Folia Neuropathologica

No association between the brain-derived neurotrophic factor 196G>A or 270C>T polymorphisms and Alzheimer's or Parkinson's disease

Marika S. Saarela[1,2], Terho Lehtimäki[1,2], Juha O. Rinne[3], Heini Huhtala[4], Riikka Rontu[1,2], Antti Hervonen[4], Matias Röyttä[5], Jukka-Pekka Ahonen[6], Kari M. Mattila[1,2]

[1]Laboratory of Atherosclerosis Genetics, Department of Clinical Chemistry, Center for Laboratory Medicine, Tampere University Hospital, Tampere, Finland; [2]Medical School, University of Tampere, Tampere, Finland; [3]Department of Neurology and PET-center, University of Turku, Turku, Finland; [4]School of Public Health, University of Tampere, Tampere, Finland; [5]Department of Pathology, University of Turku, Turku, Finland; [6]Department of Neurology, Tampere University Hospital, Tampere, Finland

Folia Neuropathol 2006; 44 (1): 12-16

Abstract

The brain-derived neurotrophic factor (BDNF) promotes survival, differentiation and maintenance of neurons in the central nervous system. BDNF 196G>A and 270C>T polymorphisms have previously been associated with Alzheimer's disease (AD) and with Parkinson's disease (PD). To study the role of BDNF 196G>A and 270C>T polymorphisms in Finnish AD and PD patients we genotyped BDNF 196G>A and 270C>T polymorphisms in 97 sporadic AD patients, 52 PD patients and 101 control subjects with polymerase chain reaction. No associations were found between the genotypes studied and AD or PD in Finnish patients. Moreover, no interaction between either BDNF polymorphism and the ε4 allele of apolipoprotein E was found. In conclusion, it seems that the BDNF gene does not contribute significantly to the risk of AD or PD in Finnish patients.

Key words: *genotype, polymerase chain reaction, apolipoprotein E.*

Introduction

Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most common neurodegenerative diseases sharing the feature of accumulation of abnormal proteins in the brain. Typical neuropathological findings in AD include β-amyloid (Aβ) containing neuritic plaques surrounded by activated microglia that release cytokines and neurofibrillary tangles composed of modified tau and reactive astrocytosis. A possibly more significant neuropathological characteristic of AD is the degeneration of the basal forebrain cholinergic neurons crucially involved in memory and cognition. The appearance of α-synuclein containing Lewy bodies and the loss of dopamine neurons in the substantia nigra are, in turn, typical neuropathological findings in PD [13].

The etiology and pathogenesis of these two conditions still remain largely unknown. The tendency to develop AD and PD runs in some families, suggesting that in these cases there is a significant genetic component elevating the risk of disease. In AD, three genes are known to be responsible for familiar forms of the disease, i.e. amyloid precursor protein and presenilins 1 and 2. Inherited forms, however, cover only 5% of all AD cases and the rest, known as sporadic AD cases, have a more complex etiology due to both environmental and genetic factors. The major known risk factor for sporadic AD is the ε4 allele of apolipoprotein E (*APOE*) [28]. In PD the mutated forms of α-synuclein gene, ubiquitin C-terminal hydrolase and parkin genes account for rare forms of familial PD.

The brain-derived neurotrophic factor (BDNF) is a neurotrophin promoting survival, differentiation and maintenance of neurons [21]. A number of studies have shown decreased BDNF protein and mRNA levels in the brains of AD and PD patients [3,5,8,9,11,18,24,26], suggesting that *BDNF* may contribute to the development of these disorders. Indeed, two single nucleotide polymorphisms of the *BDNF* gene, namely 196G>A and 270C>T, have been associated with AD [14,27,30] and PD [20,25,29].

In the present study we tested the hypothesis that the functional *BDNF* genotypes might affect the risk of AD or PD. We therefore identified the genotypes of *BDNF* 196G>A and 270C>T polymorphisms in Finnish AD, PD and control patients.

Material and methods

The AD group comprised 97 sporadic late-onset (>65 years) type AD patients (43 with probable and 54 with confirmed AD; 68 women, 29 men). The clinical diagnosis of AD was established according to the report of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) Work Group [17]. The definitive diagnosis of AD (56% of patients) was based on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria [19]. The PD group consisted of 52 PD patients (27 women, 25 men; mean age at onset 76.2±6.0 years). The pathological diagnosis of PD (100% of patients) was based on the loss of pigmented neurons in the substantia nigra with gliosis, the presence of pigmented phagocytosis and Lewy bodies, confirming the clinical diagnosis of PD. The 101 controls (56 women, 45 men; mean age at examination or at death 79.0±9.3 years; 58% neuropathologically confirmed) were cognitively healthy subjects with no clinical signs of a neurological or psychiatric disease. Ethical approval for the investigation was obtained from the local Hospital Ethical Committees.

Genomic DNA was extracted by standard methods from the whole blood and autopsy-derived frozen brain tissue. The genotyping of the *BDNF* 196G>A polymorphism was done using ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The system uses 5' nuclease assay and fluorogenic allele-specific oligonucleotide probes with conjugated minor groove binder (MGB) group. The nucleotide sequences of the primers and probes used in PCR were deduced from published sequences deposited in the GenBank database and were chosen and synthesized in conjugation with Applied Biosystems (Foster City, CA, USA). PCR was performed in 384-well plates using the standard protocol for TaqMan MGB probes in a total volume of 5 µl. Reagents included genomic DNA, 1 × Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe. Negative controls were run in parallel with unknown DNA samples. After cycling, end-point fluorescence was measured and the allelic discrimination analysis module carried out genotype identification. The genotypes of the *BDNF* 270C>T and *APOE* polymorphisms were determined as previously described [7,14,30] using polymerase chain reaction (PCR), digestion with restriction endonucleases and standard agarose gel electrophoresis.

The test for Hardy-Weinberg equilibrium was calculated using Arlequin, version 2.000 (Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland, 2000). The genotype and allele frequencies were compared between cases and controls using the χ^2 test. In view of the simultaneous comparisons, the correction factor n (m-1) (n loci with m alleles each) was applied to correct the significance level ($p<0.008$). The effects of *BDNF* 196G>A and 270C>T polymorphisms on the risk of AD were investigated by the logistic regression analysis (SPSS 12.0.1 for

Table I. Genotype and allele frequencies of the 196G>A and 270C>T polymorphisms of the brain-derived neurotrophic factor (*BDNF*) gene in Alzheimer's disease (AD), Parkinson's disease (PD) and control groups[a]

	Group		
	AD (n=97)	PD (n=52)	Controls (n=101)
BDNF 196G>A			
G/G	0.64	0.81	0.80
G/A	0.33	0.19	0.17
A/A	0.03	0.00	0.03
G	0.80	0.90	0.89
A	0.20	0.10	0.11
BDNF 270C>T			
C/C	0.91	0.83	0.80
C/T	0.09	0.14	0.19
T/T	0.00	0.03	0.01
C	0.95	0.89	0.90
T	0.05	0.11	0.10

[a]*n, the number of subjects genotyped, no differences between the groups when analyzed with Pearson's* χ^2 *test*

Windows, SPSS Inc. 2003). Age and gender were used for adjustment. The combined effects of *APOE* ε4 (ε4 carriage/non-carriage) and *BDNF* 196G>A and 270C>T polymorphisms on the risk of AD were investigated by the logistic regression analysis (SPSS 12.0.1 for Windows, SPSS Inc. 2003).

Results

All patient and control groups for both the *BDNF* polymorphisms investigated were found to be in the Hardy-Weinberg equilibrium. The distributions of genotypes and allelic frequencies of the *BDNF* polymorphisms are shown in Table I. The genotype or allele frequencies of 196G>A or 270C>T polymorphisms did not differ significantly between AD, PD and control groups after correction for multiple testing although the difference between the AD and the control groups came close to statistical significance in both of the polymorphisms analyzed (p=0.028, p=0.053). The risk of AD conferred by *APOE* ε4 was of the same order of magnitude as reported previously (OR: 10.8, CI: 5.6-20.9). No interaction was observed between either of the polymorphisms analyzed and *APOE* ε4 in AD patients.

Discussion

In this investigation we found no association between *BDNF* 196G>A (Val66Met) or *BDNF* 270C>T polymorphism and AD or PD. Our results confirm the results of another Finnish study [31], a Spanish study [2] and several North American studies [1,4] where no association with *BDNF* 196G>A (Val66Met) polymorphism was found among AD patients. In the American studies, too, no association with *BDNF* 270C>T polymorphism was found [1,4]. Moreover, the allele frequencies observed in the present study were similar to those in the studies mentioned above [1,2,4,31]. We were not able to replicate previous results where an association between AD and *BDNF* was found [14,16,23,27,30]. For PD, too, and *BDNF* 196G>A our result is consistent with another Scandinavian study [6] and one Japanese study [10]. Our result contradicts those by Japanese researchers [15,20] and the North American researchers [25]. The allele frequencies in these studies differ significantly, the G-allele frequency ranging from 0.47-0.61 [10,15,20] in Asian PD patients to a frequency of 0.82-0.90 [6] in Scandinavian PD patients. As for control patients the G-allele frequencies also differ, ranging from 0.50-0.60 in Asians and 0.82-0.90 in Scandinavians [6,10,12,15,20,22]. This might suggest that there are ethnic differences between Asians and Caucasians in *BDNF* 196G>A allele distribution. Unlike the North Americans [25] we found no association between *BDNF* 270C>T polymorphism and PD, but the number of PD patients in our study was relatively small. The C-allele frequency of PD patients in our study was 0.89 and in the North American patients 0.79 among familial PD patients and 0.85 among sporadic PD patients [25].

In the case of association studies there is always a possibility that the association found is not real but rather due to a linkage disequilibrium with a functional polymorphism in or near the gene studied. This may be the case with *BDNF*, given the different results reported by numerous researchers. False negative results may also be obtained because of the small sample size. Although our sample size was quite small we could confirm the results of another Finnish study where no association between AD and *BDNF* was found [31]. In conclusion, it seems that the *BDNF* gene is not a significant risk gene among Finnish AD patients.

Acknowledgements

The authors would like to thank Juhani Salo, neuropsychologist, for his contribution to the study. This work was supported by the Medical Research Fund of Tampere University Hospital.

References

1. Bodner SM, Berrettini W, van Deerlin V, Bennett DA, Wilson RS, Trojanowski JQ, Arnold SE. Genetic variation in the brain derived neurotrophic factor gene in Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet 2005; 134: 1-5.
2. Combarros O, Infante J, Llorca J, Berciano J. Polymorphism at codon 66 of the brain-derived neurotrophic factor gene is not associated with sporadic Alzheimer's disease. Dement Geriatr Cogn Disord 2004; 18: 55-58.
3. Connor B, Young D, Yan Q, Faull RL, Synek B, Dragunow M. Brain-derived neurotrophic factor is reduced in Alzheimer's disease. Brain Res Mol Brain Res 1997; 49: 71-81.
4. Desai P, Nebes R, DeKosky ST, Kamboh MI. Investigation of the effect of brain-derived neurotrophic factor (BDNF) polymorphisms on the risk of late-onset Alzheimer's disease (AD) and quantitative measures of AD progression. Neurosci Lett 2005; 379: 229-234.
5. Ferrer I, Ballabriga J, Marti E, Perez E, Alberch J, Arenas E. BDNF up-regulates TrkB protein and prevents the death of CA1 neurons following transient forebrain ischemia. Brain Pathol 1998; 8: 253-261.
6. Hakansson A, Melke J, Westberg L, Shahabi HN, Buervenich S, Carmine A, Klingborg K, Grundell MB, Schulhof B, Holmberg B, Ahlberg J, Eriksson E, Sydow O, Olson L, Johnels B, Nissbrandt H. Lack of association between the BDNF Val66Met polymorphism and Parkinson's disease in a Swedish population. Ann Neurol 2003; 53: 823.
7. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 1990; 31: 545-548.
8. Hock C, Heese K, Hulette C, Rosenberg C, Otten U. Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. Arch Neurol 2000; 57: 846-851.
9. Holsinger RM, Schnarr J, Henry P, Castelo VT, Fahnestock M. Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: decreased levels in Alzheimer's disease. Brain Res Mol Brain Res 2000; 76: 347-354.
10. Hong CJ, Liu HC, Liu TY, Lin CH, Cheng CY, Tsai SJ. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphisms in Parkinson's disease and age of onset. Neurosci Lett 2003; 353: 75-77.
11. Howells DW, Porritt MJ, Wong JY, Batchelor PE, Kalnins R, Hughes AJ, Donnan GA. Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. Exp Neurol 2000; 166: 127-135.
12. Itoh K, Hashimoto K, Kumakiri C, Shimizu E, Iyo M. Association between brain-derived neurotrophic factor 196 G/A polymorphism and personality traits in healthy subjects. Am J Med Genet B Neuropsychiatr Genet 2004; 124: 61-63.
13. Kosik KS. Alzheimer's disease: a cell biological perspective. Science 1992; 256: 780-783.
14. Kunugi H, Ueki A, Otsuka M, Isse K, Hirasawa H, Kato N, Nabika T, Kobayashi S, Nanko S. A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. Mol Psychiatry 2001; 6: 83-86.
15. Masaki T, Matsushita S, Arai H, Takeda A, Itoyama Y, Mochizuki H, Kamakura K, Ohara S, Higuchi S. Association between a polymorphism of brain-derived neurotrophic factor gene and sporadic Parkinson's disease. Ann Neurol 2003; 54: 276-277.
16. Matsushita S, Arai H, Matsui T, Yuzuriha T, Urakami K, Masaki T, Higuchi S. Brain-derived neurotrophic factor gene polymorphisms and Alzheimer's disease. J Neural Transm 2005; 112: 703-771.
17. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984; 34: 939-944.
18. Michalski B, Fahnestock M. Pro-brain-derived neurotrophic factor is decreased in parietal cortex in Alzheimer's disease. Brain Res Mol Brain Res 2003; 111: 148-154.
19. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991; 41: 479-486.
20. Momose Y, Murata M, Kobayashi K, Tachikawa M, Nakabayashi Y, Kanazawa I, Toda T. Association studies of multiple candidate genes for Parkinson's disease using single nucleotide polymorphisms. Ann Neurol 2002; 51: 133-136.
21. Murer MG, Yan Q, Raisman-Vozari R. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. Prog Neurobiol 2001; 63: 71-124.
22. Nakata K, Ujike H, Sakai A, Uchida N, Nomura A, Imamura T, Katsu T, Tanaka Y, Hamamura T, Kuroda S. Association study of the brain-derived neurotrophic factor (BDNF) gene with bipolar disorder. Neurosci Lett 2003; 337: 17-20.
23. Olin D, MacMurray J, Comings DE. Risk of late-onset Alzheimer's disease associated with BDNF C270T polymorphism. Neurosci Lett 2005; 381: 275-278.
24. Parain K, Murer MG, Yan Q, Faucheux B, Agid Y, Hirsch E, Raisman-Vozari R. Reduced expression of brain-derived neurotrophic factor protein in Parkinson's disease substantia nigra. Neuroreport 1999; 10: 557-561.
25. Parsian A, Sinha R, Racette B, Zhao JH, Perlmutter JS. Association of a variation in the promoter region of the brain-derived neurotrophic factor gene with familial Parkinson's disease. Parkinsonism Relat Disord 2004; 10: 213-219.
26. Phillips HS, Hains JM, Armanini M, Laramee GR, Johnson SA, Winslow JW. BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. Neuron 1991; 7: 695-702.
27. Riemenschneider M, Schwarz S, Wagenpfeil S, Diehl J, Muller U, Forstl H, Kurz A. A polymorphism of the brain-derived neurotrophic factor (BDNF) is associated with Alzheimer's

disease in patients lacking the Apolipoprotein E epsilon4 allele. Mol Psychiatry 2002; 7: 782-785.
28. Rocchi A, Pellegrini S, Siciliano G, Murri L. Causative and susceptibility genes for Alzheimer's disease: a review. Brain Res Bull 2003; 61: 1-24.
29. Toda T, Momose Y, Murata M, Tamiya G, Yamamoto M, Hattori N, Inoko H. Toward identification of susceptibility genes for sporadic Parkinson's disease. J Neurol 2003; 250 Suppl 3: III40-43.
30. Ventriglia M, Bocchio Chiavetto L, Benussi L, Binetti G, Zanetti O, Riva MA, Gennarelli M. Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. Mol Psychiatry 2002; 7: 136-137.
31. Vepsalainen S, Castren E, Helisalmi S, Iivonen S, Mannermaa A, Lehtovirta M, Hanninen T, Soininen H, Hiltunen M. Genetic analysis of BDNF and TrkB gene polymorphisms in Alzheimer's disease. J Neurol 2005; 252: 423-428.