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Interactions Among Polymorphisms of Susceptibility *Loci* for Alzheimer's Disease or Depressive Disorder

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Background: Several genetic susceptibility *loci* for major depressive disorder (MDD) or Alzheimer's disease (AD) have been described. Interactions among polymorphisms are thought to explain the differences between low- and high-risk groups. We tested for the contribution of interactions between multiple functional polymorphisms in the risk of MDD or AD.

Material/Methods: A genetic association case-control study was performed in 68 MDD cases, 84 AD cases (35 of them with comorbid depression), and 90 controls. The contribution of 7 polymorphisms from 5 genes (*APOE*, *HSPA1A*, *SLC6A4*, *HTR2A*, and *BDNF*) related to risk of MDD or AD development was analyzed.

Results: Significant associations were found between MDD and interactions among polymorphisms in *HSPA1A*, *SLC6A4*, and *BDNF* or *HSPA1A*, *BDNF*, and *APOE* genes. For polymorphisms in the *APOE* gene in AD, significant differences were confirmed on the distributions of alleles and genotype rates compared to the control or MDD. Increased probability of comorbid depression was found in patients with AD who do not carry the ε4 allele of *APOE*.

Conclusions: Assessment of the interactions among polymorphisms of susceptibility *loci* in both MDD and AD confirmed a synergistic effect of genetic factors influencing inflammatory, serotonergic, and neurotrophic pathways at these heterogenous complex diseases. The effect of interactions was greater in MDD than in AD. A presence of the ε4 allele was confirmed as a genetic susceptibility factor in AD. Our findings indicate a role of *APOE* genotype in onset of comorbid depression in a subgroup of patients with AD who are not carriers of the *APOE* ε4 allele.

MeSH Keywords: **Alzheimer Disease • Apolipoproteins E • Brain-Derived Neurotrophic Factor • Depressive Disorder • HSP70 Heat-Shock Proteins • Serotonin Plasma Membrane Transport Proteins**

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Background

Depressive disorder (unipolar depression, major depression, MDD) is a major cause of morbidity worldwide [1]. Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to dementia [2]. MDD is not a single disorder and some subtypes of MDD may be a risk factor for the onset of AD, the prodrome, and an accompanying symptom found in people with AD [3].

The pathogenesis of MDD or AD is complex, multifactorial, and not fully understood. The monoamine deficiency in MDD and the toxicity of amyloid-β (Aβ) and tau proteins in AD are the most discussed biological bases of these diseases. However, there may be common pathophysiological events underlying both AD and MDD (e.g., inflammatory changes or disturbed glucocorticoids) [4]. Preliminary evidence indicate that region-specific Aβ deposition may be present in some MDD patients, especially in those with treatment resistance, and their depressive symptoms may represent prodromal manifestations of AD [5,6]. However, there are multiple suspected associations between disturbed brain structure, development, or function and genetic, epigenetic, or environmental factors that impact apoptotic, immune-inflammatory, neurotransmitter, neurotrophic, and calcium signaling pathways, neuroplasticity, oxidative and nitrosative stress, cellular bioenergetics, and membrane or vesicular transport [7–10]. Allostatic load [11] and chronobiological changes [12,13] may participate in both the progression of a disorder and the incidence of comorbid diseases.

There is a significant genetic contribution to both MDD [14,15] and AD [16]. The effect of an individual single nucleotide polymorphism (SNP) is generally small; however, functionally relevant SNPs may additively or synergistically disturb the signaling pathways leading to increased risk of complex diseases. A polygenic model has been proposed to explain the genetic susceptibility in MDD or sporadic AD. Gene-gene interactions at 2 or more *loci* (epistasis) are assumed to contribute to the genetic risk of complex diseases [17,18].

Genetic variants that have been most frequently linked to MDD include monoaminergic genes and mutations of mitochondrial DNA [19,20]. Genes associated with the risk for MDD include *SLC6A4* for the serotonin transporter (symbolized as 5-HTT, SERT, or SLC6A4), *HTR2A* for the serotonin 2A receptor (5-HT_{2A}), *TPH2* for neuronal tryptophan hydroxylase 2, *TH* for tyrosine hydroxylase, *MAOA* for monoamine oxidase A, *COMT* for catechol-*O*-methyltransferase, *BDNF* for brain-derived neurotrophic factor, *ACE* for angiotensin-converting enzyme, *APOE* for apolipoprotein E (ApoE), *GNB3* for G protein subunit β3, and *MTHFR* for methylene tetrahydrofolate reductase [21,22]. The association of *APOE* polymorphisms with MDD is little known. Nevertheless, a significant association of the ε4 allele of *APOE* with severe depression in the elderly has been observed [23] and aberrant serum ApoE may be useful markers for assessment of post-stroke depression risk [24]. Another study found that the presence of the ApoE4 significantly enhanced the risk of cognitive decline associated with depressive symptoms [25]. Meta-analyses of genetic studies on MDD found statistically significant associations with *APOE* ε2 alleles [26]. Thus, *APOE* may be included in the list of MDD susceptibility genes. Studies to date show that variation in the serotonin transporter-linked promoter region (5-HTTLPR), *MAOA*, and the *HTR2A* gene can interact with stressful life events to increase risk for suicidal behavior [27]. However, genome-wide association studies (GWAS) of depression have not replicated associations with most *loci* previously identified in studies of individual candidates, including *APOE* [28].

A genome-wide linkage study in families with AD and association studies in patient/control cohorts identified the ε4-allele of the *APOE* gene as a major genetic risk factor for late-onset AD. The *APOE* ε4-allele also increased risk for early-onset AD [29]. More recently, an association of late-onset AD to variants in more than 2 dozen additional genes was confirmed [30,31].

Genetic polymorphisms of BDNF, ApoE, interleukin-1β, and methylenetetrahydrofolate reductase have been demonstrated to confer increased risk for both late-life depression and AD [32], suggesting common genetic pathways may underlie MDD and AD comorbidity. Common pathophysiological mechanisms of both of these disorders include deficits in nerve growth factors, inflammatory changes, and dysregulation mechanisms involving lipoproteins and folate. The most significant symptoms for a depressive disorder in AD are often the same as for patients without dementia [33]. However, it is not clear whether gene polymorphisms affecting the processes involved in the pathophysiology of MDD are the same in MDD and AD with depression. MDD and AD share some pathophysiological mechanisms leading to disturbed neurotransmission. Identified common pathways of MDD and AD include serotonergic (*SLC6A4* 5-HTTLPR, *SLC6A4* VNTR, *HTR2A*), vascular (*APOE* ε4), and neurotrophic (*BDNF*) pathways and response to cell stress (elevated cortisol) ensured by heat-shock protein 70 (HSP70) encoded by *HSPA1A* [34].

The importance of polymorphisms of functional genes *APOE*, *SLC6A4*, *HTR2A*, *BDNF*, and *HSPA1A* and pathways regulated by ApoE [30,35–45], HSP70 [46–49], the serotonin transporter [50–72], 5-HT_{2A} receptor [58,73–84], and BDNF [85–111] in the pathophysiological processes related to MDD and/or AD has been emphasized previously. Their biological interactions through SNPs have not been described for several disease states (MDD, AD, AD presenting with or without depression).

The role of ApoE in the brain is assumed in neuronal survival, plasticity, and metabolism [36]. Altered activity of this protein

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due to *APOE* gene polymorphism is supposed to be linked to the risk for a variety of vascular and neurodegenerative diseases, including AD [35,37,38] and MDD [39,40]. It was concluded that the *APOE* ε4 allele represents a major risk factor for AD across ages between 40 and 90 years and in both men and women. GWAS have confirmed that the ε4 allele of *APOE* is the genetic risk factor for late-onset AD [45]. However, the relationship between *APOE* genotype and other biomarkers or neuropsychiatric symptoms is still unclear.

HSP70 has anti-inflammatory effects in the brain [46]; it protects neurons from damage and decreases inflammatory response by inactivation of glial cells and inhibition of pro-inflammatory cytokine release. Genetic variants within the genes coding for HSP70 family proteins may affect the action of antidepressants and thus their therapeutic efficacy [47]. Increased HSP70 levels may reflect cellular distress in MDD. HSP70 may be involved in AD pathogenesis because it plays a crucial role in preventing protein misfolding and inhibiting aggregation [48,49].

Genes for the 5-HTT and serotonin receptors 5-HT_{1A} and 5-HT_{2A} have been implicated in mood and behavior and they have been widely studied in mental disorders, such as MDD and AD [54–60]. Although SERT activity in the brain could be associated with 5-HTTLPR polymorphism, whether SERT activity regulates the association between stress and depressive disorder is unclear [64]. Some meta-analyses supported the view that polymorphism of 5-HTTLPR moderates the relationship between stress and depression [65], but others do not [67,68].

Preclinical and imaging studies in humans provided support for the involvement of *HTR2A* gene in MDD [57]. The present meta-analysis did not confirm the association of the SNP rs6311 within the *HTR2A* gene with an increased risk for MDD [76]; however, polymorphisms in *HTR2A* gene may be correlated with the efficacy of antidepressants in the treatment of MDD [77,78]. Decreased frontal and temporal cortical 5-HT_{2A} receptors were found in AD patients [79]. The 5-HT_{2A} receptor polymorphism may contribute to the expression of psychosis and agitation/aggression in patients with AD [80–83]. Significant interaction effect was found between *SLC6A4* 5-HTTLPR and *HTR2A* rs6313 polymorphisms [58,84].

BDNF seems to participate in the pathophysiology of major psychiatric disorders, including MDD and AD [96–101], by supporting the activity-dependent modulation of brain networks influencing mood [102,103]. BDNF and its receptors are impaired also with aging and in AD patients [106–109]. It was demonstrated that BDNF has neuronal protective effects against Aβ neurotoxicity [110]. Serotonin and BDNF pathways co-regulate one another; thus, impaired 5-HT and BDNF signaling is central to depression and anxiety disorders, but could also play important roles in the pathogenesis of AD [111].

The genetic effect on MDD or AD development may be potentiated by interactions between various gene polymorphisms. We hypothesized that gene-based contributions to MDD and/or AD could be reflected in interactions of polymorphism of selected genes. The main objective of this study was to investigate gene × gene interactions in patients with AD or MDD by using functional polymorphisms. In a case-control association study, we tested whether interactions of genetic variants of functional genes *APOE*, *SLC6A4*, *HTR2A*, *BDNF*, and *HSPA1A* would confer susceptibility to MDD or AD. The importance of pathways regulated by serotonin, ApoE, HSP70, or BDNF in the pathophysiology of MDD or AD has been emphasized previously, but their biological interactions through SNPs have not been described for several disease states (e.g., MDD, AD, AD with depression, and AD without depression). Our approach has the potential to identify complex biological links among MDD and AD, which will ultimately improve MDD and AD risk management.

Material and Methods

Analysis of multiple SNPs was performed of selected candidate genes, which are supposed to be associated with MDD, AD, or both diseases. In all subjects (Table 1), we genotyped (1) the rs1043618 (+190G/C) and rs1008438 (–110A/C) polymorphisms in the *HSPA1A* gene for HSP70, (2) the linked polymorphic region (5-HTTLPR) and variable number of tandem repeats (STin2 VNTR) polymorphism in the *SLC6A4* gene for 5-HTT, (3) the rs7412 and rs429358 polymorphisms of the *APOE* gene, (4) the rs6265 (196G/A, Val66Met) polymorphism in the *BDNF* gene, and (5) the rs6311(1438G/A) polymorphism in the *HTR2A* gene for the 5-HT_{2A} receptor (Table 2).

Study population

Patients (white women and men) with a diagnosis of depressive disorder or Alzheimer's disease were recruited from the Department of Psychiatry of the First Faculty of Medicine, Charles University and the General University Hospital in Prague, Czech Republic. Demographic data were collected for each person, and the patients were asked to complete a dataset describing their medical history, personal habits, and use of medications (Table 1). Clinical diagnoses were established by trained specialist psychiatrists. All depressed patients were inpatients, while Alzheimer's patients were both inpatients and outpatients.

Clinical evaluation scales and recruitment criteria for participants were described in detail previously [101,112,113]. Briefly, brain magnetic resonance imaging and the NINCDS-ADRDA Alzheimer's Criteria were used in diagnosis of probable AD; patients aged over 50 years were scaled using the

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Table 1. Demographic and clinical data of participants with depressive disorder (MDD), Alzheimer's disease (AD), and controls.

Group	Number (man/ woman)	Age (years)	Education (years)	BMI (kg/m²)	MMSE	GDS	HRSD-21	CGI-S
MDD all	68 (15/53)	46.5±14.4	14.7±3.3	25.5±4.3	29.6±1.0	–	23.0±7.9	4.4±1.2
MDD responders	50 (10/40)	46.3±14.2	15.0±5.2	24.9±4.0	29.9±0.6	–	22.7±8.1	4.7±0.9
MDD nonresponders	17 (4/13)	48.6±14.2	14.6±3.0	25.6±4.5	***28.7±1.2	–	24.1±7.3	3.4±1.3
Control	90 (25/65)	47.8±16.4	14.3±2.3	26.9±4.3	29.8±0.7	–	–	–
AD all	84 (34/50)	***75.5±7.7	13.9±2.8	**23.9±3.2	***18.9±7.0	***5.9±3.7	–	–
AD without depression	49 (21/28)	***75.1±8.4	13.8±2.8	***22.9±2.4	***18.3±7.3	***3.2±1.4	–	–
AD with depression	35 (13/22)	***76.0±6.7	14.0±3.0	25.3±3.6	***19.8±6.6	***9.7±2.1	–	–
Control (>50 years)	40 (10/30)	63.8±7.7	13.9±2.0	27.8±4.2	29.5±1.0	0.4±1.0	–	–

BMI – body mass index; MMSE – mini–mental state examination; GDS – geriatric depression scale; HRSD-21–21-item Hamilton rating scale for depression; CGI-S – clinical global impression-severity scale. Variables are presented as mean ±SD. The indicated *P*-values were calculated by the unpaired *t*-test compared with controls; ****P*<0.001.

Table 2. Measured polymorphisms of genes encoding the apolipoprotein E (ApoE), heat shock 70 kDa protein 1 (HSP70), serotonin transporter (5-HTT), brain-derived neurotrophic factor (BDNF), and serotonin 2A (5-HT_{2A}) receptor.

Protein	Gene	Chromosomal location	Polymorphism	Allele	Variant	Primer – forward	Primer – revers
ApoE	*APOE*	19q13.31	rs7412-rs429358	ε2, ε3, ε4	ε2/ε2, ε2/ε3, ε3/ε3, ε2/ε4, ε3/ε4, ε4/ε4	5'ACAGAATTCGCC CCGGCCTGGTACAC3'	5'TAAGCTTGGCAC GGCTGTCCAAGGA3'
HSP70	*HSPA1A*	6p21.3	rs1043618 (+190G/C)	C, G	C/C, C/G, G/G	5'CGCCATGGA GACCAACACCC3'	5'GCGGTTCCCTG CTCTCTGTC3'
			rs1008438 (–110A/C)	A, C	A/A, A/C, C/C	5'GCCTCTGATT GGTCCAAGGAA3'	5'GCTGCCAGGTC GGGAATAT3'
5-HTT	*SLC6A4*	17q11.2	rs4795541 (5-HTTLPR)	L, S	L/L, L/S, S/S	5'GGCGTTGCC GCTCTGAATG3'	5'GAGGGACTGA GCTGGACAACCAC3'
			rs57098334 (STin2 VNTR)	9, 10, 12	9/10, 9/12, 10/10, 10/12, 12/12	5'GCTGTGGAC CTGGGCAATGT3'	5'AGTGAAGACT GAAAAGACATAATC3'
BDNF	*BDNF*	11p14.1	rs6265 (196G/A, Val66Met)	A, G	A/A, A/G, G/G	5'AGAGGCTTG ACATCATTGGCT3	5'GACTACTGAG CATCACCCTGG3'
5-HT2A receptor	*HTR2A*	13q14-q21	rs6311 (–1438G/A)	A, G	A/A, A/G, G/G	5'AAGCTGCAA GGTAGCAACAGC3'	5'AACCAACTTA TTTCCTACCAC3'

Addenbrooke's Cognitive Examination, which incorporates the Mini-Mental State Examination (MMSE), the AD Assessment Scale – Cognitive (ADAScog), the Barthel activities of daily living (ADL) scale, and the Geriatric Depression Scale (GDS). AD patients were subgrouped according to GDS to "AD with depression" (GDS >6) and "AD without depression".

In MDD patients, diagnosis of a depressive episode or a recurrent depressive episode was confirmed using a structured clinical interview according to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10); severity of depression was assessed using the 21-item Hamilton Rating Scale for Depression (HRSD) and Clinical Global Impression – Severity scale (CGI-S); and a negative screen for bipolar disorder was performed using the Mood Disorder Questionnaire (MDQ). Out of the 68 depressive patients, 47 were treated primarily with selective serotonin reuptake inhibitors (escitalopram, sertraline, paroxetine, and fluoxetine); however, antidepressants of other classes were also used (mirtazapine, venlafaxine, trazodone,

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and agomelatine). Balanced pharmacotherapy often included benzodiazepines. Response is influenced by several factors, including genetic heterogeneity. Thus, MDD patients were subgrouped according to response to treatment as "responders" (defined as >50% improvement in HRSD score compared to baseline) and "nonresponders" [114].

The whole group of controls was used in analyses in control subjects and patients with MDD. The subgroup of controls aged above 50 years was used in analyses in control subjects and patients with AD to decrease risk of undeveloped AD in the control group.

The study was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki), and the study protocol was approved by the Ethics Review Board of the First Faculty of Medicine, Charles University and the General University Hospital in Prague (No. 5/06 MŠMT–1.LFUK). Written informed consent was obtained from all subjects.

Genotyping

Genomic DNA was extracted from peripheral blood anticoagulated with EDTA using a standard salting-out procedure [115]. The concentration and quality of the isolated DNA was determined by spectrophotometer with a NanoDrop ND-1000 (Thermo Fisher Scientific, Wilmington, DE, USA). The primers used for polymerase chain reaction (PCR) are shown in Table 2.

The *APOE* ε2, ε3, and ε4 alleles can be differentiated by typing 2 non-synonymous SNPs, rs429358 and rs7412. We genotyped these SNPs in our samples using PCR and restriction fragment length polymorphism (RFLP) analysis according to the methods reported by Hixson and Vernier [116]. DNA was amplified using specific primers for *APOE* (Table 2).

We analyzed polymorphisms rs1043618 (+190G/C) and rs1008438 (–110A/C) in the gene *HSPA1A* for HSP70. Polymorphisms were detected using TaqMan SNP Genotyping Assays on the unit StepOnePlus™ Real-Time PCR Systems (Applied Biosystems, Foster City, CA, USA).

A modified methodology [117] was used for genotyping the variants of 5-HTTLPR and VNTR in the *SLC6A4* gene for the serotonin transporter. The 5-HTTLPR was amplified by PCR using primers (Table 2), which yielded short (S, 484 bp) and long (L, 528 bp) fragments. The *SLC6A4* VNTR polymorphism in the second intron was amplified using primers, which amplified 3 alleles, containing 9 (STin2.9), 10 (STin2.10), or 12 (STin2.12) copies of the 17-bp repeat element.

Genomic DNA was analyzed for the rs6311 (-1438G/A) polymorphism in the *HTR2A* gene using a PCR reaction and restriction analysis, as described by Ricca et al. [118].

Genotyping rs6265 variants in the *BDNF* gene were performed according to Chou et al. [119]. The rs6265 polymorphism of *BDNF* was analyzed by PCR amplification followed by restriction analysis.

Data analysis

The Hardy-Weinberg equilibrium was tested using the chi-square (χ^2) test. Genotype and allele frequencies of cases and controls were compared by Pearson's χ^2 test to assess departure from the null hypothesis that case and controls have the same distribution of genotype counts [120]. Pearson's *P*-value for the difference in the distribution of alleles or genotypes rates for polymorphisms between cases and control group were adjusted for multiple testing; correction of *P*-value was based on both the Šidák-Bonferroni procedure (Sidak) and the false discovery rate principle with the Benjamini-Hochberg step-up procedure (FDR) [121]. Quantitative information about the association between polymorphisms and the disease was obtained by estimation of the odds ratio (OR) and 95% confidence interval (CI) for each allele, genotype, and disease status (MDD all, MDD responders, MDD nonresponders, AD all, AD without depression, AD with depression) with respect to the reference genotype of healthy controls. ORs and 95% CIs were generated from the logistic regression model. For all associations, the false-positive report probability (FPRP) was computed [122].

Interactions between SNPs were quantified by "interaction information", which is the amount of information bound up in a set of SNPs that is not present in any subset of these SNPs [111]. The SHEsisPlus software [123,124] was used to evaluate the association between SNPs and disease status and analyze the gene interactions (epistasis detection).

Other statistical analyses were performed using the STATISTICA data analysis software system (version 12, StatSoft, Inc., Tulsa, OK, USA). Logit regression using a backward stepwise procedure and a maximum likelihood criterion was used to determine which combination of dichotomized genotypes is significantly more frequent in MDD or AD patients than in control subjects. OR, sensitivity, specificity, and confidence intervals for predicting a disease were also calculated using the DAG_Stat spreadsheet [125].

Results

Demographic and clinical data of participants with MDD, AD, and controls are summarized in Table 1. Personal and clinical

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parameters were acquired from patients and controls, including age, education, body mass index (BMI), and scores of MMSE, GDS, HRSD, and CGI-S. MDD patients and controls were similar with respect to the distribution of age, education, and BMI; AD patients had a higher age and lower BMI than controls. The MMSE score was slightly decreased in MDD nonresponders. Significantly lower MMSE and GDS scores were confirmed in AD patients compared to controls (Table 1).

In a sample of 68 MDD cases (50 MDD patients showed a clinical response, whereas 17 MDD patients did not experience a clinical response at 3–10 weeks of follow-up; response to treatment was not determined in 1 MDD patient) and in a sample of 84 AD cases (49 AD without depression, 35 AD with depression), we studied alleles, genotypes, and SNP-SNP interactions among 7 SNPs from 5 key functional candidate genes (Table 2) involved in MDD and/or AD development. Data of MDD patients were compared with 90 control subjects; a subgroup of 40 control subjects aged over 50 years (Control >50) was used for comparison with AD patients. All groups (controls, MDD patients, AD patients) showed Hardy-Weinberg equilibrium for the analyzed genetic variability except for in the *BDNF* genotypes in MDD. Deviation from Hardy-Weinberg equilibrium in the group of MDD patients can be taken as evidence of an association of *BDNF* polymorphism with depression.

Individual allele and genotype frequencies

Individual allele and genotype frequencies of polymorphisms of genes encoding ApoE, HSP70, 5-HTT, 5-HT_{2A} receptor, and BDNF in cases and control subjects are shown in Tables 3 and 4.

In MDD, a significantly lower frequency was found of (i) the C allele and C/G genotype for the rs1043618 polymorphism of the *HSPA1A* gene, (ii) the C allele and A/C genotype for the rs1008438 polymorphism of the *HSPA1A* gene, (iii) the 10/12 genotype for the rs4795541 polymorphism of the *SLC6A4* gene, and (iv) the G/A genotype for the rs6265 polymorphism of the *BDNF* gene. Similar results were obtained in the subgroup of MDD responders but not in the subgroup of MDD nonresponders, which was apparently due to the low number of patients in this subgroup. In any event, the FPRP value was not less than 0.2 (Table 3).

In the AD all or AD without depression groups, but not in the AD with depression group, significantly higher frequencies of the ε4 allele, ε3/ε4, and ε4/ε4 genotype of *APOE* were found compared to controls (the same results were obtained compared to MDD cases). In AD without depression, decreased frequency of the A/C genotype for the rs1008438 polymorphism of the *HSPA1A* gene was observed. In AD with depression, decreased frequency of the S allele for the 5-HTTLPR polymorphism of the *SLC6A4* gene was found. Based on the FPRP approach, we found that the increased frequency of the ε4 allele of *APOE* was noteworthy at the 0.2 FPRP level in AD all and AD without depression (Table 4).

Association analyses between individual alleles and genotypes

Results of association analyses between individual alleles and genotypes of *APOE*, *HSPA1A*, *SLC6A4*, *BDNF*, and *HTR2A* gene polymorphisms in cases and control subjects are summarized in Table 5 (controls versus MDD all, MDD responders, and MDD nonresponders) and in Table 6 (controls versus AD all, AD without depression, and AD with depression).

In MDD patients, Pearson's *P*-values indicate a significant difference in the distribution of alleles for both detected polymorphisms (rs1043618 and rs1008438) of the *HSPA1A* gene. In a subgroup of MDD responders, a significant difference was also found for the distribution of genotype rates for polymorphisms rs1043618 and rs1008438 of the *HSPA1A* gene, and a nearly significant difference was found for rs6265 of the *BDNF* gene. However, none of these differences was significant after correcting for multiple testing, as indicated by the Sidak and FDR *P*-values in Table 5.

In the AD all, AD without depression, and AD with depression groups, Pearson's *P*-values indicated a significant difference in the distribution of alleles ε2, ε3, and ε4 of the *APOE* gene. In the whole group of AD and the subgroup of AD without depression, a significant difference was also found for the distribution of genotype rates for polymorphisms of the *APOE* gene. In AD with depression, a significant difference was found in the distribution of the L and S alleles of the *SLC6A4* gene. In the group of all AD patients and the subgroup of AD without depression, differences in the distribution of alleles and genotypes of *APOE* remained significant after correction for multiple testing (Table 6).

For easier interpretation of results and evaluation of the sensitivity and specificity of a genetic test based on measured polymorphisms, the data were dichotomized. For each polymorphism, frequency of occurrence of a certain genotype (or genotypes) was analyzed versus the sum of the frequencies of the occurrence of all remaining genotypes (Tables 7, 8).

Compared to controls, MDD seems to be associated with increased G/G genotype of rs1043618 in *HSPA1A*, increased genotype A/A of rs1008438 in *HSPA1A*, increased 12/12 genotype of rs57098334 in *SLC6A4*, and decreased G/A genotype of rs6265 in *BDNF* (Table 7).

Logit regression and a backward stepwise method were used to perform stepwise selection of predictor variables and effects

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Table 3. Individual allele and genotype frequencies of polymorphisms of genes encoding the apolipoprotein E (ApoE), heat shock 70 kDa protein 1 (HSP70), serotonin transporter (5-HTT), brain-derived neurotrophic factor (BDNF), and serotonin 2A (5-HT_{2A}) receptor in control subjects and patients with depressive disorder (MDD).

Protein Gene Polymorphism	Variant		Control		MDD all					MDD responders					MDD nonresponders				
			N	(Freq)	N	(Freq)	OR	(95% CI)	FPRP	N	(Freq)	OR	(95% CI)	FPRP	N	(Freq)	OR	(95% CI)	FPRP
ApoE *APOE* rs429358-rs7412	Allele	ε3	146	(81.1%)	116	(85.3%)	1.00	–		82	(82.0%)	1.00	–		32	(94.1%)	1.00	–	
		ε2	12	(6.7%)	8	(5.9%)	0.84	(0.33–2.12)	0.76	7	(7.0%)	1.04	(0.39–2.74)	0.79	1	(2.9%)	0.38	(0.05–3.03)	0.78
		ε4	22	(12.2%)	12	(8.8%)	0.69	(0.33–1.45)	0.65	11	(11.0%)	0.89	(0.41–1.93)	0.75	1	(2.9%)	0.21	(0.03–1.60)	0.75
	Genotype	ε3/ε3	61	(67.8%)	50	(73.5%)	1.00	–		34	(68.0%)	1.00	–		15	(88.2%)	1.00	–	
		ε2/ε2	1	(1.1%)	0	(0.0%)	0.41	(0.02–10.18)	0.82	0	(0.0%)	0.59	(0.02–14.99)	0.83	0	(0.0%)	1.32	(0.05–34.07)	0.83
		ε2/ε3	8	(8.9%)	6	(8.8%)	0.92	(0.30–2.81)	0.79	5	(10.0%)	1.12	(0.34–3.70)	0.79	1	(5.9%)	0.51	(0.06–4.38)	0.80
		ε2/ε4	2	(2.2%)	2	(2.9%)	1.22	(0.17–8.97)	0.81	2	(4.0%)	1.79	(0.24–13.31)	0.80	0	(0.0%)	0.79	(0.04–17.39)	0.83
		ε3/ε4	16	(17.8%)	10	(14.7%)	0.76	(0.32–1.83)	0.73	9	(18.0%)	1.01	(0.40–2.53)	0.79	1	(5.9%)	0.25	(0.03–2.07)	0.77
		ε4/ε4	2	(2.2%)	0	(0.0%)	0.24	(0.01–5.19)	0.81	0	(0.0%)	0.36	(0.02–7.64)	0.82	0	(0.0%)	0.79	(0.04–17.39)	0.83
HSP70 *HSPA1A* rs1043618	Allele	G	111	(61.7%)	99	(72.8%)	1.00	–		74	(74.0%)	1.00	–		23	(67.6%)	1.00	–	
		C	69	(38.3%)	37	(27.2%)	**0.60**	**(0.37–0.97)**	0.25	26	(26.0%)	**0.56**	**(0.33–0.97)**	0.30	11	(32.4%)	0.77	(0.35–1.68)	0.71
	Genotype	G/G	33	(36.7%)	37	(54.4%)	1.00	–		29	(58.0%)	1.00	–		7	(41.2%)	1.00	–	
		C/G	45	(50.0%)	25	(36.8%)	**0.50**	**(0.25–0.98)**	0.39	16	(32.0%)	**0.40**	**(0.19–0.86)**	0.37	9	(52.9%)	0.94	(0.32–2.79)	0.79
		C/C	12	(13.3%)	6	(8.8%)	0.45	(0.15–1.32)		5	(10.0%)	0.47	(0.15–1.51)	0.69	1	(5.9%)	0.39	(0.04–3.53)	0.79
HSP70 *HSPA1A* rs1008438	Allele	A	108	(60.0%)	98	(72.1%)	1.00	–		73	(73.0%)	1.00	–		23	(67.6%)	1.00	–	
		C	72	(40.0%)	38	(27.9%)	**0.58**	**(0.36–0.94)**	0.22	27	(27.0%)	**0.55**	**(0.33–0.95)**	0.28	11	(32.4%)	0.72	(0.33–1.56)	0.68
	Genotype	A/A	31	(34.4%)	36	(52.9%)	1.00	–		28	(56.0%)	1.00	–		7	(41.2%)	1.00	–	
		A/C	46	(51.1%)	26	(38.2%)	**0.49**	**(0.25–0.96)**	0.38	17	(34.0%)	**0.41**	**(0.19–0.87)**	0.37	9	(52.9%)	0.87	(0.29–2.57)	0.78
		C/C	13	(14.4%)	6	(8.8%)	0.40	(0.13–1.17)	0.62	5	(10.0%)	0.43	(0.13–1.35)	0.66	1	(5.9%)	0.34	(0.04–3.05)	0.79
5-HTT *SLC6A4* rs4795541	Allele	L	110	(61.1%)	82	(60.3%)	1.00	–		59	(59.0%)	1.00	–		22	(64.7%)	1.00	–	
		S	70	(38.9%)	54	(39.7%)	1.03	(0.66–1.63)	0.90	41	(41.0%)	1.09	(0.66–1.80)	0.71	12	(35.3%)	0.86	(0.40–1.84)	0.74
	Genotype	L/L	31	(34.4%)	22	(32.4%)	1.00	–		17	(34.0%)	1.00	–		5	(29.4%)	1.00	–	
		L/S	48	(53.3%)	38	(55.9%)	1.12	(0.56–2.23)	0.74	25	(50.0%)	0.95	(0.44–2.04)	0.77	12	(70.6%)	1.55	(0.50–4.83)	0.74
		S/S	11	(12.2%)	8	(11.8%)	1.02	(0.35–2.96)	0.79	8	(16.0%)	1.33	(0.45–3.93)	0.76	0	(0.0%)	0.25	(0.01–4.87)	0.81

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Table 3 continued. Individual allele and genotype frequencies of polymorphisms of genes encoding the apolipoprotein E (ApoE), heat shock 70 kDa protein 1 (HSP70), serotonin transporter (5-HTT), brain-derived neurotrophic factor (BDNF), and serotonin 2A (5-HT_{2A}) receptor in control subjects and patients with depressive disorder (MDD).

Protein Gene Polymorphism	Variant		Control		MDD all					MDD responders					MDD nonresponders				
			N	(Freq)	N	(Freq)	OR	(95% CI)	FPRP	N	(Freq)	OR	(95% CI)	FPRP	N	(Freq)	OR	(95% CI)	FPRP
5-HTT *SLC6A4* rs57098334	Allele	12	101	(56.1%)	86	(63.2%)	1.00	–		66	(66.0%)	1.00	–		20	(58.8%)	1.00	–	
		10	76	(42.2%)	49	(36.0%)	0.76	(0.48–1.20)	0.50	33	(33.0%)	0.66	(0.40–1.11)	0.42	14	(41.2%)	0.93	(0.44–1.96)	0.76
		9	3	(1.7%)	1	(0.7%)	0.39	(0.04–3.83)	0.80	1	(1.0%)	0.51	(0.05–5.01)	0.81	0	(0.0%)	0.71	(0.04–14.22)	0.83
	Genotype	12/12	26	(28.9%)	30	(44.1%)	1.00	–		23	(46.0%)	1.00	–		7	(41.2%)	1.00	–	
		10/12	46	(51.1%)	25	(36.8%)	**0.47**	**(0.23–0.96)**	0.40	19	(38.0%)	0.47	(0.22–1.01)	0.46	6	(35.3%)	0.48	(0.15–1.60)	0.70
		10/10	15	(16.7%)	12	(17.6%)	0.69	(0.28–1.75)	0.72	7	(14.0%)	0.53	(0.18–1.52)	0.68	4	(23.5%)	0.99	(0.25–3.95)	0.81
		9/12	3	(3.3%)	1	(1.5%)	0.29	(0.03–2.95)	0.79	1	(2.0%)	0.38	(0.04–3.88)	0.80	0	(0.0%)	0.50	(0.02–10.90)	0.82
		9/10	0	(0.0%)	0	(0.0%)	0.87	(0.02–45.32)	0.84	0	(0.0%)	1.13	(0.02–59.10)	0.84	0	(0.0%)	3.53	(0.06–193.4)	0.83
BDNF *BDNF* rs6265	Allele	G	146	(81.1%)	117	(86.0%)	1.00	–		85	(85.0%)	1.00	–		30	(88.2%)	1.00	–	
		A	34	(18.9%)	19	(14.0%)	0.70	(0.38–1.29)	0.57	15	(15.0%)	0.76	(0.39–1.47)	0.66	4	(11.8%)	0.57	(0.19–1.73)	0.71
	Genotype	G/G	59	(65.6%)	53	(77.9%)	1.00	–		39	(78.0%)	1.00	–		13	(76.5%)	1.00	–	
		G/A	28	(31.1%)	11	(16.2%)	**0.44**	**(0.20–0.96)**	0.44	7	(14.0%)	**0.38**	**(0.15–0.95)**	0.50	4	(23.5%)	0.65	(0.19–2.17)	0.75
		A/A	3	(3.3%)	4	(5.9%)	1.48	(0.32–6.94)	0.79	4	(8.0%)	2.02	(0.43–9.51)	0.76	0	(0.0%)	0.63	(0.03–12.92)	0.83
5-HT_{2A} receptor *HTR2A* rs6311	Allele	G	108	(60.0%)	87	(64.0%)	1.00	–		65	(65.0%)	1.00	–		21	(61.8%)	1.00	–	
		A	72	(40.0%)	49	(36.0%)	0.84	(0.53–1.34)	0.63	35	(35.0%)	0.81	(0.49–1.34)	0.61	13	(38.2%)	0.93	(0.44–1.97)	0.76
	Genotype	G/G	33	(36.7%)	27	(39.7%)	1.00	–		20	(40.0%)	1.00	–		7	(41.2%)	1.00	–	
		A/G	42	(46.7%)	33	(48.5%)	0.96	(0.49–1.90)	0.76	25	(50.0%)	0.98	(0.47–2.07)	0.77	7	(41.2%)	0.79	(0.25–2.46)	0.77
		A/A	15	(16.7%)	8	(11.8%)	0.65	(0.24–1.77)	0.71	5	(10.0%)	0.55	(0.17–1.74)	0.71	3	(17.6%)	0.94	(0.21–4.16)	0.81

The odds ratio and 95% confidence interval associated with each of the 7 polymorphisms under a codominant main effect model were used to estimate the association between polymorphisms and the disease; the Haldane estimator was calculated in case of a zero frequency of a genotype. Some of the positive associations between gene variants and susceptibility to disease may be falsely positive. Thus, we also computed FPRP, the probability of no true association between an interaction and the disease status given a statistically significant result. We used the ORs and *P*-values in the FPRP computation using the Wacholder formula (at prior probability of association=0.25 and the OR that is most likely, assuming that there is a non-null association=1.5). The significant associations are in bold.

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Table 4. Individual allele and genotype frequencies of polymorphisms of genes encoding the apolipoprotein E (ApoE), heat shock 70 kDa protein 1 (HSP70), serotonin transporter (5-HTT), serotonin 2A (5-HT2A) receptor, and brain-derived neurotrophic factor (BDNF) in control subjects and patients with Alzheimer's disease (AD).

Protein Gene Polymorphism	Variant		Control >50		AD all					AD without depression					AD with depression				
			N	(Freq)	N	(Freq)	OR	(95% CI)	FPRP	N	(Freq)	OR	(95% CI)	FPRP	N	(Freq)	OR	(95% CI)	FPRP
ApoE *APOE* rs429358-rs7412	Allele	ε3	63	(78.8%)	103	(61.3%)	1.00	–		57	(58.2%)	1.00	–		46	(65.7%)	1.00	–	
		ε2	8	(10.0%)	9	(5.4%)	0.69	(0.25–1.88)	0.73	4	(4.1%)	0.55	(0.16–1.93)	0.73	5	(7.1%)	0.86	(0.26–2.79)	0.78
		ε4	9	(11.3%)	56	(33.3%)	**3.81**	**(1.76–8.22)**	0.18	37	(37.8%)	**4.54**	**(2.02–10.23)**	0.17	19	(27.1%)	2.89	(1.20–6.97)	0.43
	Genotype	ε3/ε3	26	(65.0%)	32	(38.1%)	1.00	–		16	(32.7%)	1.00	–		16	(45.7%)	1.00	–	
		ε2/ε2	1	(2.5%)	0	(0.0%)	0.27	(0.01–6.95)	0.82	0	(0.0%)	0.54	(0.02–13.93)	0.83	0	(0.0%)	0.54	(0.02–13.93)	0.83
		ε2/ε3	4	(10.0%)	6	(7.1%)	1.22	(0.31–4.78)	0.79	3	(6.1%)	1.22	(0.24–6.17)	0.80	3	(8.6%)	1.22	(0.24–6.17)	0.80
		ε2/ε4	2	(5.0%)	3	(3.6%)	1.22	(0.19–7.85)	0.81	1	(2.0%)	0.81	(0.07–9.70)	0.82	2	(5.7%)	1.63	(0.21–12.71)	0.80
		ε3/ε4	7	(17.5%)	33	(39.3%)	**3.83**	**(1.46–10.06)**	0.40	22	(44.9%)	**5.11**	**(1.78–14.66)**	0.39	11	(31.4%)	2.55	(0.82–7.94)	0.64
		ε4/ε4	0	(0.0%)	10	(11.9%)	17.1	(0.96–305.9)	0.77	7	(14.3%)	**24.1**	**(1.29–450.2)**	0.76	3	(8.6%)	11.2	(0.55–231.8)	0.79
HSP70 *HSPA1A* rs1043618	Allele	G	46	(57.5%)	111	(66.1%)	1.00	–		62	(63.3%)	1.00	–		49	(70.0%)	1.00	–	
		C	34	(42.5%)	57	(33.9%)	0.69	(0.40–1.20)	0.51	36	(36.7%)	0.79	(0.43–1.44)	0.65	21	(30.0%)	0.58	(0.29–1.14)	0.50
	Genotype	G/G	11	(27.5%)	37	(44.0%)	1.00	–		21	(42.9%)	1.00	–		16	(45.7%)	1.00	–	
		C/G	24	(60.0%)	37	(44.0%)	0.46	(0.20–1.07)	0.52	20	(40.8%)	0.44	(0.17–1.12)	0.57	17	(48.6%)	0.49	(0.18–1.31)	0.63
		C/C	5	(12.5%)	10	(11.9%)	0.59	(0.17–1.99)	0.74	8	(16.3%)	0.84	(0.22–3.18)	0.79	2	(5.7%)	0.28	(0.04–1.68)	0.74
HSP70 *HSPA1A* rs1008438	Allele	A	45	(56.3%)	109	(64.9%)	1.00	–		62	(63.3%)	1.00	–		47	(67.1%)	1.00	–	
		C	35	(43.8%)	59	(35.1%)	0.70	(0.40–1.20)	0.51	36	(36.7%)	0.75	(0.41–1.36)	0.61	23	(32.9%)	0.63	(0.32–1.22)	0.54
	Genotype	A/A	10	(25.0%)	35	(41.7%)	1.00	–		21	(42.9%)	1.00	–		14	(40.0%)	1.00	–	
		A/C	25	(62.5%)	39	(46.4%)	0.45	(0.19–1.06)	0.52	20	(40.8%)	**0.38**	**(0.15–0.99)**	0.53	19	(54.3%)	0.54	(0.20–1.49)	0.67
		C/C	5	(12.5%)	10	(11.9%)	0.57	(0.16–2.06)	0.74	8	(16.3%)	0.76	(0.20–2.93)	0.78	2	(5.7%)	0.29	(0.05–1.78)	0.75
5-HTT *SLC6A4* rs4795541	Allele	L	45	(56.3%)	115	(68.5%)	1.00	–		63	(64.3%)	1.00	–		52	(74.3%)	1.00	–	
		S	35	(43.8%)	53	(31.5%)	0.59	(0.34–1.03)	0.36	35	(35.7%)	0.71	(0.39–1.31)	0.59	18	(25.7%)	**0.44**	**(0.22–0.89)**	0.34
	Genotype	L/L	12	(30.0%)	40	(47.6%)	1.00	–		21	(42.9%)	1.00	–		19	(54.3%)	1.00	–	
		L/S	21	(52.5%)	35	(41.7%)	0.50	(0.22–1.16)	0.56	21	(42.9%)	0.57	(0.22–1.45)	0.66	14	(40.0%)	0.42	(0.16–1.13)	0.59
		S/S	7	(17.5%)	9	(10.7%)	0.39	(0.12–1.25)	0.65	7	(14.3%)	0.57	(0.16–2.02)	0.74	2	(5.7%)	0.18	(0.03–1.02)	0.69

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Table 4. continued. Individual allele and genotype frequencies of polymorphisms of genes encoding the apolipoprotein E (ApoE), heat shock 70 kDa protein 1 (HSP70), serotonin transporter (5-HTT), serotonin 2A (5-HT2A) receptor, and brain-derived neurotrophic factor (BDNF) in control subjects and patients with Alzheimer's disease (AD).

Protein Gene Polymorphism	Variant		Control		MDD all					MDD responders					MDD nonresponders				
			N	(Freq)	N	(Freq)	OR	(95% CI)	FPRP	N	(Freq)	OR	(95% CI)	FPRP	N	(Freq)	OR	(95% CI)	FPRP
5-HTT *SLC6A4* rs57098334	Allele	12	42	(52.5%)	104	(61.9%)	1.00	–		59	(60.2%)	1.00	–		45	(64.3%)	1.00	–	
		10	36	(45.0%)	61	(36.3%)	0.70	(0.29–1.73)	0.71	39	(39.8%)	0.77	(0.42–1.41)	0.64	22	(31.4%)	0.57	(0.29–1.12)	0.49
		9	2	(2.5%)	3	(1.8%)	0.17	(0.03–1.14)	0.72	0	(0.0%)	0.14	(0.01–3.05)	0.80	3	(4.3%)	1.40	(0.22–8.80)	0.80
	Genotype	12/12	9	(22.5%)	33	(39.3%)	1.00	–		18	(36.7%)	1.00	–		15	(42.9%)	1.00	–	
		10/12	22	(55.0%)	37	(44.0%)	0.46	(0.19–1.14)	0.57	23	(46.9%)	0.52	(0.19–1.41)	0.66	14	(40.0%)	0.38	(0.13–1.11)	0.60
		10/10	7	(17.5%)	11	(13.1%)	0.43	(0.13–1.42)	0.68	8	(16.3%)	0.57	(0.16–2.08)	0.75	3	(8.6%)	0.26	(0.05–1.25)	0.70
		9/12	2	(5.0%)	1	(1.2%)	0.14	(0.01–1.68)	0.77	0	(0.0%)	0.10	(0.00–2.36)	0.79	1	(2.9%)	0.30	(0.02–3.80)	0.80
		9/10	0	(0.0%)	2	(2.4%)	1.42	(0.06–32.13)	0.83	0	(0.0%)	0.51	(0.01–27.96)	0.83	2	(5.7%)	3.06	(0.13–70.94)	0.82
BDNF *BDNF* rs6265	Allele	G	66	(82.5%)	132	(78.6%)	1.00	–		72	(73.5%)	1.00	–		60	(85.7%)	1.00	–	
		A	14	(17.5%)	36	(21.4%)	1.29	(0.65–2.55)	0.68	26	(26.5%)	1.70	(0.82–3.53)	0.56	10	(14.3%)	0.79	(0.32–1.90)	0.74
	Genotype	G/G	27	(67.5%)	52	(61.9%)	1.00	–		27	(55.1%)	1.00	–		25	(71.4%)	1.00	–	
		G/A	12	(30.0%)	28	(33.3%)	1.21	(0.53–2.75)	0.74	18	(36.7%)	1.50	(0.61–3.71)	0.70	10	(28.6%)	0.91	(0.34–2.42)	0.78
		A/A	1	(2.5%)	4	(4.8%)	2.08	(0.22–19.51)	0.80	4	(8.2%)	4.00	(0.42–38.15)	0.78	0	(0.0%)	0.36	(0.01–9.23)	0.82
5-HT$_{2A}$ receptor *HTR2A* rs6311	Allele	G	47	(58.8%)	92	(54.8%)	1.00	–		52	(53.1%)	1.00	–		40	(57.1%)	1.00	–	
		A	33	(41.3%)	76	(45.2%)	1.18	(0.69–2.02)	0.67	46	(46.9%)	1.26	(0.69–2.29)	0.65	30	(42.9%)	1.07	(0.56–2.04)	0.75
	Genotype	G/G	15	(37.5%)	23	(27.4%)	1.00	–		12	(24.5%)	1.00	–		11	(31.4%)	1.00	–	
		A/G	17	(42.5%)	46	(54.8%)	1.76	(0.75–4.15)	0.62	28	(57.1%)	2.06	(0.78–5.43)	0.62	18	(51.4%)	1.44	(0.52–3.89)	0.73
		A/A	8	(20.0%)	15	(17.9%)	1.22	(0.42–3.59)	0.77	9	(18.4%)	1.41	(0.42–4.75)	0.76	6	(17.1%)	1.03	(0.29–3.69)	0.80

The odds ratio and 95% confidence interval associated with each of the 7 polymorphisms under a codominant main effect model were used to estimate the association between polymorphisms and the disease; the Haldane estimator was calculated in case of a zero frequency of a genotype. Some of the positive associations between gene variants and susceptibility to disease may be falsely positive. Thus, we also computed FPRP, the probability of no true association between an interaction and the disease status given a statistically significant result. We used the ORs and *P*-values in the FPRP computation using the Wacholder formula (at prior probability of association=0.25 and the OR that is most likely, assuming that there is a non-null association=1.5). The significant associations are in bold.

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Table 5. Association analyses between individual alleles and genotypes of genes *APOE* (encoding the apolipoprotein E), *HSPA1A* (encoding heat shock 70 kDa protein 1), *SLC6A4* (encoding serotonin transporter), *BDNF* (encoding brain-derived neurotrophic factor), and *HTR2A* (encoding serotonin 2A receptor) in control subjects and patients with depressive disorder (MDD).

Gene	Polymorphism	Variant		MDD all					MDD responders					MDD nonresponders				
				χ^2	df	P	Sidak	FDR	χ^2	df	P	Sidak	FDR	χ^2	df	P	Sidak	FDR
APOE	rs7412-rs429358	Alleles	ε2, ε3, ε4	1.070	2	0.586	0.852	0.683	0.098	2	0.952	0.952	0.952	3.527	2	0.171	0.731	0.847
		Genotypes	ε2/ε2, ε2/ε3, ε3/ε3, ε2/ε4, ε3/ε4, ε4/ε4	2.750	5	0.738	0.968	0.861	2.066	5	0.840	0.965	0.840	3.214	5	0.667	0.986	0.799
HSPA1A	rs1043618	Alleles	G, C	4.303	1	**0.038**	0.207	0.133	4.362	1	**0.037**	0.201	0.128	0.437	1	0.509	0.941	0.847
		Genotypes	G/G, C/G, C/C	4.976	2	0.083	0.405	0.200	5.988	2	**0.050**	0.284	0.123	0.756	2	0.685	0.986	0.799
HSPA1A	rs1008438	Alleles	A, C	4.964	1	**0.026**	0.167	0.133	4.753	1	**0.029**	0.187	0.128	0.704	1	0.401	0.923	0.847
		Genotypes	A/A, A/C, C/C	5.552	2	0.062	0.362	0.200	6.129	2	**0.047**	0.284	0.123	0.993	2	0.609	0.986	0.799
SLC6A4	5-HTTLPR	Alleles	L, S	0.022	1	0.883	0.882	0.882	0.120	1	0.729	0.926	0.850	0.156	1	0.693	0.970	0.847
		Genotypes	L/L, L/S, S/S	0.103	2	0.949	0.968	0.949	0.408	2	0.815	0.965	0.839	2.945	2	0.229	0.838	0.799
SLC6A4	STin2 VNTR	Alleles	12, 10, 9	1.946	1	0.377	0.850	0.661	2.658	1	0.264	0.785	0.576	0.611	1	0.736	0.970	0.847
		Genotypes	12/12, 10/12, 10/10, 9/12, 9/10	4.861	2	0.182	0.552	0.318	4.224	2	0.238	0.663	0.416	2.382	2	0.496	0.983	0.799
BDNF	rs6265	Alleles	G, A	1.342	1	0.247	0.757	0.575	0.673	1	0.412	0.878	0.576	0.994	1	0.319	0.900	0.847
		Genotypes	G/G, G/A, A/A	4.906	2	0.086	0.405	0.200	5.876	2	0.053	0.284	0.123	1.095	2	0.578	0.986	0.799
HTR2A	rs6311	Alleles	G, A	0.517	1	0.472	0.852	0.661	0.681	1	0.409	0.878	0.576	0.037	1	0.847	0.970	0.847
		Genotypes	G/G, A/G, A/A	0.762	2	0.683	0.968	0.861	1.169	2	0.557	0.913	0.780	0.180	2	0.914	0.986	0.914

5-HTTLPR - serotonin transporter gene-linked polymorphic region; STin2 VNTR – variable number of tandem repeats (VNTR) polymorphism in the functional second intron; χ^2 – Pearson's chi-square test; *P* – Pearson's *P*-value for difference in the distribution of alleles or genotypes rates in polymorphisms between MDD and control group; Sidak – Šidák-Bonferroni step-down adjusted *P*-value for strong control of the family-wise Type I error rate; FDR – *P*-value corrected for multiple testing based on false discovery rate principle with the Benjamini-Hochberg step-up procedure. Confounding factor: age. The significant associations are in bold.

(i.e., to determine which combination of dichotomized genotypes is more frequent in MDD patients than in control subjects). We found that the simultaneous presence of the A/A genotype of rs1008438 in *HSPA1A* and the absence of the G/A genotype of rs6265 in *BDNF* may be responsible for increased risk of MDD development. Sensitivity and specificity of the genetic test "genotype A/A of rs1008438 in *HSPA1A* is present AND genotype G/A of rs6265 in *BDNF* is absent" were 0.44 and 0.81, respectively, for MDD all and 0.50 and 0.81, respectively, for MDD responders.

Compared to controls, AD was associated with decreased ε3/ε3 genotype of *APOE*, increased presence of genotypes containing the ε4 allele of *APOE*, or increased presence of genotypes containing the ε4 allele and not containing the ε2 allele of APOE (Table 8). AD without depression seems to be associated with a decreased presence of the A/C genotype of rs1008438 in *HSPA1A*. AD with depression seems to be associated with increased L/L genotype of rs4795541 in *SLC6A4*. Note that when comparing AD with depression with AD without depression, we did not find any significant difference in studied genotypes.

Using logit regression and a backward stepwise method, we found that the simultaneous presence of the ε4/ε4 or ε3/ε4 genotype of *APOE* and the A/A genotype of rs1008438 in *HSPA1A* may be associated with AD development. Sensitivity and specificity of the genetic test "allele ε4 of *APOE* is present

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Table 6. Association analyses between individual alleles and genotypes of genes *APOE* (encoding the apolipoprotein E), *HSPA1A* (encoding heat shock 70 kDa protein 1), *SLC6A4* (encoding serotonin transporter), *BDNF* (encoding brain-derived neurotrophic factor), and *HTR2A* (encoding serotonin 2A receptor) in control subjects and patients with Alzheimer's disease (AD).

Gene	Polymorphism	Variant		AD all					AD without depression					AD with depression				
				χ^2	df	P	Sidak	FDR	χ^2	df	P	Sidak	FDR	χ^2	df	P	Sidak	FDR
APOE	rs7412-rs429358	Alleles	ε2, ε3, ε4	14.250	2	**0.0008**	**0.005**	**0.005**	17.031	2	**0.0002**	**0.001**	**0.001**	6.276	2	**0.043**	0.233	0.151
		Genotypes	ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4	15.454	5	**0.0086**	0.058	0.060	17.889	5	**0.0031**	**0.021**	**0.021**	7.111	5	0.213	0.697	0.373
HSPA1A	rs1043618	Alleles	G, C	1.714	1	0.190	0.651	0.333	0.614	1	0.433	0.723	0.447	2.512	1	0.113	0.450	0.263
		Genotypes	G/G, C/G, C/C	3.326	2	0.190	0.646	0.291	3.305	2	0.192	0.654	0.405	3.087	2	0.214	0.697	0.373
HSPA1A	rs1008438	Alleles	A, C	1.715	1	0.190	0.651	0.333	0.904	1	0.342	0.723	0.447	1.868	1	0.172	0.529	0.300
		Genotypes	A/A, A/C, C/C	3.438	2	0.179	0.646	0.291	4.285	2	0.117	0.527	0.405	2.448	2	0.294	0.697	0.411
SLC6A4	5-HTTLPR	Alleles	L, S	3.525	1	0.060	0.312	0.211	1.192	1	0.275	0.723	0.447	5.315	1	**0.021**	0.138	0.148
		Genotypes	L/L, L/S, S/S	3.677	2	0.159	0.646	0.291	1.560	2	0.458	0.704	0.458	5.449	2	0.066	0.377	0.373
SLC6A4	STin2 VNTR	Alleles	12, 10, 9	1.997	1	0.368	0.747	0.515	3.193	1	0.202	0.677	0.447	3.029	1	0.219	0.529	0.307
		Genotypes	12/12, 10/12, 10/10, 9/12, 9/10	5.877	2	0.208	0.646	0.291	4.221	2	0.238	0.663	0.405	6.908	2	0.140	0.597	0.373
BDNF	rs6265	Alleles	G, A	0.520	1	0.471	0.747	0.549	2.062	1	0.151	0.625	0.447	0.287	1	0.592	0.833	0.690
		Genotypes	G/G, G/A, A/A	0.570	2	0.752	0.751	0.751	2.111	2	0.348	0.704	0.405	0.930	2	0.628	0.861	0.732
HTR2A	rs6311	Alleles	G, A	0.350	1	0.554	0.747	0.554	0.577	1	0.447	0.723	0.447	0.040	1	0.841	0.842	0.842
		Genotypes	G/G, A/G, A/A	1.774	2	0.412	0.654	0.480	2.193	2	0.334	0.704	0.405	0.599	2	0.741	0.861	0.741

5-HTTLPR - serotonin transporter gene-linked polymorphic region; STin2 VNTR – variable number of tandem repeats (VNTR) polymorphism in the functional second intron; χ^2 – Pearson's chi-square test; *P* – Pearson's *P*-value for difference in the distribution of alleles or genotypes rates in polymorphisms between AD and control group; Sidak – Šidák-Bonferroni step-down adjusted *P*-value for strong control of the family-wise Type I error rate; FDR – *P*-value corrected for multiple testing based on false discovery rate principle with the Benjamini-Hochberg step-up procedure. Confounding factor: age. The significant associations are in bold.

AND allele ε2 is absent" was 0.51 and 0.83, respectively, for AD all, 0.59 and 0.83, respectively, for AD without depression, and 0.40 and 0.83, respectively, for AD with depression. Sensitivity and specificity of the genetic test "genotype ε4/ε4 OR ε3/ε4 of *APOE* is present AND genotype A/A of rs1008438 in *HSPA1A* is present" was 0.73 and 0.58, respectively, for AD all, 0.76 and 0.58, respectively, for AD without depression, and 0.69 and 0.58, respectively, for AD with depression.

Interactions between polymorphisms

To investigate whether the MDD- or AD-associated SNPs might interact with each other, we analyzed all 7 SNPs in 5 candidate genes. Epistasis was evaluated by SHEsisPlus software that can calculate the multi-way interaction. The results of the multi-way interaction analyses of the *APOE*, *HSPA1A*, *SLC6A4*, *BDNF*, and *HTR2A* gene polymorphisms in cases compared to controls are shown in Tables 9 and 10. Only those multi-way interactions between gene polymorphisms with a crude *P*-value <0.05 were selected. The stepwise selection procedure detected the following numbers of significant two- to five-way interactions between polymorphisms in various groups of cases: 6 in MDD all, 11 in MDD responders, 2 in MDD nonresponders, 2 in AD all, 7 in AD without depression, and 6 in AD with depression. After correction for multiple testing using the Šidák-Bonferroni or FDR principle, none of the multi-way interactions remained significant at the 0.05 level (for adjusted *P*-values) in AD patients, and only 2 interactions remained significant in

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Table 7. Association of dichotomized genotypes of genes *APOE*, *HSPA1A*, *SLC6A4*, *BDNF*, and *HTR2A* with depressive disorder (MDD) when compared to controls.

Gene Polymorphism	Compared variants			MDD all		MDD responders		MDD nonresponders	
				(95% CI)	OR	(95% CI)	OR	(95% CI)	OR
APOE rs7412-rs429358	ε3/ε3	*vs.*	ε2/ε2+ε2/ε3+ ε2/ε4+ε3/ε4+ ε4/ε4	1.32	(0.66–2.65)	1.01	(0.48–2.12)	3.57	(0.76–16.64)
	ε4/ε4+ε3/ ε4+ε2/ε4	*vs.*	ε3/ε3+ε2/ε3+ ε2/ε2	0.75	(0.34–1.66)	0.99	(0.43–2.27)	0.22	(0.03–1.75)
	ε4/ε4+ ε3/ε4	*vs.*	ε3/ε3+ε2/ε3+ ε2/ε2+ε2/ε4	0.69	(0.30–1.61)	0.88	(0.36–2.13)	0.25	(0.03–2.01)
HSPA1A rs1043618	G/G	*vs.*	C/G+C/C	**2.06**	**(1.09–3.92)**	**2.39**	**(1.18–4.83)**	1.21	(0.42–3.48)
	C/G	*vs.*	G/G+C/C	0.58	(0.31–1.11)	0.47	(0.23–0.97)	1.13	(0.40–3.18)
	C/C	*vs.*	G/G+C/G	0.62	(0.22–1.75)	0.72	(0.24–2.18)	0.41	(0.05–3.35)
HSPA1A rs1008438	A/A	*vs.*	A/C+C/C	**2.14**	**(1.12–4.08)**	**2.42**	**(1.19–4.92)**	1.33	(0.46–3.84)
	A/C	*vs.*	A/A+C/C	0.59	(0.31–1.12)	0.49	(0.24–1.01)	1.08	(0.39–3.04)
	C/C	*vs.*	A/A+A/C	0.57	(0.21–1.60)	0.66	(0.22–1.97)	0.37	(0.05–3.04)
SLC6A4 rs4795541	L/L	*vs.*	L/S+S/S	0.91	(0.47–1.78)	0.98	(0.47–2.03)	0.79	(0.26–2.46)
	L/S	*vs.*	L/L+S/S	1.11	(0.59–2.09)	0.88	(0.44–1.75)	2.10	(0.68–6.45)
	S/S	*vs.*	L/L+L/S	0.96	(0.36–2.53)	1.37	(0.51–3.66)	0.20	(0.01–3.51)
SLC6A4 rs57098334	12/12	*vs.*	10/12+10/10+ 9/12+9/10	**1.94**	**(1.00–3.76)**	**2.10**	**(1.02–4.30)**	1.72	(0.59–5.01)
	10/12	*vs.*	12/12+10/10+ 9/12+9/10	0.56	(0.29–1.06)	0.59	(0.29–1.19)	0.52	(0.18–1.53)
	10/10	*vs.*	12/12+10/12+ 9/12+ 9/10	1.07	(0.47–2.47)	0.81	(0.31–2.15)	1.54	(0.44–5.37)
BDNF rs6265	G/G	*vs.*	G/A+A/A	1.86	(0.90–3.81)	1.86	(0.84–4.14)	1.71	(0.51–5.68)
	G/A	*vs.*	G/G+A/A	**0.43**	**(0.19–0.94)**	**0.36**	**(0.14–0.90)**	0.68	(0.20–2.28)
	A/A	*vs.*	G/G+G/A	1.81	(0.39–8.38)	2.52	(0.54–11.75)	0.71	(0.04–14.45)
HTR2A rs6311	G/G	*vs.*	A/G+A/A	1.14	(0.60–2.17)	1.15	(0.57–2.34)	1.21	(0.42–3.48)
	A/G	*vs.*	G/G+A/A	1.08	(0.57–2.02)	1.14	(0.57–2.28)	0.80	(0.28–2.29)
	A/A	*vs.*	G/G+A/G	0.67	(0.26–1.68)	0.56	(0.19–1.63)	1.07	(0.27–4.19)
HSPA1A, rs1008438 *BDNF*, rs6265	A/A and G/G+A/A	*vs.*	All others	**3.39**	**(1.66–6.91)**	**4.29**	**(2.00–9.23)**	1.32	(0.38–4.56)

Estimated odds ratio (OR) and 95% confidence interval (CI); Haldane estimator was calculated at zero frequency. The significant associations are in bold. "+" is used to denote the logic operation "disjunction". Confounding factor: age.

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Table 8. Association of dichotomized genotypes of genes *APOE*, *HSPA1A*, *SLC6A4*, *BDNF*, and *HTR2A* with Alzheimer's disease (AD) when compared to controls.

Gene Polymorphism	Compared variants			MDD all		MDD responders		MDD nonresponders	
				(95% CI)	OR	(95% CI)	OR	(95% CI)	OR
APOE rs7412-rs429358	ε3/ε3	*vs.*	ε2/ε2+ε2/ε3+ε2/ε4+ε3/ε4+ε4/ε4	**0.33**	**(0.15–0.73)**	**0.26**	**(0.11–0.63)**	0.45	(0.18–1.15)
	ε4/ε4+ε3/ε4+ε2/ε4	*vs.*	ε3/ε3+ε2/ε3+ε2/ε2	**4.17**	**(1.77–9.83)**	**5.44**	**(2.13–13.90)**	**2.90**	**(1.07–7.86)**
	ε4/ε4+ε3/ε4	*vs.*	ε3/ε3+ε2/ε3+ε2/ε2+ε2/ε4	**4.94**	**(1.97–12.42)**	**6.84**	**(2.53–18.49)**	**3.14**	**(1.09–9.07)**
HSPA1A rs1043618	G/G	*vs.*	C/G+C/C	2.08	(0.92–4.70)	1.98	(0.81–4.84)	2.22	(0.85–5.81)
	C/G	*vs.*	G/G+C/C	0.52	(0.24–1.13)	0.46	(0.20–1.08)	0.63	(0.25–1.57)
	C/C	*vs.*	G/G+C/G	0.95	(0.30–2.98)	1.37	(0.41–4.56)	0.42	(0.08–2.34)
HSPA1A rs1008438	A/A	*vs.*	A/C+C/C	2.14	(0.93–4.95)	2.25	(0.90–5.60)	2.00	(0.75–5.35)
	A/C	*vs.*	A/A+C/C	0.52	(0.24–1.12)	**0.41**	**(0.18–0.97)**	0.71	(0.28–1.79)
	C/C	*vs.*	A/A+A/C	0.95	(0.30–2.98)	1.37	(0.41–4.56)	0.42	(0.08–2.34)
SLC6A4 rs4795541	L/L	*vs.*	L/S+S/S	2.12	(0.95–4.72)	1.75	(0.72–4.23)	**2.77**	**(1.07–7.15)**
	L/S	*vs.*	L/L+S/S	0.65	(0.30–1.38)	0.68	(0.29–1.57)	0.60	(0.24–1.51)
	S/S	*vs.*	L/L+L/S	0.57	(0.19–1.65)	0.79	(0.25–2.46)	0.29	(0.06–1.48)
SLC6A4 rs57098334	12/12	*vs.*	10/12+10/10+9/12+9/10	2.23	(0.94–5.28)	2.00	(0.78–5.13)	2.58	(0.95–7.02)
	10/12	*vs.*	12/12+10/10+9/12+9/10	0.64	(0.30–1.37)	0.72	(0.31–1.67)	0.55	(0.22–1.37)
	10/10	*vs.*	12/12+10/12+9/12+ 9/10	0.71	(0.25–2.00)	0.92	(0.30–2.80)	0.44	(0.11–1.86)
BDNF rs6265	G/G	*vs.*	G/A+A/A	0.78	(0.35–1.73)	0.59	(0.25–1.41)	1.20	(0.45–3.23)
	G/A	*vs.*	G/G+A/A	1.17	(0.52–2.63)	1.35	(0.56–3.30)	0.93	(0.34–2.53)
	A/A	*vs.*	G/G+G/A	1.95	(0.21–18.04)	3.47	(0.37–32.33)	0.37	(0.01–9.40)
HTR2A rs6311	G/G	*vs.*	A/G+A/A	0.63	(0.28–1.40)	0.54	(0.22–1.35)	0.76	(0.29–1.99)
	A/G	*vs.*	G/G+A/A	1.64	(0.77–3.50)	1.80	(0.78–4.20)	1.43	(0.58–3.57)
	A/A	*vs.*	G/G+A/G	0.87	(0.33–2.26)	0.90	(0.31–2.60)	0.83	(0.26–2.67)
APOE, rs7412-rs429358 *HSPA1A*, rs1008438	ε4/ε4+ε3/ε4 and A/A	*vs.*	All others	**3.59**	**(1.63–7.90)**	**4.17**	**(1.69–10.30)**	**2.95**	**(1.14–7.63)**

Estimated odds ratio (OR) and 95% confidence interval (CI); Haldane estimator was calculated at zero frequency. The significant associations are in bold. "+" is used to denote the logic operation "disjunction". Confounding factor: age.

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Table 9. Analysis of multi-way interaction effects of *APOE*, *HSPA1A*, *SLC6A4*, *BDNF*, and *HTR2A* gene polymorphisms in patients with depressive disorder (MDD) compared to controls.

Group	Multi-way interactions between gene polymorphisms							*P*-value		
	APOE	***HSPA1A***	***HSPA1A***	***SLC6A4***	***SLC6A4***	***BDNF***	***HTR2A***	**Crude**	**Sidak**	**FDR**
MDD all					rs57098334		rs6311	**0.047**	0.994	0.643
	rs7412-rs429358	rs1043618				rs6265		**0.002**	0.215	0.242
	rs7412-rs429358		rs1008438			rs6265		**0.018**	0.874	0.527
	rs7412-rs429358			rs4795541	rs57098334			**0.006**	0.522	0.280
	rs7412-rs429358	rs1043618	rs1008438			rs6265		**0.007**	0.564	0.280
	rs7412-rs429358	rs1043618	rs1008438			rs6265	rs6311	**0.045**	0.993	0.643
MDD responders	rs7412-rs429358	rs1043618					rs6311	**0.007**	0.562	0.211
		rs1043618		rs4795541		rs6265		**0.044**	0.990	0.456
		rs1043618			rs57098334		rs6311	**0.019**	0.879	0.279
			rs1008438	**rs4795541**		**rs6265**		**0.0002**	**0.021**	**0.016**
	rs7412-rs429358			rs4795541	rs57098334			**0.015**	0.815	0.252
	rs7412-rs429358	rs1043618	rs1008438	rs4795541				**0.032**	0.966	0.363
	rs7412-rs429358	**rs1043618**	**rs1008438**			rs6265		**0.0003**	**0.032**	**0.016**
	rs7412-rs429358	rs1043618				rs6265	rs6311	**0.023**	0.913	0.289
	rs7412-rs429358		rs1008438			rs6265	rs6311	**0.004**	0.378	0.161
			rs1008438	rs4795541	rs57098334	rs6265		**0.009**	0.640	0.211
	rs7412-rs429358	rs1043618	rs1008438			rs6265	rs6311	**0.013**	0.769	0.252
MDD nonresponders			rs1008438				rs6311	**0.049**	0.996	0.881
					rs57098334	rs6265		**0.001**	0.147	0.159

Sidak – Šidák-Bonferroni step-down adjusted *P*-value for strong control of the family-wise Type I error rate; FDR – *P*-value corrected for multiple testing based on false discovery rate principle with the Benjamini–Hochberg step-up procedure. The significant interactions are in bold.

the subgroup of MDD responders (see Sidak-adjusted and FDR-adjusted *P*-values in Tables 9 and 10): (1) rs1008438 (–110A/C) of *HSPA1A*: rs4795541 (5-HTTLPR) of *SLC6A4*: rs6265 (196G/A) of *BDNF*; (2) rs7412-rs429358 (ε2/ε2, ε2/ε3, ε3/ε3, ε2/ε4, ε3/ε4, ε4/ε4) of *APOE*: rs1043618 (+190G/C) of *HSPA1A*: rs1008438 (–110A/C) of *HSPA1A*: rs6265 (196G/A) of *BDNF*.

Discussion

Recently, the possibility of an increased risk of AD in individuals with previous depression and an effect of current depression on the progression of AD has been discussed. To confirm whether a significant association exists between selected

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Table 10. Analysis of multi-way interaction effects of *APOE*, *HSPA1A*, *SLC6A4*, *BDNF*, and *HTR2A* gene polymorphisms in patients with Alzheimer's disease (AD) compared to controls (above 50 years old).

Group	Multi-way interactions between gene polymorphisms							*P*-value		
	APOE	***HSPA1A***	***HSPA1A***	***SLC6A4***	***SLC6A4***	***BDNF***	***HTR2A***	**Crude**	**Sidak**	**FDR**
AD all		rs1043618	rs1008438	rs4795541		rs6265	rs6311	**0.023**	0.932	0.801
	rs7412-rs429358		rs1008438	rs4795541		rs6265	rs6311	**0.030**	0.968	0.801
AD with depression			rs1008438	rs4795541				**0.021**	0.911	0.847
	rs7412-rs429358				rs57098334		rs6311	**0.008**	0.618	0.847
				rs4795541	rs57098334		rs6311	**0.039**	0.988	0.847
		rs1043618		rs4795541	rs57098334		rs6311	**0.045**	0.993	0.847
				rs4795541	rs57098334	rs6265	rs6311	**0.026**	0.946	0.847
		rs1043618	rs1008438	rs4795541	rs57098334		rs6311	**0.043**	0.991	0.847
AD without depression		rs1043618			rs57098334			**0.045**	0.992	0.737
	rs7412-s429358	rs1043618			rs57098334		rs6311	**0.039**	0.987	0.737
	rs7412-rs429358	rs1043618		rs4795541		rs6265	rs6311	**0.020**	0.900	0.737
	rs7412-rs429358		rs1008438	rs4795541	rs57098334		rs6311	**0.046**	0.993	0.737
	rs7412-rs429358		rs1008438	rs4795541		rs6265	rs6311	**0.020**	0.902	0.737
	rs7412-rs429358	rs1043618			rs57098334	rs6265	rs6311	**0.043**	0.991	0.737
	rs7412-rs429358			rs4795541	rs57098334	rs6265	rs6311	**0.030**	0.967	0.737

Sidak – Šidák-Bonferroni step-down adjusted *P*-value for strong control of the family-wise Type I error rate; FDR – *P*-value corrected for multiple testing based on false discovery rate principle with the Benjamini–Hochberg step-up procedure. The significant interactions are in bold.

genetic markers in patients with MDD, AD, and AD presenting with or without depression, a genetic association case-control study was performed. To estimate MDD or AD risk conferred by individual SNPs and SNP-SNP interactions, we studied 7 SNPs from 5 key genes involved in serotonergic, ApoE, HSP70, and neurotrophic pathways (Table 2) in individuals from a population of the Czech Republic with and without diagnosis of AD or MDD. Our study supports the view that cross-talk between different signaling pathways in complex diseases, such as MDD and AD, may be associated with multi-way interactions between polymorphisms of candidate genes (*HSPA1A*, *APOE*, *SLC6A4*, and *BDNF* in MDD; *APOE*, *SLC6A4*, and *HTR2A* in AD). This is in agreement with the known data on the common pathophysiological changes in AD and MDD due to disturbances in signaling pathways regulated by ApoE, inflammatory cytokines, neurotrophins, and serotonin.

Depressive disorder

An association analysis found significant differences in the distribution of alleles for polymorphisms in the *HSPA1A* gene, which supported the role of HSP70 proteins in the pathophysiology of depression, probably due to protective effects of HSP70 in neuroinflammation [126,127]. Using odds ratios to

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estimate the association between individual polymorphisms and MDD, we specified that differences in the distribution of alleles and genotypes of the gene for HSP70 in MDD versus controls were caused by a significantly lower frequency of the C allele and C/G genotype for the rs1043618 polymorphism in *HSPA1A* and a lower frequency of the C allele and A/C genotype for the rs1008438 polymorphism in the *HSPA1A* gene.

No significant differences were observed in the frequency of the polymorphism in *APOE*, *HTR2A*, or 5-HTTLPR in MDD cases compared to controls. The detailed analysis showed a significantly lower frequency of the 10/12 genotype for the rs4795541 polymorphism in the *SLC6A4* gene and a lower frequency of the G/A genotype for the rs6265 polymorphism in the *BDNF* gene. All these associations were slightly more significant in the subgroup of depressive patients that respond to pharmacotherapy. This result confirmed the view that response to treatment of MDD is a complex phenotype in which different factors are involved, including genetic factors. However, FPRP values were not less than 0.2, which indicates that the results of our association analysis between individual allele and genotypes in MDD cannot be described as truly significant ("noteworthy").

The role of polymorphisms in genes encoding 5-HTT, BDNF, and HSP70 was confirmed in the susceptibility to depression (Table 7). In our set of polymorphisms, the contemporary absence of genotype G/A (for rs6265 in *BDNF*) and presence of genotype A/A (for rs1008438 in *HSPA1A*) led to maximum OR, sensitivity, and specificity. We concluded that the absence of genotype G/A for rs6265 in *BDNF* and simultaneous presence of genotype A/A for rs1008438 in *HSPA1A* may be considered a genetic marker initiating an increased risk of MDD development, with very low sensitivity and sufficient specificity.

Alzheimer's disease

A significant difference, which remained significant after correction for multiple testing, was found in the distribution of alleles and genotype rates for polymorphisms of the *APOE* gene in AD compared to controls. Thus, the role of ApoE proteins in the pathophysiology of late-onset AD [128,129] was confirmed. We specified that differences in the distribution of alleles and genotypes of the gene for ApoE in AD compared to both controls and MDD cases were caused by significantly higher frequencies of the ε4 allele and the ε3/ε4 and ε4/ε4 genotypes, respectively. This finding was valid in the all AD group and in the subgroup of AD without depression but not in the AD with depression group. This result indicates increased probability that depressive disorder is comorbid with AD or may contribute to development of AD in the subgroup of AD patients without the ε4 allele. Previous findings also supported that depressive symptoms before the onset of AD result in an increased risk of developing AD [130,131].

In AD with depression, but not in AD without depression, a significant difference was found in the distribution of alleles L and S of the gene for 5-HTT, which indicates a role of the serotonin transporter activity in depression comorbid with AD [132,133]. We specified that decreased frequency of the S allele for the 5-HTTLPR polymorphism in the *SLC6A4* gene is responsible for this effect. However, the difference was not significant after correcting for multiple testing, and our results did not contradict the previous conclusion that 5-HTT polymorphisms are unlikely to play any substantial role in susceptibility to AD [134]. Moreover, decreased frequency of the A/C genotype for the rs1008438 polymorphism in the *HSPA1A* gene was observed in AD without depression but not in AD with depression. We found that polymorphisms in *HSPA1A* may be applied through SNP-SNP interactions (Table 10).

For dichotomized results, the best predictor of risk for AD development is the presence of genetic variations of *APOE* containing one or 2 ε4 alleles but not ε2 alleles (i.e., the presence of the ε4/ε4 or ε3/ε4 genotype of *APOE*) (Table 8). The odds ratio, sensitivity, and specificity were only slightly affected when simultaneous occurrence was considered of the ε4/ε4 or ε3/ε4 genotypes of *APOE* and the A/A genotype for rs1008438 in *HSPA1A*; both sensitivity and specificity of the test remained low. Although the simultaneous occurrence of risk genotypes of *APOE* and *HSPA1A* did not lead to a significant increase in sensitivity and specificity of the genetic test, these results indicate the role of changes in activity of HSP70 proteins, not only in depression, but also in AD.

Gene-gene interactions

It is very unlikely that individual SNPs are highly associated with the development of complex diseases such as MDD or AD. Interactions of SNPs are thought to explain the differences between low- and high-risk groups; therefore, epistasis should be considered in complex diseases such as MDD or AD.

The multi-way interaction analyses of *APOE*, *HSPA1A*, *SLC6A4*, *BDNF*, and *HTR2A* gene polymorphisms in MDD cases compared to controls showed that all measured polymorphisms were included in significant interactions, the most frequent being the *HSPA1A*, *APOE*, and *BDNF* polymorphisms. Sidak-adjusted, as well as FDR-adjusted *P*-values, remained lower than 0.05 in MDD responders for the interaction between polymorphisms of genes for (1) HSP70, 5-HTT, and BDNF, and (2) HSP70, ApoE, and BDNF (Table 9). It appears that these interactions are associated with common disturbances in neuroinflammatory, serotonergic, and neurotrophic pathways in MDD. Association of the ε4 allele of *APOE* with MDD or geriatric depression is little known. Recently, it was found that the ε4 allele of *APOE* predicted future depression even after excluding depressed patients who later developed dementia [135]. Our

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data indicate that polymorphisms of *APOE* may be associated with MDD rather indirectly, through interaction with other polymorphisms in *HSPA1A* and *BDNF*.

In AD cases compared to controls, there were interactions of all tested polymorphisms, which indicate participation of serotonergic, neurotrophic, and neuroinflammatory pathways in AD. However, none of these interactions remained significant after correction for multiple testing using the Šidák-Bonferroni or FDR principle. Multi-way interaction between polymorphisms in AD with the lowest crude *P*-value included polymorphisms in *APOE*, *SLC6A4*, and *HTR2A* (i.e., genetic variations with potential impact on activity of ApoE, 5-HTT and the 5-HT_{2A} receptor) (Table 10). Nevertheless, the increased frequency of the ε4 allele of *APOE* and genotypes containing the ε4 allele and not containing the ε2 allele remained the only significant observations after correction for multiple testing in AD, and *APOE* polymorphism was confirmed to contribute significantly to AD.

This study is limited by the small number of participants, which was inadequate for genetic analysis. Therefore, the results obtained must be interpreted with caution. While sample sizes were relatively small, we could detect evidence for a genetic association between selected polymorphisms and MDD or AD. Moreover, it was shown that replication is more effective in distinguishing spurious from true associations compared to increasing the power of individual studies [136]. Candidate genes with a high prior probability of association with disease were selected [137]. Caution should be used when interpreting our results because statistical modelling of interactions might not correspond to any physiological interaction, and an association study has limitations that come with selecting participants. Since the numbers in both MDD and AD groups were not large, our conclusions should be confirmed by other studies, including a longitudinal study to explain the risk represented by an episode or more of MDD for subsequent AD development. Qualified diagnostics and data analysis for patients with MDD or AD presenting with or without depression can be considered an advantage of this study.

The novelty of our study is the demonstration of statistically significant interactions between SNPs that did not have a significant effect on MDD risk individually; we found significant interaction between (1) rs1008438, rs4795541, and rs6265 located in genes *HSPA1A*, *SLC6A4*, and *BDNF*, respectively; and (2) rs1043618 and rs1008438 located in *HSPA1A* and rs7412-rs429358 and rs6265 located in *APOE* and *BDNF*, respectively. A significantly increased frequency of the ε4 allele of *APOE* in AD is only a confirmation of previous results [44,138]. However, our results show a potential role of interactions between polymorphisms in the increased risk of AD development (e.g., rs7412-rs429358, rs57098334, and rs6311, located in genes *APOE*, *SLC6A4*, and *HTR2A*, respectively). Our study does not definitively prove the association is real, but it provides some solid evidence that it is.

Conclusions

In MDD cases, frequencies of the C allele and G/C genotype for the rs1043618 polymorphism in *HSPA1A*, the C allele and A/C genotype for the rs1008438 polymorphism in *HSPA1A*, the 10/12 genotype for the rs4795541 polymorphism in *SLC6A4*, and the G/A genotype for the rs6265 polymorphism in *BDNF* were found to be significantly lower, which indicates protective effects of these genotypes in MDD. Moreover, assessment of the combined influence of the 2 to 5 polymorphisms demonstrated a significant effect, whereby the combination of polymorphisms (1) rs1008438 in *HSPA1A*, rs4795541 in *SLC6A4*, and rs6265 in *BDNF*, or (2) rs1043618 and rs1008438 in *HSPA1A*, rs7412-rs429358 in *APOE*, and rs6265 in *BDNF* was associated with MDD. These results indicate a possible synergistic effect of genetic factors influencing inflammatory, serotonergic, and neurotrophic pathways in MDD.

In AD cases, especially in AD without depression, frequencies of the ε4 allele and the ε3/ε4 and ε4/ε4 genotypes of *APOE* were confirmed to be significantly higher than that in controls, even after correction for multiple testing. There is increased probability that depressive disorder is comorbid with AD in the subgroup of AD patients without the ε4 allele of *APOE*. This suggests that the ε4 allele may act as a genetic susceptibility factor in AD. In AD with depression, the decreased frequency of the S allele for the 5-HTTLPR polymorphism in *SLC6A4* indicated an involvement of genetically regulated 5-HTT activity in depression comorbid to AD. The A/C genotype of the rs1008438 polymorphism in *HSPA1A* was less frequent in AD without depression, which indicates protective effects of this genotype in the subgroup of AD patients. Contrary to MDD, an association of *HSPA1A* polymorphisms with depression in AD was not found. Analysis of multi-way interactions between SNPs supported a possible low synergistic effect of genetic variations influencing serotonergic neurotransmission and activity of ApoE, BDNF, and HSP70 in AD. However, the increased frequency of the ε4 allele and ε3/ε4 and ε4/ε4 genotypes of *APOE* remained the only significant observations after correction for multiple testing.

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Conflict of interest

None.

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