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ELECTRONIC LETTER

Genetic variants in brain-derived neurotrophic factor associated with Alzheimer's disease

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Background: Alzheimer's disease is complex, with variants in multiple genes contributing to interactions increasing risk for the disease. Brain-derived neurotrophic factor (BDNF) promotes neuronal survival and modulates hippocampal-dependent memory.

Methods: We examined 11 SNPs that spanned the gene on chromosome 11p14 in 220 Alzheimer's patients and 128 control spouses.

Results: Not all of the SNPs were informative, due to minor allele frequencies of <2%. Neither C270T nor two SNPs that reside proximal to exon V had significant association with the disease. However, we did find that the heterozygous form of the rs6265 SNP (Val66Met), as well as the diplotype of three SNPs (rs6265, rs11030104, rs2049045; H1-GTC/H2-ACG) all were highly significant in APOE 4 non-carriers (OR=2.734; $p=0.0096$).

Conclusion: The combination of the diplotypes for three SNPs exhibited significant p values for Alzheimer's APOE 4 non-carriers. The two SNPs (rs11030104 and rs2049045) are found between exons VI and VII, while the Val66Met polymorphism is located in the coding exon VIII; the total distance for the three SNPs is 14308 bp. Whether the SNPs are involved with alternative splicing of the VII/VIII transcript is of considerable interest.

Alzheimer's disease is an age-related progressive neurodegenerative disorder characterised by severe cognitive impairment. The E4 allele of apolipoprotein E (APOE) is the most prevalent identified risk factor and accounts for 40–50% of the risk as a single factor. The disease is complex with numerous systems, cells and molecules affected. Several genes may interact to cause the disease or serve as risk factors. Measurement of any one component may be small and variable, depending upon the presence of other factors. Brain-derived neurotrophic factor (BDNF) promotes neuronal survival and is known to be reduced in affected cortical regions in Alzheimer's disease.^{1–2} Several polymorphisms in the gene have been identified that are associated with Alzheimer's disease, but the reports are conflicting (www.alzgene.org).

The gene for BDNF, located on chromosome 11p14, consists of eight exons, with the coding region in the last exon. There

are two transcripts, due to alternative splicing. There are seven non-coding exons for one expressed transcript and 11 non-coding exons for the second transcript which may be non-protein coding.³ The type I transcript uses a single splice acceptor site in the main protein coding exon VIII. One of seven transcript classes may be formed when transcription is initiated at either exon I, II, ... VII with the donor site spliced to the major coding exon VIII acceptor site. Four predicted translation products have been identified thus far.³

The two most common polymorphisms studied in Alzheimer's disease are the most terminal polymorphism (rs6265) with a G/A (Val/Met) at codon 66 in the coding exon VIII and the C270T polymorphism (corrected to C132T)⁴ which is untranslated in exon 5, contributing to an mRNA transcript class. The Val66Met polymorphism was associated with Alzheimer's disease in an Italian population⁵ but not in other studies.^{6–15} Likewise, the C270T polymorphism was associated with the disease in Japanese and European patients^{13–16–17} but not in other groups.^{10–12}

To verify a potential role for BDNF polymorphisms in Alzheimer's disease, we analysed 11 SNPs (five tagSNPs and five non-synonymous SNPs including the Val66Met, as well as the C270T polymorphism) that spanned the gene. We also analysed the data according to APOE status.

METHODS

Subjects

The patients and family members were recruited from communities in Texas and Georgia, as well as from the Memory Disorders Clinic at the Medical College of Georgia. Medical records were obtained on each patient and the clinical diagnosis of probable Alzheimer's disease was made according to NINCDS-ADRDA criteria.¹⁸ This included a documented progressive decline in cognitive function and appropriate blood work to rule out other medical conditions, including thyroid and vitamin B12 deficiencies. In addition, we included in the diagnosis, results from a CT or MRI scan of the brain which indicated the presence of cortical atrophy but no evidence of strokes or tumours. The patients were Caucasian and of European descent. Spouses of patients and of siblings of similar age, ethnic background and similar environmental exposure served as controls. All participants or the authorised representatives of the patients gave consent for the study, in accordance with the Institutional Review Board guidelines. We also included 36 patients with the diagnosis of Alzheimer's disease from the National Cell Repository. The groups were analysed separately for population stratification with Structure 2.1 software (<http://pritch.bsd.uchicago.edu/software.html>) and no significant differences were found.¹⁹

There were 220 patients (155 females and 65 males) with an average age of onset of 71.0 ± 8.0 years (range 50–92 years; 175

Key points

- Alzheimer's disease is complex; multiple genes may interact, increasing risk for the disease.
- Brain-derived neurotrophic factor promotes neuronal survival and modulates hippocampal-dependent memory.
- The combination of diplotypes for three SNPs increased risk for Alzheimer's disease in APOE 4 non-carriers.

Abbreviations: APOE, apolipoprotein E; BDNF, brain-derived neurotrophic factor

Table 1 SNP positions and variation

dbSNP ID	Chromosome position*	AF411339 position†	Alleles	Tagging SNPs in HapMap Project
rs11030121	26523448	39149	G-A	Yes
rs2049046	26511016	51579	A-T	Yes
C270T	26508860	53620	C-T	
rs2049045	26481482	81114	C-G	Yes
rs11030104	26471758	90836	T-C	Yes
rs8192466‡	26467348	95231	C-T (Thr2Ile)	
rs6265	26467157	95422	A-G (Val66Met)	
rs1048218‡	26467128	95451	G-T (Gln75His)	
rs1048220‡	26466979	95600	G-T (Arg125Met)	
rs1048221‡	26466973	95606	G-T (Arg127Leu)	
rs7124442	26464282	98296	G-A	Yes

*Chromosome position from the NCBI SNP database; †GenBank AF411339 position; ‡minor allele frequencies of <2% in our samples.

with age of onset >64 years) and 128 control spouses (75 females and 53 males) with an average age at enrolment of 72.0 ± 8.0 years (range 50–88 years).

Genotyping SNP selection

Using the 10/04 HapMap Project (www.hapmap.org), there were 15 SNPs that spanned 59 kb across the BDNF gene, from exon III to exon VIII, the coding exon. All 15 of the SNPs were in tight linkage disequilibrium and were in one haplotype block.²⁰ Eleven SNPs were selected that spanned the gene from intron 3 to exon VIII: five tagSNPs, five non-synonymous SNPs and the C127T polymorphism. The SNPs are listed in table 1 and were genotyped using fluorescent-detected single base extension²¹ with the SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA) as described.²² APOE and the BDNF polymorphisms (Val66Met and C270T) were genotyped as described previously.^{5 17 23}

Statistical analysis

Hardy-Weinberg equilibrium tests were performed separately among patient and control groups for each SNP, using

Arlequin²⁴; no significant deviations were observed. Power analysis was performed for the 225 patients and 127 controls, using G*Power and the power was 0.9978.²⁵ When the samples were divided into APOE 4 carriers and non-carriers, the power was 0.8109 for the carriers and 0.9354 for the non-carriers. Logistic regression was performed using SAS 9.1 to investigate the association between the SNPs and Alzheimer's disease. Pairwise linkage disequilibrium for the SNPs was evaluated with Arlequin. Haplotypes were inferred for each individual using PHASE.²⁶ The haplotype data for the individuals with EM probabilities greater than 85% were exported to SAS for logistic regression analysis to determine the risk associated with particular haplotypes.

RESULTS

Of the 11 BDNF SNPs that were analysed (table 1), four had minor allele frequencies lower than 2% in our samples and even though they were in the coding region, they were excluded from further analysis (rs1048221, rs1048220, rs1048218 and rs8192466). Comparisons of the allele frequencies or the genotype frequencies between patients and controls did not reveal significant differences, although the p values for the heterozygous genotypes for the patients with the three adjacent

Table 2 Allele and genotype frequencies*

SNP ID	Genotypic comparison			p Value†	Allelic comparison			
		Alzheimer's disease	Control			Alzheimer's disease	Control	
		n (%)	n (%)		n (%)	n (%)	p Value	
rs7124442	AA	95 (43.18)	55 (43.31)	0.9884	A	287 (65.23)	163 (64.17)	0.8779
	GA	97 (44.09)	53 (41.73)		G	163 (34.77)	91 (35.83)	
	GG	28 (12.73)	19 (14.96)					
rs6265	AA	4 (1.82)	5 (3.91)	0.0956	A	74 (16.82)	35 (13.67)	0.1909
	AG	66 (30.00)	25 (19.53)		G	366 (83.18)	221 (86.33)	
	GG	150 (68.18)	98 (76.56)					
rs11030104	CC	5 (2.27)	5 (3.91)	0.0749	C	83 (18.86)	38 (14.84)	0.1338
	CT	73 (33.18)	28 (21.88)		T	357 (81.14)	218 (85.16)	
	TT	142 (64.55)	95 (74.21)					
rs2049045	CC	153 (69.54)	100 (78.12)	0.0561	C	369 (83.86)	223 (87.11)	0.1304
	CG	63 (28.64)	23 (17.97)		G	71 (16.14)	33 (12.89)	
	GG	4 (1.82)	5 (3.91)					
C270T	CC	202 (91.82)	113 (88.28)	0.6545	C	420 (95.45)	241 (94.14)	0.7298
	CT	16 (7.27)	15 (11.72)		T	20 (4.55)	15 (5.86)	
	TT	2 (0.91)	0					
rs2049046	AA	61 (27.73)	36 (28.13)	0.3662	A	239 (54.32)	133 (51.95)	0.2969
	AT	117 (53.18)	61 (47.65)		T	201 (45.68)	123 (48.05)	
	TT	42 (19.09)	31 (24.22)					
rs11030121	AA	22 (10.00)	13 (10.16)	0.9142	A	143 (32.50)	83 (32.42)	0.7252
	GA	99 (45.00)	57 (44.53)		G	297 (67.50)	173 (67.58)	
	GG	99 (45.00)	58 (45.31)					

*The SNPs with minor allele frequency of <2% are not listed here; †overall genotypic p value by logistic regression, adjusted for age, gender and APOE 4 status.

Table 3 APOE 4 status for risk SNPs

SNP ID	APOE 4 carriers			p Value	APOE 4 non-carriers		
	Alzheimer's disease	Control			Alzheimer's disease	Control	p Value
rs6265	AA	3 (1.99)	0	0.9324	1 (1.45)	5 (5.15)	0.0327*
	AG	43 (28.48)	8 (25.81)		23 (33.33)	17 (17.53)	
	GG	105 (69.53)	23 (74.19)		45 (65.22)	75 (77.32)	
	A	49 (16.23)	8 (12.90)		25 (18.12)	27 (13.92)	
rs11030104	G	253 (83.77)	54 (87.10)	0.5118	113 (81.88)	167 (86.08)	0.2766
	CC	4 (2.65)	0		1 (1.45)	5 (5.15)	
	CT	48 (31.79)	8 (25.81)		25 (36.23)	20 (20.62)	
	TT	99 (65.56)	23 (74.19)		43 (62.32)	72 (74.23)	
rs2049045	C	56 (18.54)	8 (12.90)	0.8726	27 (19.57)	30 (15.46)	0.3457
	T	246 (81.46)	54 (87.10)		111 (80.43)	164 (85.54)	
	CC	108 (71.52)	24 (77.42)		45 (65.22)	76 (78.36)	
	CG	40 (26.49)	7 (22.58)		23 (33.33)	16 (16.49)	
	GG	3 (1.99)	0		1 (1.45)	5 (5.15)	
	G	256 (84.77)	55 (88.71)		113 (81.88)	168 (86.60)	
		46 (15.23)	7 (11.29)	25 (18.12)	26 (13.40)	0.0226*	0.0525

Results: n (%)
*p<0.05

SNPs (rs6265, rs11030104 and rs2049045) approached significance ($p = 0.096$, 0.075 and 0.056 , respectively) (table 2). When the patients and controls were divided according to APOE 4 status, the genotype frequencies of two of the three SNPs were significantly different between APOE 4 non-carrier patients and controls (table 3). Comparisons of the genotype frequencies between patients and controls in APOE 4 non-carriers for SNPs rs6265 (Val66Met) and rs2049045 gave p values of 0.0327 and 0.0226, respectively, while that for SNP rs11030104 was 0.0534. The p values for APOE 4 carriers and for the remaining four SNPs in either group were not significant.

To further investigate the possible association of the three SNPs (rs6265, rs11030104 and rs2049045) with the disease, we examined their genotype combinations. The heterozygous combination of AG/CT/CG had a significantly higher frequency among the patients when compared with the controls (28.64% v 17.97%). When compared with the homozygous genotype, GG/TT/CC, there was an increased risk for the disease ($p = 0.0283$, OR = 2.01) (table 4). The p value was even more significant in APOE 4 non-carriers (33.33% v 16.49%) when heterozygous genotype combinations were compared with homozygous genotypes ($p = 0.0160$, OR = 2.57).

Haplotypes

The seven SNPs described in table 2 are in linkage disequilibrium and reside in one haplotype block. Within that block, four haplotypes had frequencies higher than 5% (data not shown). Each haplotype was compared with the combination of the others. As the allelic associations were not significant, the

haplotypes were also not significant. However, when the diplotypes for the three SNPs (rs6265, rs11030104 and rs2049045) were compared, the diplotype H1 (GTC)/H2 (ACG) had an odds ratio of 2.734 ($p = 0.0096$) when compared with all others in APOE 4 non-carriers (table 5). Even with Bonferroni correction of five multiple tests, the p value remains significant at $p = 0.048$.

DISCUSSION

Of the 22 studies listed on the www.alzgene.org website regarding polymorphisms in the BDNF gene associated with Alzheimer's disease, eight reported positive findings. The studies primarily involved the C270T and the rs6265 polymorphisms. Meta-analysis reported on the same website (www.alzgene.org) of the seven studies of the C270T polymorphism using Caucasian subjects revealed odds ratios of 1.09 (95% CI: 0.7 to 1.68). However, meta-analysis for the rs6265 (Val66Met) SNP in 11 studies of Caucasian subjects showed an odds ratio of 1.08 (95% CI: 0.98 to 1.19). Our study did not show any allelic association with the two polymorphisms with the T or A alleles. However, when additional SNPs were analysed, the combination of the heterozygous genotype of the rs6265 SNP with the heterozygous genotypes of the flanking SNPs (rs11030104 and rs2049045) was significantly associated with the disease in APOE 4 non-carriers in our sample. To our knowledge we are the first group to examine the entire gene by genotyping all the tagSNPs as well as all the non-synonymous SNPs currently listed. This strategy provided additional power to detect the association between the SNPs and the disease.

Table 4 Combined genotypes of the risk SNPs: rs6265/rs11030104/rs2049045

	All subjects		APOE 4 carriers		APOE 4 non-carriers	
	Alzheimer's disease	Control	Alzheimer's disease	Control	Alzheimer's disease	Control
AA/CC/GG	4 (1.82)	5 (3.91)	3 (1.99)	0	1 (1.45)	5 (5.15)
AG/CT/CC	3 (1.36)	1 (0.78)	3 (1.99)	0	0	1 (1.03)
AG/CT/CG	63 (28.64)	23 (17.97)	40 (26.49)	7 (22.58)	23 (33.33)	16 (16.49)
AG/TT/CC	0	1 (0.78)	0	1 (3.23)	0	0
GG/CC/CC	1 (0.45)	0	1 (0.66)	0	0	0
GG/CT/CC	7 (3.18)	4 (3.13)	5 (3.31)	1 (3.23)	2 (2.90)	3 (3.09)
GG/TT/CC	142 (64.55)	94 (73.43)	99 (65.56)	22 (70.96)	43 (62.32)	72 (74.24)
AG/CT/CG v GG/TT/CC	$p = 0.0238^*$		$p = 0.5934$		$p = 0.0160^*$	
	OR = 2.01		OR = 1.29		OR = 2.57	
	95% CI: 1.10 to 3.67		95% CI: 0.51 to 3.28		95% CI: 1.193 to 5.55	

Results: n (%)
*p<0.05

Table 5 SNP haplotypes and diplotypes: rs6265/rs11030104/rs2049045

	All subjects		APOE 4 carriers		APOE 4 non-carriers	
	Alzheimer's disease	Control	Alzheimer's disease	Control	Alzheimer's disease	Control
Haplotype						
H1	357 (81.2)	217 (84.8)	246 (81.5)	53 (85.5)	111 (80.4)	164 (84.5)
H2	71 (16.1)	33 (12.9)	46 (15.2)	7 (11.3)	25 (18.1)	26 (13.4)
Other	12 (2.7)	6 (2.3)	10 (3.3)	2 (3.2)	2 (1.5)	4 (2.1)
Total	440	256	302	62	138	194
H1 v others	p=0.1941 OR=0.737 95% CI: 0.465 to 1.168		p=0.4350 OR=0.737 95% CI: 0.342 to 1.586		p=0.3457 OR=0.754 95% CI: 0.419 to 1.356	
H2 v others	p=0.1304 OR=1.462 95% CI=0.894 to 2.391		p=0.4243 OR=1.414 95% CI: 0.605 to 3.304		p=0.2254 OR=1.463 95% CI: 0.791 to 2.706	
Diplotype						
H1H1	142 (64.5)	94 (73.4)	99 (65.5)	22 (70.9)	43 (62.3)	72 (74.2)
H1H2	63 (28.6)	23 (18.0)	40 (26.5)	7 (22.6)	23 (33.3)	16 (16.5)
H1Other	10 (4.6)	6 (4.7)	8 (5.3)	2 (6.5)	2 (2.9)	4 (4.1)
H2H2	4 (1.8)	5 (3.9)	3 (2.0)	0	1 (1.5)	5 (5.2)
OtherOther	1 (0.5)	0	1 (0.7)	0	0	0
Total	220	128	151	31	69	97
H1H2 v all others	p=0.0190* OR=2.040 95% CI: 1.124 to 3.7		p=0.6444 OR=1.242 95% CI: 0.494 to 3.123		p=0.0096* OR=2.734 95% CI: 1.277 to 5.852	

H1: GTC; H2: ACG.
Results: n (%)
*p<0.05

The gene for BDNF has been extensively studied recently and was found to consist of seven non-coding exons and one major coding exon in type 1 BDNF and 11 exons of the reverse non-coding type 2 BDNF gene.³ This assembly suggests multiple splicing patterns and transcripts providing heterogeneity in expression in different brain regions. The SNPs in our study are found between exons III and IV (rs11030121 and rs2049046), in exon V (C270T), between exon VIA and VII (rs2049045 and rs1130104), in the coding exon VIII (rs6265) and in the coding exon VIII after the polyadenylation site (rs7124442). The distance between rs2049045 and rs6265, including rs11030104, is approximately 14 308 bp. It is interesting that our analysis of the heterozygous genotypes of the three SNPs (rs6265:rs11030104:rs2049045; AG/CT/CG) produced an odds ratio of 2.57 (p=0.0160; 95% CI: 1.193 to 5.55) in APOE 4 non-carriers. Whether the two SNPs before exon VII are involved with alternative splicing of the VII/VIII transcript is unknown.

The BDNF Val66Met polymorphism has been associated with variations in the performance of episodic memory, with those carrying the Met allele performing worse than those homozygous for the Val genotype.²⁷ In MRI studies of the hippocampus in healthy adults, those with the Val/Val genotypes had larger hippocampal volumes than those who carried the Val/Met genotypes.^{28–31} Heterozygous expression of the Val/Met genotypes formed heterodimers in cultured neurons which impaired regulated BDNF secretion.³² Since BDNF supports basal forebrain cholinergic projections to the hippocampus and neocortex, the downregulation of BDNF secretion may contribute to the neuronal loss found in Alzheimer's disease.

ELECTRONIC DATABASE INFORMATION

The webpage of the Alzheimer Research Forum is at <http://www.alzgene.org> and details of the International HapMap Project can be found at <http://www.hapmap.org>

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