Brain derived neurotrophic factor Val66Met polymorphism and psychotic symptoms in Alzheimer's disease

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Abstract

Objective: Alzheimer’s disease (AD) is an irreversible, progressive neurodegenerative disorder with a high prevalence. Since behavioral disturbances, such as psychotic symptoms, represent a key feature of AD, genes related to dopamine, serotonin and brain derived neurotrophic factor (BDNF), are considered as candidate genes for AD. BDNF is a neurotrophin that regulates neurodevelopment, neuroplasticity, and neuronal functions. BDNF is involved in the etiopathogenesis of psychiatric and neurodegenerative disorders. A single base pair polymorphism (BDNF Val66Met) was reported to be associated with AD and/or schizophrenia, as well as other psychoses, although some studies failed to replicate these findings.

Method: BDNF Val66Met was analyzed in 211 patients with AD and in 402 aged healthy control subjects. All subjects were ethnically homogenous Caucasians from Croatia, and were subdivided according to the gender, onset of AD, and presence of psychotic symptoms. A χ² test, with Bonferroni correction and standardized residuals were used to evaluate the data.

Results: Distribution of the BDNF Val66Met genotypes differed significantly between male and female AD patients with or without psychotic symptoms. This difference was due to the significant contribution of the Met/Val genotype and the combined Met/Met and Met/Val genotypes between psychotic and non-psychotic symptoms in male, but not in female patients with AD. The frequency of the gene variants of the BDNF Val66Met did not differ significantly among male and female patients with AD and control subjects, or between male and female patients with early or late onset AD. There were significant sex related differences in age, duration of illness and scores of dementia between patients with AD.

Conclusion: Our male patients were younger, had shorter duration of illness, and had less severe dementia and higher cognitive performance than female AD patients. The gene variants of the BDNF Val66Met polymorphism were significantly associated with the presence of psychotic symptoms in male, but not in female patients with AD. The results had adequate statistical power to suggest that BDNF Val66Met was not related to susceptibility to AD or the onset of AD, but that presence of one or two Met alleles of BDNF Val66Met polymorphism might present a risk factor for psychosis in AD.

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1. Introduction

Alzheimer’s disease (AD) is an irreversible, progressive neurodegenerative disorder with a high prevalence. AD is the most frequent cause of dementia (Cummings, 2004; Fratiglioni et al., 2000). With the progression of illness the severity of symptoms increases, and clinical features of AD include heavy memory loss, reduction of the cognitive function, complete alteration of behavior including the presence of psychotic symptoms in later stages (Mega et al., 1996), as well as the impairment of decision-making and speech impairment (Cummings, 2004; Kawas, 2003). The characteristic neuropathological hallmarks of AD are the presence of senile plaques, neurofibrillary tangles, and loss of synapses (Findeis, 2007; Wenk, 2003). The risk factors for AD include aging, genetic factors, oxidative damage to neurons from overproduction of free radicals, and inflammation.
of toxic free radicals, serious head injuries, brain inflammation and various environmental factors (Frey et al., 2005; Grunblatt et al., 2009). Genetic factors associated with the development of AD include mutations of genes coding for amyloid precursor protein, presenilin-1 and presenilin-2, the ε4 allele of apolipoprotein E (APOE 4), cystatin C, ubiquitin-1, as well as genes involved in oxidative stress and inflammatory response (Serretti et al., 2007). Since behavioral disturbances, depression and psychotic symptoms are also key features of AD (Borroni et al., 2009; 2010; Schneider and Dagerman, 2004), genes related to brain derived neurotrophic factor (BDNF), catechol-o-methyl-transferase (COMT), serotonin transporter and dopaminergic receptors (Borroni et al., 2010; Serretti et al., 2007) are also considered as candidate genes for AD. There are two subtypes of AD: early onset AD, a hereditary form characterized by the disease onset before the age of 65, and sporadic or late onset AD, characterized by the disease onset after the age of 65, which is the most prevalent form of AD (WHO, 1992; APA, 1994). Clinically, AD can be further subdivided into two subtypes, depending on the presence of psychotic features (psychotic and non-psychotic subtype of AD). Psychotic symptoms (delusions, hallucinations, fear, hostility, anxiety, paranoia, agitation, aggression and verbal outbursts) occur in 40–80% of patients with AD. These symptoms are associated with rapid cognitive decline, heavy burden to caregivers and early institutionalization of AD patients (Borroni et al., 2010; Schneider and Dagerman, 2004). Psychotic symptoms in AD have a variable course and respond to lower doses of antipsychotic medication (Jeste and Finkel, 2000).

BDNF is a neurotrophin that regulates neuronal survival, proliferation, regeneration, connectivity, plasticity, neuronal development and function (Russo-Neustadt, 2003). In addition, BDNF modulates cholinergic, dopaminergic, and serotoninergic neurotransmission, and has a modulatory role in cognition, memory formation and processing, learning, mood, behavior, and stress response (Huang and Reichardt, 2001). Consequently, BDNF has been implicated in the etiopathogenesis of affective disorders, schizophrenia, substance dependence, posttraumatic stress disorders, attention deficit hyperactivity disorder, eating disorders and neurodegenerative diseases such as AD (Gratacos et al., 2007; Russo-Neustadt, 2003). Reduced BDNF levels are found in patients with dementia and cognitive decline, while AD is associated with the reduced BDNF mRNA levels, as well as reduced BDNF/TrkB signaling in the postmortem brain samples (Tapia-Arancibia et al., 2008). These collective data point to impairment of BDNF and its receptors in AD. Diminished serum and cerebrospinal fluid concentrations of BDNF have also been reported in AD (Laske et al., 2007; Yasutake et al., 2006). Reduced BDNF protein levels were found in hippocampus and cortical areas of the AD brains compared to control brains (Hock et al., 2000; Lee et al., 2005). Because of the diverse functions of BDNF (Goldberg and Weinberger, 2004), its gene is a good candidate for the susceptibility to various cognitive disturbances occurring in different neuropsychiatric disorders including AD. A common single nucleotide polymorphism Val66Met produces an amino acid substitution of valine (Val) to methionine (Met) at codon 66 in the 5′-prodomain in the BDNF gene. It has been reported that BDNF Val66Met affects intracellular packaging and trafficking, which might alter the secretion of the mature protein (Egan et al., 2003). This BDNF Val66Met polymorphism has been implicated in lower depolarization-induced production of BDNF, decreased n-acetyl aspartate content, and reduced hippocampal activation during memory processing (Bueller et al., 2006; Hariri et al., 2003; Pezawas et al., 2004). The Met allele has been associated with reductions of hippocampal gray matter volume (Pezawas et al., 2004), reduced delayed episodic memory or working memory performance (Egan et al., 2003; Yamada et al., 2002), but also with enhanced verbal reasoning ability (Goldberg and Weinberger, 2004; Harris et al., 2005). A significant association between BDNF Val66Met and AD was reported previously, but several studies failed to confirm that finding (Lee et al., 2005; Li et al., 2005; Vepsäläinen et al., 2005). However, a recent meta-analysis confirmed the significant association between the Met allele of the BDNF Val66Met and AD in female patients (Fukumoto et al., 2010), stressing the need to subdivide AD patients according to gender. Namely, Met allele was significantly associated with susceptibility to AD in women, but not in men (Fukumoto et al., 2010). Since AD occurs more frequently in women (Candore et al., 2006) and BDNF Val66Met was reported to have sexually dimorphic effect on susceptibility to AD (Fukumoto et al., 2010), we subdivided all patients according to gender. Given that psychotic symptoms occur frequently in AD (Borroni et al., 2010; Cummings et al., 1994; Schneider and Dagerman, 2004), and BDNF Val66Met was reported to be associated with psychosis (Rosa et al., 2006), all subjects were further subdivided according to the presence or absence of psychotic features. Due to the ethnic differences found in BDNF Val66Met (Pivac et al., 2009), we evaluated BDNF Val66Met and the susceptibility to AD, onset of AD and psychotic symptoms in ethnically homogenous population of male and female Caucasians living in Croatia.

2. Methods

2.1. Patient population

The study included 211 patients with AD who were 74.1±11.2 years old (54 male and 157 female patients), recruited at the Psychiatric Hospital Vrapce and Department of Neurology, Clinical Hospital Centre Zagreb. All AD patients met the diagnostic criteria of probable AD according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (APA, 1994), and the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINDS-ADRDA), (McKhan et al., 1984). The exclusion criteria for all patients were diagnosis of severe organic disease (cancer, heart disease, epilepsy, and brain trauma), major functional psychiatric disorders (depression, schizophrenia, and mania), and alcoholism. The severity of dementia was assessed by Mini Mental Status Examination (MMSE) (Folstein et al., 1975). All patients were subdivided into those with early and late onset AD: AD onset before or after 65 years of age, according to the International Classification of Diseases, 10th revision—ICD–10 (WHO, 1992), categorized as F00.0 (Dementia in Alzheimer’s disease with early onset) and F00.1 (Dementia in Alzheimer’s disease with late onset). In addition, AD patients were divided into groups according to the presence of psychotic symptoms. Psychotic features in patients with AD were evaluated by means of the Neuropsychiatric Inventory (NPI, Cummings et al., 1994). Information for the NPI was obtained from a caregiver familiar with the patient behavior. The NPI was applied to all AD patients. Since the NPI consists of several different parts focusing on delusions, hallucinations, dysphoria, anxiety, agitation/aggression, euphoria, disinhibition, irritability, apathy and aberrant motor activity, only patients with positive answers under items “Delusions” and/or “Hallucinations” were rated as exhibiting psychotic symptoms. The psychotic symptoms had to be present in the past month, and their severity was rated as “moderate” or “severe”. The exclusion criteria included the presence of delusions and hallucinations due to other psychiatric disorders with psychotic symptoms which have different manifestation compared to AD. Participants or their guardians gave informed consent.

2.2. Control subjects

The control group included 402 elderly healthy subjects 75.7±7.4 years old (187 male and 215 female), consisting of unrelated Croatian male and female individuals from the local senior centers, with no personal or familiar history of psychiatric illnesses. All subjects filled out a questionnaire containing the questions about their medical history, smoking and drinking habits. Portion of the healthy subjects (N=215) was also assessed with MMSE, and male

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and female control subjects had similar mean MMSE scores. Control subjects and patients with drug abuse, head injury, kidney, liver or cardiovascular disease and diabetes were excluded. All participants gave informed consent.

All subjects were ethnically homogenous population of Caucasians living in Croatia. The study design was approved by the local Ethics Committees. All positive laws of the Republic of Croatia and ethical norms, as well as the anonymity of the participants, were respected. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.3. Genotyping

The DNA was isolated from blood using the ‘salting-out’ method (Miller et al., 1988), or using the DNeasy Blood and Tissue Kit (Qiagen). Genotyping of BDNF Val66Met was carried out in a total volume of 10 µL containing 30–100 ng of DNA by measuring the fluorescence intensity of reporters at PCR endpoint with an ABI Prism 7000 Sequence Detection System apparatus (ABI, Foster City, USA), using Taqman-based allele-specific polymerase chain reaction assay, according to the manufacturer’s instructions. The primers and probes were purchased from Applied Biosystems as well (ABI, Foster City, USA).

2.4. Statistical analysis

The results regarding age, duration of AD and MMSE scores were presented as means ± SD and were evaluated using one-way analysis of variance (ANOVA) followed by a Tukey’s test. The frequency of the genotypes and alleles was evaluated using a χ² or Fischer’s exact test, and the Yates correction for continuity when appropriate, with Sigma Stat 3.5 (Jandel Scientific Corp. San Rafael, California, USA). Hardy–Weinberg equilibrium (HWE) of the genotype frequencies in all sub-samples was evaluated. The power of calculation (power), which should be ≥0.800, was evaluated using Sigma Stat 3.5. To determine which genotype had the major influence on the significant χ²-test statistics, standardized residuals (R) were evaluated using Microsoft Excel (http://www.acastat.com/Statbook/chisqresid.htm). All subjects were additionally sub-grouped as the Met carriers (combined Met/Met and Met/Val genotypes) and the homozygous Val/Val genotype. The level of significance was set to α = 0.0125, with 2-sided p values, after a Bonferroni correction for multiple testing (4 comparisons: 4 AD subgroups/early onset AD, late onset AD, psychotic AD and non-psychotic AD).

3. Results

3.1. Age, MMSE scores and duration of AD in male and female patients with AD and in healthy subjects

The mean MMSE scores, describing the severity of dementia, differed significantly (F = 203.470; d.f. = 3.422; P < 0.001, one-way ANOVA, power = 1.000) between male (16.5 ± 7.1) and female (12.8 ± 8.5) patients with AD compared to healthy men (27.7 ± 1.8) and women (27.9 ± 1.6). As expected, healthy men and women assessed using MMSE had significantly (P < 0.001, Tukey’s test) higher mean MMSE scores than male or female patients with AD. Female patients with AD had significantly (P < 0.001, Tukey’s test) lower mean MMSE scores compared to all other groups.

Although healthy male or female subjects were matched for age (in years) with female AD patients, mean age of the subjects differed significantly (F = 27.744; d.f. = 3.609; P < 0.001, one-way ANOVA, power = 1.000). Male patients with AD were significantly (P < 0.001, Tukey’s test) younger (65.1 ± 10.8) than female patients with AD (77.1 ± 9.7), or than healthy male (75.0 ± 8.6) or female (76.3 ± 6.2) subjects.

The mean duration of AD (in years) was significantly different (F = 75.458; d.f. = 1.209; P < 0.001, one-way ANOVA, power = 1.000) between male (3.7 ± 1.9) and female (5.7 ± 1.4) patients with AD, since female patients exhibited significantly (P < 0.001, Tukey’s test) longer duration of illness than male patients.

3.2. BDNF Val66Met polymorphism allele and genotype frequencies in male and female patients with AD with or without psychotic symptoms

The detailed distribution of the gene variants of the BDNF Val66Met polymorphism, consisting of count and frequency (%) of the Met/Met, Met/Val and Val/Val genotypes, or Met or Val alleles, or Met carriers [the combined Met/Met and Met/Val genotypes] versus the Val/Val homozygous genotype in male and female control subjects and patients with AD (subdivided further according to the onset of AD and presence of psychotic symptoms) is shown in Tables 1–3. The deviation from HWE in the Met/Met, Met/Val and Val/Val genotypes of the BDNF Val66Met was detected in female patients with AD (χ² = 11.924; P = 0.005), female patients with late onset AD (χ² = 11.969; P = 0.0005), and in female patients with psychotic AD (χ² = 14.829; P = 0.0001). Other groups did not show any deviation from the HWE (data not shown). When patients with AD were subdivided according to the presence or absence of psychotic symptoms, significant differences in the frequency of the Met/Met, Met/Val and Val/Val genotypes (P = 0.008), Met and Val alleles (P = 0.008) and the combined Met/Met, Met/Val genotypes when compared to the homozygous Val/Val genotype (P = 0.006) were found in male AD patients with or without psychotic symptoms (Table 1). To find out which genotype significantly contributed to this significant χ² statistics, standardized residuals were calculated. The highest R values were found in male psychotic AD patients for the Met/Val genotype (R = 2.61), for the Met allele (R = 2.09) and for the combined Met/Met and Met/Val genotypes (R = 2.30), confirming the major influence of one or two Met alleles of BDNF Val66Met on the differences between psychotic and non-psychotic male patients. In contrast to male patients, there were no significant differences in the frequency of the gene variants of the BDNF Val66Met among female patients with AD with or without psychotic symptoms (Table 1).

3.3. BDNF Val66Met polymorphism allele and genotype frequencies in male and female patients with early and late onset AD

When male and female patients with early or late onset AD were compared, no significant difference was detected in the frequency of the Met/Met, Met/Val and Val/Val genotypes, Met and Val alleles and the combined Met/Met, Met/Val genotypes versus the Val/Val homozygous genotype between male patients with early or late onset AD, or between female patients with early or late onset AD (Table 2).

3.4. BDNF Val66Met polymorphism allele and genotype frequencies in male and female control subjects and patients with AD

When groups were compared separately according to gender, there was no significant difference in the frequency of the Met/Met, Met/Val and Val/Val genotypes, Met and Val alleles and the combined Met/Met, Met/Val genotypes when compared to Val/Val homozygous genotype between male patients with AD and male control subjects, or between female patients with AD and female control subjects (Table 3).

3.5. Sex related differences in the BDNF Val66Met variants between male and female patients with AD and male and female control subjects

In the group of patients with psychotic symptoms in AD, significant sex related differences were found between male and female patients in the frequency of the BDNF Val66Met genotypes...
Table 1
BDNF genotype and allele count and frequencies (percentages) in male and female patients with Alzheimer’s disease (AD) subdivided in patients with psychotic features (psychotic) or without psychotic features (non-psychotic).

<table>
<thead>
<tr>
<th>BDNF Val66Met</th>
<th>Genotype</th>
<th>Allele</th>
<th>Met carriers</th>
<th>Val homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Val/Val</td>
<td>Val/Met</td>
<td>Met/Met</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>(N)</td>
<td>(N)</td>
<td>(N)</td>
<td>(N)</td>
</tr>
<tr>
<td>Psychotic</td>
<td>3 (25.0)</td>
<td>8 (66.7)</td>
<td>1 (8.3)</td>
<td>14 (58.3)</td>
</tr>
<tr>
<td></td>
<td>(χ² = 9.573; df = 2; P = 0.003; power = 0.551)</td>
<td></td>
<td>(10 (41.7)</td>
<td>(75.0)</td>
</tr>
<tr>
<td>Non-psychotic</td>
<td>29 (74.4)</td>
<td>9 (23.1)</td>
<td>1 (2.5)</td>
<td>67 (85.9)</td>
</tr>
<tr>
<td></td>
<td>(χ² = 6.926; df = 1; P = 0.008; power = 0.759)</td>
<td></td>
<td>(11 (14.1)</td>
<td>(25.6)</td>
</tr>
<tr>
<td>Female</td>
<td>32 (76.2)</td>
<td>5 (11.9)</td>
<td>5 (11.9)</td>
<td>69 (82.1)</td>
</tr>
<tr>
<td>Psychotic</td>
<td>(71 (61.2)</td>
<td>35 (30.2)</td>
<td>10 (8.6)</td>
<td>177 (76.3)</td>
</tr>
<tr>
<td></td>
<td>(χ² = 5.477; df = 2; P = 0.065; power = 0.533)</td>
<td></td>
<td>(15 (17.9)</td>
<td>(38.8)</td>
</tr>
<tr>
<td>Non-psychotic</td>
<td>55 (29.8)</td>
<td>10 (5.9)</td>
<td>2 (0.7)</td>
<td>55 (10)</td>
</tr>
<tr>
<td></td>
<td>(χ² = 0.086; df = 1; P = 0.770; power = 0.014)</td>
<td></td>
<td>(23.7)</td>
<td>(38)</td>
</tr>
</tbody>
</table>

(χ² = 15.507; df = 2; P = 0.001; power = 0.962), and in the frequency of Met carriers versus Val homozygous genotype (χ² = 8.587; df = 1; P = 0.003; power = 0.551), while the difference in the allelic (Met or Val) frequency (χ² = 4.685; df = 1; P = 0.030; power = 0.799) disappeared after Bonferroni correction. No significant sex related differences in the frequency of the genotypes, alleles or Met carriers versus Val homozygous genotype were detected between male and female control subjects, male and female patients with AD, male and female patients with late onset AD, as well as male and female patients with non-psychotic subtype of AD (data available on request).

4. Discussion

The results of the present study showed that 1) BDNF Val66Met was significantly associated with the presence of psychotic symptoms in male, but not in female patients with AD; and 2) the gene variants of the BDNF Val66Met polymorphism were not significantly associated with AD or onset of AD in ethnically homogenous Caucasian male or female subjects from Croatia.

To the best of our knowledge, this is the first study to show a significant association between the gene variants of the BDNF Val66Met polymorphism and psychotic symptoms in male patients with AD. The psychotic symptoms, frequent complication of AD (Borroni et al., 2010; Cummings et al., 1994; Schneider and Dagerman, 2004), include mostly delusions, hallucinations, aggression, and these symptoms cause complete disruption in patient’s functioning (Schneider and Dagerman, 2004), induce rapid cognitive decrease, and result in institutionalization of AD patients (Borroni et al., 2010; Cummings et al., 1994). Psychotic symptoms were associated with decreased cerebrospinal fluid and plasma BDNF levels in the first episode of psychosis (Pillai et al., 2010). Psychotic symptoms were

Table 2
BDNF genotype and allele count and frequencies (percentages) in male and female patients with Alzheimer’s disease (AD) subdivided according to the early and late onset AD.

<table>
<thead>
<tr>
<th>BDNF Val66Met</th>
<th>Genotype</th>
<th>Allele</th>
<th>Met carriers</th>
<th>Val homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Val/Val</td>
<td>Val/Met</td>
<td>Met/Met</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>(N)</td>
<td>(N)</td>
<td>(N)</td>
<td>(N)</td>
</tr>
<tr>
<td>Early onset AD</td>
<td>22 (64.7)</td>
<td>10 (29.4)</td>
<td>2 (5.9)</td>
<td>54 (79.4)</td>
</tr>
<tr>
<td></td>
<td>(χ² = 1.077; df = 2; P = 0.215; power = 0.315)</td>
<td></td>
<td>(20.6)</td>
<td>(35.3)</td>
</tr>
<tr>
<td>Late onset AD</td>
<td>10 (50.0)</td>
<td>0 (50.0)</td>
<td>0 (0.07)</td>
<td>30 (75.0)</td>
</tr>
<tr>
<td></td>
<td>(χ² = 0.086; df = 1; P = 0.770; power = 0.438)</td>
<td></td>
<td>(25.0)</td>
<td>(50.0)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (63.0)</td>
<td>8 (29.6)</td>
<td>2 (7.4)</td>
<td>42 (77.8)</td>
</tr>
<tr>
<td>Early onset AD</td>
<td>13 (66.2)</td>
<td>3 (23.8)</td>
<td>13 (10.0)</td>
<td>203 (78.0)</td>
</tr>
<tr>
<td></td>
<td>(χ² = 0.097; df = 1; P = 0.924; power = 0.048)</td>
<td></td>
<td>(22.0)</td>
<td>(33.8)</td>
</tr>
<tr>
<td>Late onset AD</td>
<td>44 (63.0)</td>
<td>13 (23.8)</td>
<td>13 (10.0)</td>
<td>86 (66.2)</td>
</tr>
<tr>
<td></td>
<td>(χ² = 0.009; df = 1; P = 0.924; power = 0.048)</td>
<td></td>
<td>(33.8)</td>
<td>(66.2)</td>
</tr>
</tbody>
</table>
related to gender induced differences in regional perfusion alterations in AD patients, showing different cerebral physiology in male and female patients with AD (Moran et al., 2008). A significant association between the BDNF Val66Met genotypes and the risk for psychosis (Rosa et al., 2006), onset of schizophrenia (Zhou et al., 2010) and the positive symptoms (conceptual disorganization, hallucinatory behavior, unusual thought content and grandiosity) of schizophrenia (Numata et al., 2006) was found. In our study a significant effect of one or two Met alleles of BDNF Val66Met on psychotic symptoms was detected in male, but not female patients with AD. Although smaller number of male compared to female patients was included, all comparisons in male patients, in contrast to female patients, had adequate power to detect significant association between the risk genotypes and psychosis in AD. Since the Met allele is assumed to be associated with the reduced BDNF concentration, and lower concentration of BDNF was found in schizophrenia (Pillai et al., 2010), our results agree with these findings, and with the findings that carriers of the Met allele had an increased risk for schizophrenia, other psychotic disorders (Gratacos et al., 2007) or schizoaffective and psychotic mood disorder (Jonsson et al., 2006). In line with the finding that Met carriers experienced greater social-stress-induced paranoia than subjects with the Val/Val genotype (Simons et al., 2009), or more frequently develop depression (Verhagen et al., 2010), our psychotic male patients with AD were more frequently Met carriers than carriers of the Val/Val homozygous genotype. Since BDNF Val66Met was reported to be related to altered intracellular trafficking of the pro-BDNF, and therefore the altered secretion of the matured BDNF protein (Chen et al., 2004; Egan et al., 2003), its effects on the psychotic features in AD might have been achieved via influence on BDNF expression and protein production. If BDNF promotes the survival of cholinergic, dopaminergic, and serotonergic neurotransmission (Fumagalli et al., 2006; Huang and Reichardt, 2001), and their function is altered in AD, while Met allele of the BDNF Val66Met is associated with psychoses (Gratacos et al., 2007), we expected that Met allele would be associated with severe deficits, such as psychotic symptoms in AD. The reason for the sexually dimorphic effect of BDNF Val66Met on psychotic symptoms in AD at present unclear, but might be due to the gender differences in AD (Candore et al., 2006), gender differences in the brain function in patients with or without psychotic symptoms in AD (Moran et al., 2008), or gender differences in BDNF Val66Met in AD (Fukumoto et al., 2010). This sexually dimorphic effect might be due to the departure from HWE in female but not in male patients, or in the lack of power to detect significant difference in the frequency of the BDNF Val66Met genotypes in psychotic and non-psychotic female AD patients. The observed gender differences might also be explained by the high incidence of delusions and hallucinations that occur early in the course of AD (Paulsen et al., 2000), and accordingly our male patients were younger, had AD for a shorter time, and had less severe dementia than female patients with AD.

In addition to the significant associations between psychotic symptoms in AD and variants of genes encoding for the COMT, dopamine receptors (DRD1 and DRD3), serotonergic receptors (5-HT2A and 5-HT2C), serotonin transporter, interleukin 1, and alpha 7 nicotinic acetylcholine receptor, reviewed in details recently (Borroni et al., 2010), we have detected significant association between psychotic symptoms in AD and BDNF Val66Met in male but not female AD patients. The role of APOE4 in psychotic AD is still unclear (Borroni et al., 2006; Borroni et al., 2010). Namely, the frequency of the APOE4 carriers or non-carriers did not differ significantly between our psychotic and non-psychotic AD patients (unpublished data), and no significant association between APOE genotype and “psychosis” endophenotype, i.e. hallucinations, delusions, sleep disturbances and aberrant motor behavior was found (Borroni et al., 2006). On the other hand, other reports showed a significant association between ApoE allele and psychotic symptoms, mostly delusions (Spalletta et al., 2006; Zdanys et al., 2007).

The observed distribution of the gene variants of the BDNF Val66Met between patients with AD subdivided according to gender and onset of AD and elderly control men and women is in accordance with the findings from previous studies (Bian et al., 2005; He et al., 2007; Lee et al., 2005; Li et al., 2005; Desai et al., 2005; Forero et al., 2006; Nacmias et al., 2004; Vepsalainen et al., 2005; Tsai et al., 2004; Zhang et al., 2006), although conflicting reports have also been published (Fukumoto et al., 2010; Tsai et al., 2006; Ventriglia et al., 2002). To exclude the effect of the ethnic differences in BDNF Val66Met (Pivac et al., 2009; Fukumoto et al., 2010), and to explain the departure from HWE in our female patients with AD, we compared the BDNF Val66Met genotype distribution, separately in our male and female patients with AD, with the reported distribution in Caucasian patients with AD (Fukumoto et al., 2010). This comparison showed a similar frequency of the BDNF Val66Met

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**Table 3**

BDNF genotype and allele count and frequencies (percentages) in male and female control subjects and patients with Alzheimer’s disease (AD).

<table>
<thead>
<tr>
<th></th>
<th>BDNF Val66Met</th>
<th>Allele</th>
<th>Met carrier</th>
<th>Val homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Patients with AD</td>
<td>122 (65.2)</td>
<td>59 (31.6)</td>
<td>6 (3.2)</td>
<td>303 (81.0)</td>
</tr>
<tr>
<td></td>
<td>6 (3.2)</td>
<td>2 (3.7)</td>
<td>71 (19.0)</td>
<td>(19.0)</td>
</tr>
<tr>
<td></td>
<td>(27.8)</td>
<td>(22.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.722</td>
<td>power = 0.098</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>146 (67.9)</td>
<td>59 (27.4)</td>
<td>10 (4.7)</td>
<td>351 (81.0)</td>
</tr>
<tr>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Patients with AD</td>
<td>103 (65.6)</td>
<td>39 (24.8)</td>
<td>15 (9.6)</td>
<td>245 (78.0)</td>
</tr>
<tr>
<td></td>
<td>15 (9.6)</td>
<td>2 (3.2)</td>
<td>69 (19.0)</td>
<td>(19.0)</td>
</tr>
<tr>
<td></td>
<td>(22.0)</td>
<td>(26.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.169</td>
<td>power = 0.360</td>
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</tr>
</tbody>
</table>

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genotypes within male groups with AD (χ² = 17.868; df = 14; P = 0.213; power = 0.781). Within female subjects there was a slight, but significant difference in the frequency of the genotypes between our and reported data (χ² = 24.736; df = 14; P = 0.037; power = 0.924). Although our female patients were older, had AD for a longer time, and had more severe dementia and lower cognitive performance than male AD patients, as evidenced by the lower MMSE scores, we failed to replicate the finding that BDNF Met66 allele contributed to AD susceptibility in female patients (Fukumoto et al., 2010). The deviation from the HWE in female AD patients might be due to genotyping error (Xu et al., 2002). However, we have excluded this possibility by repeating the genotyping. The apparent non-replication might be explained by the lack of statistical power and consequent type II error, or the influence of the APOE4 status. Namely, BDNF Val66Met was reported to be a risk factor for AD in non-APOE4 carriers (Tsai et al., 2006), however our unpublished data and other reports did not confirm this association (Matsushita et al., 2005; Nacmias et al., 2004; Ventriglia et al., 2002).

The limitations of the present study are the small number of patients with AD, the lack of the statistical power in female patients with AD, and the determination of only BDNF Val66Met polymorphism. However, the other most frequently studied BDNF polymorphism, C270T, was not associated with susceptibility to AD (Fukumoto et al., 2010; Zhang et al., 2006). The advances of this study are in ethnically uniform Caucasian patients and adequate number of elderly control subjects, in subdivision of patients according to the gender, onset of AD, presence or psychotic symptoms, in the adequate statistical power to detect differences between psychotic and non-psychotic male patients with AD, and in the first significant association found between the gene variants of the BDNF Val66Met polymorphism and psychosis in AD.

5. Conclusion

In conclusion, we have detected a significant association between the genetic variants in the BDNF Val66Met polymorphism and psychotic symptoms, with significant differences in the distribution of the Met/Met, Met/Val and Val/Val genotypes, and the combined Met/Met and Met/Val genotypes versus the homozygous Val/Val genotype, between male patients with AD, with or without psychotic symptoms. This difference was due to the major contribution of the presence of one or two Met allele to development of psychotic symptoms in male, but not in female patients with AD. Our results suggest that carrying one or two Met alleles of BDNF Val66Met might present a risk factor for development of psychotic symptoms in AD in male patients, and confirm the important role of BDNF in neurodegenerative, synaptic plasticity and development of psychosis.

Acknowledgments

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References


