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Brain-derived neurotrophic factor gene polymorphisms, neurotransmitter levels, and depressive symptoms in an elderly population

Maria E. Czira · Heike Wersching · Bernhard T. Baune · Klaus Berger

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Abstract A large number of studies have examined associations between brain-derived neurotrophic factor (BDNF) gene polymorphisms and depressive symptoms. However, results still remain controversial. Recent studies suggested a significant age and gender effect on the heritability of depression. The potential neurobiological pathways that could possibly mediate this relationship have not been examined so far. Since BDNF is involved in the regulation of neurotransmitter production, a mediating role of neurotransmitters seems plausible. The present study aims to examine the association between three common BDNF single-nucleotid polymorphisms (SNPs; rs7103411, rs7124442, and rs6265) and depressive symptoms in a community-based elderly population taking into account the serum levels of four neurotransmitters, serotonin, dopamine, adrenalin, and noradrenalin, as potential mediating factors. We also examined whether age and gender had a modifying effect on this association. We collected and analyzed the genetic and laboratory data as well as Center for Epidemiologic Studies-Depression scores of 350 community-dwelling elderly individuals (aged 65+ years). We found that the BDNF rs6265 polymorphism was related to the severity of depressive symptoms, and that this association was independent of neurotransmitter levels. Stratified analyses showed that this association was restricted to older individuals (≥74 years) and men. The associations of SNPs rs7103411 or rs7124442 SNP with depressive symptoms were not statistically significant. This study importantly adds to the existing literature by affirming previous assumptions on an age and gender difference in the relation between BDNF genotype and depression. We moreover first-time report a missing mediating role of neurotransmitters in this association.

Keywords Elderly · Depression · Brain-derived neurotrophic factor · Gene polymorphism · Neurotransmitter

M. E. Czira (✉) · H. Wersching · K. Berger
Institute of Epidemiology and Social Medicine,
University of Münster,
Domagkstr. 3.,
48149 Münster, Germany
e-mail: cziramaria@yahoo.com

M. E. Czira
Institute of Behavioral Sciences, Semmelweis University,
Nagyvarad ter 4.,
1089 Budapest, Hungary

B. T. Baune
Discipline of Psychiatry, School of Medicine,
University of Adelaide,
Adelaide, SA 5005, Australia

Introduction

Depression is a multifactorial disorder with genetic and environmental factors contributing to its development (Ebmeier et al. 2006). One of the candidate genes discussed in association with depression is the gene encoding the brain-derived neurotrophic factor

(BDNF) (Levinson 2006). BDNF is highly expressed in the central nervous system, especially in the hippocampus, and is involved in neuronal proliferation, survival and activity-dependent plasticity (McAllister et al. 1999; Bath and Lee 2006). The most extensive research had been done on a single-nucleotide polymorphism (dbSNP reference number rs6265) in the coding region of exon V of the BDNF gene, producing an amino acid substitution (valine to methionine) at codon 66 (Val66Met). This amino acid change is associated with abnormal intracellular packaging of BDNF resulting in a reduced production of the functional neurotrophin in the central nervous system (Duman 2004). According to the "neurotrophic hypothesis of depression" (Duman 2004; Duman et al. 1997) a reduction of BDNF production is involved in the pathophysiology of depression (Schumacher et al. 2005; Angelucci et al. 2005; Karege et al. 2002). The meta-analysis by Kato and Serretti (2008) found a better response to antidepressants in subjects with the Met variant within the BDNF Val66Met polymorphism. A recent paper by Domschke et al. (2010) suggested associations of two other BDNF polymorphisms, rs7124442 (position: chr11:27,633,617) and an intronic SNP rs7103411 (position: chr11:27,656,701) with the treatment response to antidepressants in melancholic depression. The study by Zhang et al. (2010) also reported that SNPs rs7124442 and rs6265 conferred increased risk for major depression in two- and three-locus gene interactions with a Glycogen synthase kinase 3 beta polymorphism (rs6782799) in a Chinese population. Additionally, rs7124442 has shown to be functionally related to BDNF plasma levels in eating disorder subjects (Mercader et al. 2007). As missense SNPs (e.g., Val66Met) make amino acid sequence changes, they can directly affect protein expression and function. However, it is difficult to explain the expected effects of intron SNPs, which do not affect directly the amino acid sequences. Recently, it has been suggested that some intronic changes had an effect on the efficiency of normal splicing and structural stabilization of pre-mRNA, the expression of exons, and protection from degradation (Malisic et al. 2010; Ying et al. 2010). However, rs7103411 and rs7124442 polymorphisms have not been extensively examined yet in relation with depressive symptoms.

Although a large number of studies examined the associations between BDNF gene polymorphisms and depressive symptoms, results in the published literature on this relationship remain controversial. Some studies have found conclusive relationships between BDNF gene polymorphisms and depressive symptoms (Taylor et al. 2007; Hwang et al. 2006; Ribeiro et al. 2007) while others found only slight or no association (Oswald et al. 2005; Surtees et al. 2007; Schumacher et al. 2005; Hong et al. 2003; Tsai et al. 2003). A recent meta-analysis summarizing the results of 14 case–control studies also reported no association between the rs6265 (Val66Met) polymorphism and depression (Verhagen et al. 2010). When Verhagen et al. stratified data by gender, they found a significant association between BDNF Val66Met polymorphism and major depressive disorder (MDD) in male subjects: male MDD cases carried the Met allele significantly more often than male controls (32.5% in MDD cases versus 22.2% in controls; $p=0.001$), and male MDD cases carried the Met/Met genotype significantly more often than male controls (21.9% in MDD cases versus 7.9% in controls; $p=0.003$).

The same paper also reported that the magnitude of odds ratios for this relationship was the highest in studies carried out in the oldest age groups (Hwang et al. 2006; Taylor et al. 2007). Other studies have also found gender differences and age effects in the relationship between BDNF polymorphisms and depression (Chen et al. 2008). In the etiology of geriatric depression, the involvement of the hippocampus is stronger (Steffens et al. 2000). During chronic stress, the hippocampus suffers glucocorticoid-induced damage (Sheline et al. 1996; Sapolsky 2000). A reduction of hippocampal volume has been consistently associated with depression (Videbech and Ravnkilde 2004; Campbell et al. 2004; Steffens et al. 2000), although findings about directionality are controversial. As the main site of BDNF production is the hippocampus, this might serve as a possible explanation. BDNF is highly concentrated in the hippocampus (Bohlen and Halbach 2011), thus it can protect hippocampal cells from glucocorticoid-induced damage. However, altered neutrophin production might result in a decreased ability for neuronal repair and plasticity. Older participants are also more likely to suffer from late-onset depression, which often precedes the onset of dementia, especially Alzheimer's disease (Saczynski et al. 2010; Dal Forno et al. 2005). Alzheimer's disease is also associated with substantial hippocampal degeneration (Laakso et al. 1998; Ryu et al. 2010), and BDNF polymorphisms (rs6265 and

rs7124442) have also been examined in relation to it (Borroni et al. 2009; Huang et al. 2007).

When examining the relationship between BDNF polymorphisms and depression, most epidemiological studies used a genetic approach without analyzing further neurobiological factors that could possibly mediate this association. The often criticized but still not rejected "monoamine hypothesis of depression", that has been established decades ago, postulates an association between serotonin, noradrenalin, and/or dopamine levels and depression (Schildkraut 1965). BDNF is also involved in the release of neurotransmitters (McAllister et al. 1999), and evidence has been found for a biological epistasis between BDNF and the serotonin transporter gene (SLC6A4) (Pezawas et al. 2008). However, to date, the complex, multi-dimensional relationship between BDNF gene polymorphisms, neurotransmitter levels, and depressive symptoms has not been studied.

In this paper, our aim was to examine the association between three common BDNF polymorphisms, (rs6265, rs7103411, and rs7124442) and depressive symptoms in a community-based elderly population, taking into account the serum levels of four neurotransmitters, serotonin, dopamine, adrenalin, and noradrenalin, as potential mediating factors for the assumed association. We also aimed to examine whether age and gender have a modifying effect on this association.

Methods

Participants

Participants of the second MONICA survey Augsburg, Germany (1989/1990 WHO, Monitoring Trends and Determinants in Cardiovascular Disease) (Keil et al. 1998) aged 65 years and older were re-contacted as part of a follow-up study, the Memory and Morbidity in Augsburg Elderly (MEMO) study (Schmidt et al. 2004). Of those re-contacted, 60.6% responded positively to the MEMO study invitation, resulting in a total of 385 participants. Five participants failed to complete the Center for Epidemiologic Studies-Depression (CES-D) questionnaire, and 30 lacked either all genotyping data or neurotransmitter level; therefore, the analyses presented in this paper are based on the data of 350 participants. Before recruitment into the MEMO study, participants received detailed information regarding the aims and protocol of the study and gave written consent to participate. Demographic data, risk factors, and details of the medical history were collected by interview in the MEMO study center. The study was approved by the Ethics Committee of the University of Münster, Germany.

Assessment of depressive symptoms

To assess the severity of depressive symptoms, all participants completed the CES-D scale in face-to-face interviews. This 20-item scale was designed to measure depressive symptoms during the previous week (summary scores ranges from 0 to 60 points; higher scores indicate more depressive symptoms) (Radloff 1977). The CES-D scale has shown to be a valid and reliable instrument in older populations (Lopez-Leon et al. 2008). To determine the proportion with clinically relevant depression, we applied the commonly used cut-off value of 16 points on the CES-D sale, which has good criterion validity for major depression (Lopez-Leon et al. 2008). To simplify description in this analysis participants with CES-D≥16 are referred to as "high risk for depression" or "depressed" while participants with CES-D<16 are called "low risk for depression" or "non-depressed." The German version of the CES-D scale was used which had been validated in the German general population (Hautzinger and Bailer 1993).

Screening for dementia

Mini–mental state examination (MMSE) (Folstein et al. 1975) was completed for all participants by a trained physician. MMSE is a widely used 30-item standardized tool to assess several aspects of cognitive performance, including orientation, attention, immediate and short-term recall, language, and the ability to follow simple verbal and written commands. Each item is scored as 0 or one, thus the total score ranges between 0 and 30, lower scores indicating a more severe cognitive impairment. MMSE is a brief screening method and does not provide diagnosis or identify specific disorders.

Genotyping

Genotyping of the selected three BDNF tagging SNPs (rs6265, rs7103411, and rs7124442) was carried out

following published protocols for the multiplex genotyping assay iPLEX™ for use with the MassARRAY® platform (Sequenom, San Diego, CA, USA). The genotyping completion rate was 96% for rs6265, 94% for rs7103411, and 91% for rs7124442 due to genotyping errors. Genotypes were determined by investigators blinded to the purposes of this study. Linkage disequilibrium was moderately high between SNPs rs6265 and rs7103411 (D'=0.968, r^2=0.762) and low between SNPs rs6265 and rs7124442 (D'=0.936, r^2=0.078) and SNPs rs7103411 and rs7124442 (D'=0.906, r^2=0.088; Haploview 4.2).

Laboratory data

Non-fasting blood samples were taken from each participant after the interview. After centrifugation serum samples were kept frozen at −80°C until further use. Neurotransmitter levels were measured by the same laboratory with enzyme-linked immunosorbent assay (ELISA), according to standard procedures (Serotonin-ELISA, CAT (AD/NAD/DA)-ELISA, DLD Gesellschaft für Diagnostika und Medizinische GmbH, Hamburg, Germany, www.dld-diagnostika.de).

Co-morbidities and medication

Data about co-morbidities, such as diabetes mellitus, hypertension, previous stroke, history of myocardial infarction, and bone- and joint-related disorders were collected as self-reports of a physician diagnosis in each participant's history. Information about the use of antidepressant medication was collected from the patients.

Statistical analyses

Hardy–Weinberg equilibrium was examined using the program Finetti provided as an online source (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl; Wienker and Strom). SNPs were used as categorical variables coded as 0 for wild type, 1 for heterozygous variant, and 2 for homozygous variant genotype. Data were summarized using proportions, means (±standard deviation) or median (interquartile range (IQR)) as appropriate. Categorical variables were analyzed using Chi-square test, and continuous variables were compared using Student's t test, Mann–Whitney U test, or Kruskal–Wallis test as appropriate. Trends for CES-D score and neurotransmitter levels across genotypes were tested by Cuzick's test for median trend (Cuzick 1985) for each SNPs.

As the distribution of CES-D score was skewed, we did a natural logarithmic transformation of the variable to obtain a normal distribution, thus in subsequent linear regression models the dependent variable was ln(CES-D+1). We examined the association of all three SNPs with CES-D score in multivariate linear regression models adjusted for age and gender. In subsequent analyses, we only included those SNPs which had a significant association with the CES-D score in the simple model. Using multivariable linear regression models we further determined if the association of these SNPs with the CES-D score was independent of other important covariates and neurotransmitter levels. Four distinct models with the transformed depression score as the outcome variable were analyzed. Each model included age, gender, education, antidepressant treatment, co-morbidities, the rs6265 or the rs7103411 SNP, and one of the neurotransmitters (serotonin, dopamine, noradrenalin or adrenalin) as the independent variables. Subsequently we included all four neurotransmitters simultaneously in the same model. To examine whether age and gender interact with the association between the SNP and depression, we repeated the analyses in gender stratified and age-stratified groups. For the latter purpose, participants were classified into two age groups according to the median of age (65–73 and ≥74 years).

Finally, as a sensitivity analysis, we repeated all multivariate analyses including the MMSE score in the models as independent variable to see if cognitive function had any effect on the examined relationship. We also repeated all analyses by excluding those participants who were receiving antidepressant treatment to eliminate the confounding effect of antidepressants on the examined relationship.

Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, USA) and STATA version 11.1 (STATA Corporation, College Station, TX, USA) software packages.

Results

Baseline characteristics of the sample are shown in Table 1. The average age was 72.7±4.5 years, and 47% of the participants were women. The median (IQR) CES-D score in the total sample was 6.7 (7.8). Twelve

Table 1 Characteristics of the sample

	Total (N=350)
Age (mean±SD)	72.7±4.5
Women (%)	47
Years of education (median; IQR)	10; 1
CES-D score (median; IQR)	6.7; 7.8
Known diabetes mellitus (%)	10.6
History of stroke (%)	6.6
History of acute myocardial infarction (%)	8.3
Known hypertension (%)	47.9
Antidepressant medication use (%)	5.7
Mini–mental state exam score (median; IQR)	26; 3

IQR interquartile range

percent of the participants had CES-D scores indicating a high risk for depression (CES-D≥16). There were more women in the depressed group compared with the non-depressed (61% vs 45%; $p<0.047$). We found no significant difference between the two groups regarding age, educational level, cognitive function (MMSE score), and percentage of individuals with known histories of diabetes, hypertension, acute myocardial infarction or stroke (data not shown).

The distributions of rs6265, rs7103411, and rs7124442 genotypes did not significantly differ from the expected numbers calculated on the basis of observed allele frequencies according to the Hardy–Weinberg equilibrium. The distribution of genetic variants and neurotransmitter levels and the comparison between the groups with CES-D<16 and ≥16 in these parameters are shown in Table 2. There was no significant difference between the two groups neither in median levels of the four neurotransmitters nor in the distribution of the three genetic variants.

The distribution of dopamine and adrenalin significantly differed between men and women. There was no difference in the distribution of serotonin and noradrenalin. We found no difference in the distribution of neurotransmitter levels between the two age groups below and above the median age (data not shown).

The relationship between the three examined BDNF SNPs, the CES-D score and neurotransmitter levels is shown in Table 3. There was a significantly increasing trend of the CES-D score across the three genotypes of SNP rs6265 (5.9, 7.8, and 10.0, respectively). We found no significant trend in the levels of any of the four neurotransmitters for neither of the examined genotypes.

In linear regression analyses SNP rs6265 and rs7103411 showed a significant association with the depression score, adjusted for age and gender (B=0.211, p=0.003 and B=0.138, p=0.04, respectively). The SNP rs7124442 showed no association with CES-D score in any of the performed analyses (Table 3.).

To assess whether the associations between the two SNPs (rs6265 and rs7103411) and CES-D score are independent of other important covariates and neurotransmitter levels, we built separate models for both

Table 2 Distribution of neurotransmitter levels and BDNF gene polymorphisms in the depressed and non-depressed groups

Neurotransmitters[a]	Total	Non-depressed CES-D<16 N=314	Depressed CES-D≥16 N=36	p[a]
Serotonin (ng/mL)	22.1; 22.2	21.5; 21.8	26.8; 26.3	0.37
Dopamine (pg/mL)	0; 448	0; 447	0; 433	0.26
Noradrenalin (pg/mL)	772.1; 801.4	803; 777	690; 960	0.49
Adrenaline (pg/mL)	0; 53.4	0; 55.8	0; 43.5	0.29
Genotypes				
Rs6265 wild type (% (N))	67 (234)	68 (214)	56 (20)	0.33
Heterozygote (% (N))	25 (87)	24 (75)	33 (12)	
Homozygote (% (N))	4 (14)	4 (12)	6 (2)	
Rs7103411 wild-type (% (N))	61 (212)	61 (193)	53 (19)	0.70
Heterozygote (% (N))	29 (102)	29 (90)	33 (12)	
Homozygote (% (N))	5 (16)	5 (14)	6 (2)	
Rs7124442 wild-type (% (N))	46 (160)	46 (144)	44 (16)	0.88
Heterozygote (% (N))	36 (127)	36 (113)	39 (14)	
Homozygote (% (N))	9 (31)	9 (27)	11 (4)	

[a] Median; interquartile range

[b] p for difference between non-depressed and depressed groups

Table 3 Association between three BDNF polymorphisms, depressive symptoms and neurotransmitter levels

	CES-D score (median; IQR)	Linear regression model[a]		Serotonin (ng/ml (median; IQR))	Dopamine (pg/ml (median; IQR))	Noradrenalin (pg/ml (median; IQR))	Adrenalin (pg/ml (median; IQR))
		B	*p*				
Rs6265							
Wild type	5.9; 7.8	0.21	0.003	22.3; 23.3	0; 459.8	766.3; 810.2	0; 53.2
Heterozygote	7.8; 6.7			19.6; 23.1	0; 403.1	722.8; 800.7	0; 58.3
Homozygote	10.0; 10.3*			19.6; 23.0	0; 345.9	823.4; 545.4	0; 17.5
Rs7103411							
Wild type	6.3; 7.5	0.14	0.04	22.4; 20.0	0; 457.8	812.1; 838.7	0; 52.8
Heterozygote	6.7; 6.7			19.7; 23.0	0; 446.7	706.6; 801.1	0; 59.8
Homozygote	7.8; 8.6			21.8; 21.6	0; 253.0	850.2; 1046.5	0; 0
Rs7124442							
Wild type	6.7; 7.8	−0.43	0.49	20.1; 22.5	0; 470.3	896.0; 837.9	0; 57.5
Heterozygote	6.7; 7.8			22.5; 23.5	0; 456.3	736.0; 809.3	0; 53.5
Homozygote	6.7; 7.8			20.7; 14.5	0; 450.6	808.4; 797.5	0; 51.0

*p=0.02 in Cuzick's test for median trend across genotype

[a] Unstandardized coefficients and p values of each SNP in a linear regression model corrected for age and gender; the dependent variable is ln(CES-D+1)

SNPs with the log-transformed depression score as the outcome variable (Table 4). Each model included age, gender, education, antidepressant treatment, co-morbidities, the respective SNP, and one of the four neurotransmitters at a time as independent variables. Gender, antidepressant treatment and SNP rs6265 were significantly associated with the CES-D score independent from the covariates (Table 4). The association of education with the depression score was very weak and failed to reach statistical significance. Of the four neurotransmitters only adrenalin had a very weak association with the depression score. When all four neurotransmitters were included in the model simultaneously, SNP rs6265 kept its significant relationship with CES-D score with a coefficient of the same size as in the model without neurotransmitters (data not shown).

In the multivariable linear regression model, SNP rs7103411 was not significant any more after the other covariates had been adjusted for (Table 5). When all four neurotransmitters were included in the model, the beta-coefficient for rs7103411 SNP was comparable in

Table 4 Association between rs6265 SNP and depressive symptoms in linear regression model

	Model serotonin		Model dopamine		Model noradrenalin		Model adrenalin	
	B	*p*	*B*	*p*	*B*	*p*	*B*	*p*
Age	0.01	0.17	0.01	0.14	0.01	0.18	0.01	0.15
Gender	0.36	<0.001	0.37	<0.001	0.36	<0.001	0.34	<0.001
Education	−0.03	0.07	−0.03	0.05	−0.03	0.07	−0.03	0.12
Cardiovascular morbidity	0.21	0.01	0.22	0.01	0.21	0.01	0.21	0.02
Bone and joint diseases	−0.03	0.83	−0.03	0.82	−0.03	0.80	0.003	0.98
Antidepressant use	0.67	<0.001	0.66	<0.001	0.67	<0.001	0.67	<0.001
Rs6265	0.17	0.01	0.18	0.009	0.17	0.005	0.17	0.011
Neurotransmitter	0.001	0.33	8*10E−5	0.05	10E−5	0.89	−0.002	0.02

R^2 =0.154 for model serotonin; 0.161 for model dopamine; 0.152 for model noradrenalin; 0.165 for model adrenalin

Table 5 Association between rs7103411 SNP and depressive symptoms in linear regression model

	Model serotonin		Model dopamine		Model noradrenalin		Model adrenalin	
	B	*p*	*B*	*p*	*B*	*p*	*B*	*p*
Age	0.01	0.11	0.02	0.08	0.01	0.12	0.01	0.09
Gender	0.33	<0.001	0.35	<0.001	0.33	<0.001	0.31	<0.001
Education	−0.03	0.05	−0.04	0.03	−0.03	0.05	−0.03	0.10
Cardiovascular morbidity	0.22	0.01	0.24	0.007	0.22	0.01	0.21	0.01
Bone and joint diseases	−0.08	0.52	−0.07	0.54	−0.08	0.49	−0.05	0.67
Antidepressant use	0.66	<0.001	0.64	<0.001	0.65	<0.001	0.66	<0.001
Rs7103411	0.09	0.17	0.10	0.12	0.09	0.16	0.09	0.16
Neurotransmitter	<0.001	0.40	10E−5	0.02	5*10E−5	0.94	−0.002	0.05

R^2 =0.139 for model serotonin; 0.151 for model dopamine; 0.137 for model noradrenalin; 0.147 for model adrenalin

size to the model without neurotransmitters, but did not match statistical significance (p=0.051).

To examine if the relationships of SNPs rs6265 and rs7103411 with the depression score differed by gender, we repeated the linear regression analyses stratified for men and women. In male participants, rs6265 had a strong, significant relationship with the depression score in all four models, corrected for age, education, antidepressant treatment, co-morbidities, and serum levels of neurotransmitters (see Table 6). In women, however, we found no relationship between the rs6265 SNP and the depression score. The regression coefficients were about five-fold higher in men than in women indicating an increase in the lnCES-D score of about 0.3 with each additional variant allele. In men SNP rs7103411 also had a stronger relationship with the lnCES-D score than in women, but these associations failed to reach statistical significance (data not shown).

We also examined the effect of age on the aforementioned relationships in groups stratified according to the

Table 6 Association between rs6265 SNP and depressive symptoms in gender stratified groups

	Model serotonin		Model dopamine		Model noradrenalin		Model adrenalin	
	B	*p*	*B*	*p*	*B*	*p*	*B*	*P*
Men (*N*=187)								
Age	0.01	0.64	0.01	0.52	0.01	0.62	0.01	0.50
Education	−0.02	0.29	−0.02	0.23	−0.02	0.28	−0.01	0.54
Cardiovascular morbidity	0.26	0.03	0.27	0.02	0.25	0.03	0.21	0.06
Bone and joint diseases	−0.002	0.99	0.03	0.85	0.002	0.99	0.07	0.64
Antidepressant use	0.91	0.003	0.94	0.002	0.92	0.003	0.92	0.002
Rs6265	0.29	0.004	0.30	0.003	0.29	0.004	0.32	0.002
Neurotransmitter	<0.001	0.87	10E−4	0.04	3*10E−5	0.77	−0.002	0.005
Women (*N*=163)								
Age	0.02	0.09	0.02	0.12	0.02	0.13	0.02	0.13
Education	−0.05	0.06	−0.05	0.09	−0.05	0.09	−0.05	0.09
Cardiovascular morbidity	0.15	0.27	0.13	0.33	0.14	0.29	0.12	0.36
Bone and joint diseases	−0.07	0.71	−0.12	0.54	−0.09	0.65	−0.10	0.59
Antidepressant use	0.63	0.002	0.57	0.006	0.60	0.005	0.58	0.005
Rs6265	0.06	0.49	0.06	0.51	0.06	0.52	0.06	0.51
Neurotransmitter	0.004	0.02	4*10E−5	0.41	3*10E−5	0.73	<0.001	0.86

median of age. In the older age group (≥74 years, mean age=76.8±2.3 years) we found a strong relationship between both BDNF polymorphisms and the depression score, independent of gender, education, antidepressant treatment, co-morbidities and neurotransmitter levels while in the younger group (65–73 years, mean age=69.4±2.6 years), neither of the SNPs showed association with the CES-D score (see Table 7 for results including the rs6265 SNP).

Finally, we repeated all multivariate analyses including the MMSE score in the models as independent variable. The relationship between MMSE score and the CES-D score was not significant in any of the models, and the association between the examined BDNF polymorphisms and depression remained the same after controlling for cognitive function in the total population and also in the gender and age-stratified subgroups (data not shown). Similarly, excluding participants on antidepressant treatment did not affect our results (data not shown).

Discussion

In this cross-sectional study of an elderly community-based population, we found a positive association between BDNF rs6265 genotype and the CES-D score. This association was independent of serum levels of four selected neurotransmitters. Stratified analyses showed that this association was restricted to men and older individuals. The association of a second SNP (rs7103411) with the severity of depressive symptoms was not statistically significant in the final models, except for in the older age group. We found no association between rs7124442 SNP and CES-D score in any of the performed analyses. Median serum levels of serotonin, dopamine, noradrenalin, and adrenalin did not differ significantly between genotypes of the three examined BDNF polymorphisms and were also independent of age. However, the distributions of dopamine and adrenalin levels were different in men and women.

Depression is one of the most common psychiatric disorders in the elderly, causing impairment in several domains of daily life. According to the "neurotrophic hypothesis of depression" decreased circulating levels of neurotrophins, such as brain-derived neurotrophic factor are claimed to be associated with depressive symptoms (Duman 2004; Duman et al. 1997). There is a current debate in the literature about the association between BDNF gene polymorphisms and depression. The most extensively examined gene

Table 7 Association between rs6265 SNP and depressive symptoms in age-stratified groups

	Model serotonin		Model dopamine		Model noradrenalin		Model adrenalin	
	B	*p*	*B*	*p*	*B*	*p*	*B*	*p*
65–73 years (*N*=195)								
Gender	0.35	0.002	0.38	0.001	0.36	0.002	0.34	0.003
Education	−0.04	0.05	−0.04	0.03	−0.04	0.05	−0.03	0.11
Cardiovascular morbidity	0.23	0.08	0.24	0.06	0.22	0.09	0.23	0.08
Bone and joint disease	0.01	0.94	0.02	0.92	−0.01	0.97	0.07	0.68
Antidepressant use	0.40	0.08	0.35	0.13	0.37	0.11	0.41	0.08
Rs6265	0.004	0.96	0.01	0.92	0.001	0.99	−0.001	0.99
Neurotransmitter	0.001	0.37	10E−4	0.01	3*10E−5	0.72	−0.002	0.07
≥74 years (*N*=155)								
Gender	0.42	0.001	0.42	<0.001	0.42	<0.001	0.40	0.001
Education	−0.003	0.92	0.004	0.90	−0.002	0.94	0.004	0.90
Cardiovascular morbidity	0.20	0.08	0.20	0.07	0.20	0.08	0.19	0.10
Bone and joint disease	−0.02	0.87	−0.03	0.85	−0.03	0.86	−0.03	0.84
Antidepressant use	0.94	<0.001	0.95	<0.001	0.93	<0.001	0.91	<0.001
Rs6265	0.42	<0.001	0.42	<0.001	0.42	<0.001	0.43	<0.001
Neurotransmitter	<0.001	0.78	6*10E−5	0.50	−10E−6	0.99	−0.002	0.17

variant in this field is the Val66Met (rs6265) polymorphism. While several publications reported conclusive associations between BDNF Val66Met polymorphism and depressive symptoms (Taylor et al. 2007; Hwang et al. 2006; Suchanek et al. 2011), others found slight or no association (Oswald et al. 2005; Surtees et al. 2007; Schumacher et al. 2005; Hong et al. 2003; Tsai et al. 2003). In this study, apart from the well documented predictors of depression like female gender and cardiovascular disease, we found BDNF Val66Met polymorphism to be in a significant relationship with the depression score even after adjusting for covariates.

Differences in age and gender distributions between studies might have contributed to the previous controversial results. The paper by Chen et al. (2008) reported no evidence for the association between BDNF polymorphisms and indicators of depression in relatively young women. These results support our findings that the association might be only present in men and in older individuals. A recent meta-analysis (Verhagen et al. 2010) draws the same conclusion. Although, the authors found no association between the BDNF gene polymorphism and depression in the total sample, they emphasized the gender and age differences between the examined studies. In those studies, in which gender information was available, depressed men were carrying the Met allele and the Met/Met genotype of the BDNF Val66Met polymorphism more often than male controls. The odds ratio for this relationship was highest in studies carried out in the oldest age groups (Hwang et al. 2006; Taylor et al. 2007). In contrast, the results of a large, community-based survey of Surtees et al. (2007) disagree with these findings, as they reported no association of the Val66Met polymorphism with depression in older adults. However, even in our study of elderly individuals we did not observe the association in the "younger" age group (65–73 years). Thus, it is possible that the "older" study population (mean age=60.2±9.2 years) in the Surtees et al. study was still too young to find a relationship.

Potential biological pathways for the interaction of depression with age are the possible structural changes of the brain, particularly of the hippocampus. According to the stress-induced glucocortioid toxicity model, during chronic stress the hippocampus suffers glucocorticoid-induced damage (Sheline et al. 1996; Sapolsky 2000). A possible explanation for the age-related reduction in hippocampal volume (Raz et al. 2005) lies in the longer time exposed to stress. Several lines of evidence suggest an association between smaller hippocampal volume and depression; however, the directionality of this association is unclear. Previous studies consistently reported smaller hippocampal volume for depressed individuals compared with the non-depressed controls (Videbech and Ravnkilde 2004; Campbell et al. 2004; Steffens et al. 2000). Frodl et al. (2002) found reduced hippocampal volume in male participants with first-ever depressive episode, suggesting that hippocampal degeneration precedes the onset of depression. Structures, such as the prefrontal cortex, the amygdala, and nucleus accumbens receive inputs from the hippocampus, thus providing a possibility for altered hippocampal function to affect emotionality (Maren and Hobin 2007; O'Donnell and Grace 1995). A recent prospective study, however, disagrees with this theory, as they reported that depression might lead to a faster rate of hippocampal volume decline, but they found no evidence for smaller hippocampal volume to precede the development of depression (den Heijer et al. 2011). Unfortunately, those participants who were lost during follow-up in this study were the older ones, and the authors also failed to perform gender and age-stratified analyses, which might have shown different result. Either way, it is reasonable to suggest that BDNF has a protective effect on the hippocampal cells, thus by mitigating the glucocorticoid-induced toxicity the neurotrophin either delays the development of depression or reduces the hippocampal damage during depressive symptoms. In case of impaired production of the functioning neurotrophin, it is probably unable to prevent neuronal degeneration, thus either providing a site of vulnerability for the development of depressive symptoms, or enabling a higher rate of hippocampal volume reduction during depression. The findings of a recent meta-analysis (Hajek et al. 2011) support this theory as they reported that even in healthy carriers of the BDNF Val66Met polymorphism hippocampal volume was reduced compared to Val/Val homozygotes, although the effect sizes in the individual studies were so small, that only the meta-analysis could show the actual difference, supposedly because participants with smaller hippocampal volumes were already excluded for being depressed. Older participants are also more likely to suffer from late-onset depression, which has been suggested to often precede the onset of demen-

tia, especially Alzheimer's disease (Saczynski et al. 2010; Dal Forno et al. 2005), which is also associated with a more pronounced hippocampal degeneration (Laakso et al. 1998; Ryu et al. 2010). BDNF Val66Met polymorphism has been previously associated with depressive symptoms in patients with Alzheimer's disease (Borroni et al. 2009). In our sensitivity analyses, however, we found no association between depression and MMSE score, nor did entering the MMSE score in the multivariate model change the association between the BDNF polymorphisms and depression. It is possible that dementia-associated depression was less frequent in our sample.

It is well known from the literature that the prevalence of depressive symptoms is higher in women than in men, suggesting a possible gender difference in the pathophysiology and development of depression. Although some theories exist with explanations, the specific mechanisms behind these differences are still unclear. In the last decades, several studies have suggested gender differences in the anatomy, chemistry and function of different parts of the brain, including the hippocampus (Cahill 2006). Animal studies have reported evidence for the sex differences in the hippocampal neurotransmitter systems (Madeira and Lieberman 1995) and also in the stress-response of the locus coeruleus (Curtis et al. 2006). An early study reported gender differences in monoamine and monoamine oxidase-levels in several brain regions (Robinson et al. 1977). These also might serve as a basis for the different distribution of neurotransmitters in men and women reported in this paper. Another explanation is based on the difference in genetic linkage associated with depression. Evidence shows that different gene regions are linked to depression in women than in men (Zubenko et al. 2002; Zubenko et al. 2003). A third possible explanation might be the influence of environmental factors. Several studies reported that despite a similar number of experienced life events, women subjectively perceive more stress and have higher acute stress vulnerability in relation to those life events compared with men of the same age (Becker et al. 2007; Troisi 2001). On the other hand, evidence from animal studies suggests that chronic stress causes substantial hippocampal damage in males, but not so much, if any in females (McEwen 2000). The interplay between these structural differences and the genetically determined alteration of BDNF production might serve as a basis for a different vulnerability in the two genders. It appears that the genetically determined vulnerability to chronic stress plays a greater role in the development of depression in men while life events and social circumstances have higher impact in women.

Most previous studies focused on the relationship between BDNF polymorphisms and depression and did not examine potential neurobiological pathways that could possibly mediate this relationship. According to the monoamine hypothesis, depressive symptoms result from monoamine deficiency in the central nervous system (Delgado 2000). Although, this theory has often been criticized, depression is still considered to be associated with impairment of the serotonergic, noradrenergic and/or dopaminergic pathways. Since BDNF is involved in the regulation of neurotransmitter production (McAllister et al. 1999), and associated with the expression of a serotonin transporter gene (Pezawas et al. 2008), it seems obvious to question whether the BDNF-depression association is mediated by their relationship with the neurotransmitters. We tried to examine this complex, multi-dimensional relationship by additionally analyzing and accounting for different neurotransmitters. Our results do not support the monoamine hypothesis since we neither found significant differences in the levels of serotonin, dopamine, noradrenalin, and adrenalin between depressed and non-depressed participants nor between the genotypes of the three examined polymorphisms. Results of the additional subgroup analyses have to be viewed with great caution due to a relatively small sample size. Here, dopamine and adrenaline in men and in older individuals showed very weak associations with depressive symptoms while in women, only serotonin behaved this way.

BDNF rs7124442 SNP has also been examined previously with respect to Alzheimer's disease (Huang et al. 2007) and has shown to be functionally related to BDNF plasma levels in eating disorder subjects (Mercader et al. 2007). The study by Zhang et al. reported an increased risk for major depression in two- and three-locus gene interactions in Chinese population for SNPs rs7124442 and rs6265 (Zhang et al. 2008). In a recent paper (Domschke et al. 2010), the authors found no association of rs7124442 or rs7103411 with the prevalence of depression in a German sample. Since their study population was much younger than ours, the lack of association might have been the result of the age difference. However,

they proposed a potential association of these polymorphisms with the treatment response to antidepressants in melancholic subtype of depression, especially in women.

As antidepressant medication might alter the serum levels of neurotransmitters, we repeated all analyses in the antidepressant medication free participants to rule out any possible effect of antidepressant use on our results. In these analyses, we found very similar results as in the total sample (data not shown).

Rs7103411 SNP is an intronic SNP therefore it is very difficult to explain the possible effects of a base change on the protein production or function. Some introns have been reported to affect the efficiency of normal splicing and the stabilization of pre-mRNA (Malisic et al. 2010; Ying et al. 2010). Intronic regions can also have an overall effect on protein production by controlling the expression of exons, and protecting from degradation. However, as linkage disequilibrium was moderately high between SNPs rs6265 and rs7103411, it is possible that the marginal association observed between the intronic SNP and depressive symptoms was a result of its linkage with SNP rs6265. Supposedly the same explanation stands for the lack of association between CES-D score and SNP rs7124442, as it was in a weaker linkage association with rs6265.

There are several strengths and limitations of our study. Among the limitations is the notion that our results are not to be generalized without further considerations. In the MEMO study 60.6% of the contacted individuals responded, which is high for the included age group, but might have caused a selection bias in our sample. Our study sample consisted of Caucasian individuals only. Assessment of depressive symptoms was based on an established and validated scale, but no clinical diagnosis could be made in this setting. Depressive symptoms were assessed at one point of time; therefore, we have no data about the time of onset or the possible recurring nature of the mood disorder. However, questionnaires remain valuable tools to assess depressive symptoms in epidemiologic surveys. A main strength of our study is the measurement and adjustment for neurotransmitter levels that has not been done before in similar context in human studies. Laboratory analyses and interpretation of these levels, however, are subject to several potential limitations. It was a one-time measurement from peripheral blood drawn at different times between 12 and 4 pm. Serum had been frozen at −80°C for several years before the analyses, and although the refrigeration was continuous, we cannot exclude the possible degradation of neurotransmitters in a frozen sample over time. Although at −80°C generally no degradation occurs in the first few years, there is no published data about such a long storage time as ours; therefore, our results regarding the neurotransmitter levels should be interpreted with caution. BDNF level was not available in our dataset, thus we have no information about the actual quantity of the functional neurotrophin.

In summary, we found that the BDNF rs6265 polymorphism is related to depressive symptoms in men, and in elderly individuals, independently of neurotransmitter levels. We found no association between serum neurotransmitter levels and BDNF gene variants. Further examinations of the potential pathophysiological mechanisms behind the age and gender differences in this relation are needed.

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