7 (−3 to −9)	−6 (−2 to −9)	−5 (0 to −8)	−9 (−8 to −10)
Minimal or no disability at 3 months (proportion of subjects with mRS score of 0–1 at 3 months)	46 (34%)	20 (31%)	6 (67%)	61 (39%)	10 (21%)	1 (33%)

BDNF, brain-derived neurotrophic factor.

Primary analysis focussed on the 197 subjects with ischaemic stroke who had 0–1 copies of either polymorphism and found that recovery over the first month ($P = 0.047$) and the proportion of subjects with minimal or no disability at 3 months ($P = 0.018$) each varied according to ApoE ϵ4 status. A secondary analysis of all 208 subjects with ischaemic stroke found that presence of ApoE ϵ4 was associated with a lower proportion of subjects with minimal or no disability at 3 months as compared to subjects in whom this polymorphism was absent ($P = 0.025$). Note that for none of the three outcome measures did results differ according to whether the stroke was ischaemic or hemorrhagic. A negative value for NIHSS score change indicates clinical improvement. NIHSS values are median (IQR); mRS values, number of subjects (percent genotype).

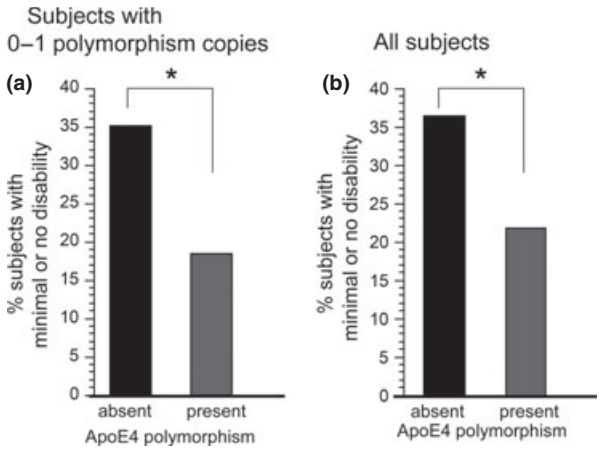

Figure 1 ApoE ϵ4 is associated with higher risk of disability after stroke. (a) In primary analysis of the 241 subjects with 0–1 polymorphism copies, the proportion of subjects with minimal or no disability (mRS score 0–1) 3 months post-stroke varied significantly ($P = 0.01$) in relation to ApoE ϵ4 status (absolute risk reduction for minimal or no disability with ApoE ϵ4 polymorphism present = 17.3%). (b) This remained true for a secondary analysis that included all 255 subjects for polymorphism presence versus absence (absolute risk reduction for ApoE ϵ4 absent, 14.7%, $P = 0.029$). *$P < 0.03$. Note that in neither case did the proportion of subjects with minimal or no disability 3 months post-stroke vary with brain-derived neurotrophic factor val^{66}met polymorphism status.

polymorphism absent]. Secondary analysis of all 255 subjects was also consistent ($P = 0.029$), finding that the proportion of subjects with mRS score of 0–1 at 3 months remained lower in those with the ApoE ϵ4 polymorphism present, compared to those with polymorphism absent [21.7% (13/60 patients) when polymorphism present; 36.4% (71/195 patients) when polymorphism absent, see Fig. 1b]. The proportion of subjects with minimal or no disability at 3 months did not vary according to the presence versus absence of the val^{66}met BDNF polymorphism amongst the 241 subjects with 0–1 copies of either polymorphism, amongst the 197 patients with ischaemic stroke, or amongst all 255 patients. Regarding the other secondary end-point, change in NIHSS score from acute stroke to 3 months, none of the analyses found a significant association with either polymorphism.

Predictive model

A model directly compared ApoE ϵ4 status and BDNF val^{66}met polymorphism status whilst controlling for nine baseline and demographic measures. The outcome measure used for this model was the proportion of patients with mRS score of 0–1 at 3 months post-stroke. In primary analysis that focused on the 241 subjects with 0–1 copies of either polymorphism, two variables survived as significant predictors, ApoE ϵ4 status ($P = 0.006$, odds ratio for ApE4 present, 0.31, 95% CI = 0.12–0.73) and baseline stroke severity ($P < 0.0001$, odds ratio per point in NIHSS, 0.78, 95% CI = 0.71–0.84). Note that r^2 for this model was 0.26, higher than the r^2 value of 0.21 found with the nine covariates alone without any genetics data. Results were virtually

identical when only the 197 patients with ischaemic stroke were examined in this model. Results were also near identical in a secondary analysis that included all 255 subjects, where ApoE ϵ4 status ($P = 0.026$, odds ratio for ApoE4 present, 0.42, 95% CI = 0.18–0.90) and baseline stroke severity ($P < 0.0001$, odds ratio per point in NIHSS, 0.79, 95% CI = 0.73–0.84) again remained as the significant predictors.

Mitochondrial DNA haplotype and stroke outcome

The mtDNA haplogroup could be determined in 227 subjects, as insufficient DNA was available to identify the haplotype in 14 cases, and were U (14.9%), R0 (42.3%), N (13.3%), M (2.5%), L (4.6%), and JT (16.6%). The mtDNA haplogroup was not related to ApoE ϵ4 status ($P = 0.18$), but was significantly related to the presence of the BDNF val^{66}met polymorphism ($P = 0.0004$). The latter was attributed to subjects carrying the BDNF val^{66}met polymorphism having a higher frequency of mtDNA haplotypes M and N, as expected, as both are more common in Asian populations [18,19]. Two measures varied at baseline in relation to mtDNA haplogroup: ethnicity, as expected ($P < 0.0001$; JT, R0, and U were 99–100% Caucasian; M and N were mainly Asian) [19], and the proportion of subjects whose stroke was intracerebral hemorrhage ($P = 0.037$), being highest in M and N haplotypes. Across the six mtDNA haplotypes, no significant relationship was found for stroke recovery or minimal disability end-points. Exploratory *post hoc* analysis examining each haplotype separately suggested an effect in one case: subjects with the R0 haplotype had a median 5 point [IQR 3–9] change in NIHSS score from acute to 1 month post-stroke, compared to a 4 point [IQR 0–6] change in non-R0 ($P = 0.004$).

Discussion

This study suggested that genetic polymorphisms may be related to differences in outcome after stroke, independent of baseline deficits. The results were strongest for patients carrying the ApoE ϵ4 polymorphism. Genetic variability may account for some of the inter-subject variability in stroke recovery and might be a source of molecular insights into stroke outcomes in humans.

ApoE is the most abundant brain lipoprotein, and its ApoE ϵ4 polymorphism adversely affects processes related to repair and recovery such as neuronal remodeling [6] and synaptic turnover [7]. Consistent with this, the current analysis found that presence of the ApoE ϵ4 polymorphism was associated with poorer recovery during the time of maximum remodeling, from acute presentation to 1 month after stroke. The magnitude was modest but could be important to some patients. ApoE ϵ4 was also associated with an absolute risk reduction of 17.3% for achieving minimal or no disability at 3 months post-stroke (Fig. 1a), a finding that is substantial given that absolute risk reduction with administration of IV tPA for the same end-point (mRS 0–1) ranges from 7.3% [20] to 13% [21]. The ApoE ϵ4 polymorphism results remained significant when only patients with ischaemic stroke were assessed and when patients with two polymorphism copies were examined in secondary analyses.

ApoE ϵ4 has previously been associated with poorer outcome after traumatic brain injury [8] or hemorrhagic stroke [9,10,22], but this association has not been found in prior studies that examined patients with ischaemic stroke. There are several possible reasons for the disparity between prior reports and the current findings. First, unlike most prior studies, outcomes in the current study were highly standardized, being performed by trained examiners in a phase III trial setting. Second, the influence of the ApoE ϵ4 polymorphism after stroke might interact with gender [23], with results varying according to the population under study; however, this interaction was not found in the current study (Table 2). Third, many prior studies focused on measuring the proportion of subjects with a poor outcome (death or dependency) [22] rather than with a good outcome as in the current analysis. Consistent with this, when current results were re-examined looking at proportion of subjects reaching mRS score of 4–6 (death or dependency) at 3 months, no difference was seen in relation to ApoE ϵ4 status. A notable exception [24] examined ApoE ϵ4 status in relation to minimal or no disability in the setting of a Phase III clinical trial that enrolled patients with stroke of <3 h duration at US sites and did not find an association of ApoE ϵ4 status with outcome. The reasons for discrepancy with current findings are unclear but might include differences in the entry criteria and in the populations under study, for example, the GAIN studies excluded patients who were unresponsive but the prior study [24] did not.

The current study also suggested the possibility that genetic variations in BDNF and in mitochondrial DNA might be related to stroke outcome. BDNF is the most abundant neurotrophin in the brain, has increased brain levels after stroke, and affects neuronal survival, differentiation, and use-dependent plasticity. The BDNF val^{66}met polymorphism is associated with impaired activity-dependent BDNF release, which is tied to many forms of learning and plasticity. This study suggested a possible relationship between the presence of the BDNF val^{66}met polymorphism and poorer recovery from acute to 1 month after stroke, the period

of maximum repair and clinical recovery [3]. Although this *P* value (0.036) did not survive correction for multiple comparisons, this finding is consistent with a prior study [25] that found that outcome after subarachnoid hemorrhage is poorer in the presence of the BDNF val^{66}met polymorphism, although interestingly this association might be most pronounced in those patients with subarachnoid hemorrhage who have no associated cerebral infarct [26]. The current results also suggested a potential relationship between the R0 mtDNA haplotype and outcome after stroke. Mitochondria generate cellular energy as well as reactive oxygen species [27], and so mtDNA has a very high mutation rate, and has been divided on this basis into several haplogroups. The current study hypothesized that mtDNA haplotype might be related to outcome after stroke, as cellular energetics might be important to recovery, and indeed some mitochondrial haplogroups have shown significant clinical associations such as with longevity [28], degenerative disease risk [13], and stroke risk [14,15]. Although both the BDNF val^{66}met polymorphism and the R0 mtDNA haplotype findings might reflect type I error, current results do suggest avenues for future research.

Strengths of this study include measurement of within-subject change beginning with the acute presentation for the measure of recovery and collection of outcome measures by trained examiners in a phase III clinical trial setting. Collection of data across multiple sites and countries, whilst potentially resulting in underestimation of associations, increases confidence in the results and their generalization. Recruitment of subjects within hours of stroke avoids a selection bias that might affect studies enrolling late after stroke, where the sickest patients might not be enrolled because of early death. Weaknesses include other possible sources of bias, such as the clinical trials' inclusion criteria and obtaining DNA from only a subset of the entire cohort of patients treated in the GAIN studies, although, as mentioned previously, baseline features of the current group were similar to the overall GAIN study cohort. Sample size was small by genetic study standards. For example, the study was not powered to detect a gene dose effect, and indeed there were very few subjects with two copies of either polymorphism. For this reason, it is unclear whether having two copies of either polymorphism is truly protective, as might be inferred from Table 2, or whether this is simply because of chance. The GAIN studies excluded patients who were unresponsive or had rapidly improving symptoms, and so the mildest and the worst strokes may been excluded from the current analyses; as mentioned previously, such differences in entry criteria might contribute to inconsistencies between the current and prior studies [24] regarding ApoE ϵ4 polymorphism effects. Also, no data were available on the rehabilitation therapy subjects received during recovery, a covariate of interest to any study of recovery, although variability in rehabilitation might be expected to dilute rather than overestimate polymorphism effects. Finally, although the molecules examined are involved with repair, the current data do not rule out that that the BDNF val^{66}met polymorphism and the ApoE ϵ4 polymorphism influence outcomes via other mechanisms such as secondary stroke complications, cognitive effects, or response to preventative medicines.

The current findings build on prior studies that suggest that genetic variation can influence clinical course after stroke independent of initial injury. These findings might be of value to stroke therapy trials, for example, informing entry criteria and stratification, and might suggest future avenues for individualized medical treatment after stroke.

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Disclosure of conflict of interest

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