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Replicative Association Analysis of Genetic Markers of Cognitive Traits with Alzheimer's Disease in the Russian Population

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Abstract—A replicative association analysis of Alzheimer's disease (AD) was carried out for 15 genetic markers that have been associated with cognitive disorders in genome-wide association studies. In the Russian population, AD was associated with *CSMD1* rs2616984 (OR = 1.50, 95% CI 1.07–2.09, *p*-value = 0.018) and, potentially, with *NOTCH4* rs313296 (OR = 1.53, 95% CI 0.98–2.39, *p*-value = 0.06) and *NRIP1* rs2229741 (OR = 1.35, 95% CI 0.99–1.85, *p*-value = 0.061). Combinations of epistatically interacting genes (*CSMD1* and *NRIP1*; *NOTCH4*, *CSMD1*, and *NRIP1*; and *TLR4*, *CSMD1*, and *NRIP1*) were identified, along with their genotype combinations that showed a significant association with AD and the highest predictive values. Possible molecular mechanisms of the gene involvement in AD pathogenesis are discussed. A bioinformatics analysis of the biological processes, molecular functions, and protein–protein interactions for the AD genes indicated that the genes may play a modulating or modifying role, acting together in various regulatory and signaling pathways involved in AD.

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that affects middle-aged and elderly people. Cognitive defects varying in severity are main signs of AD. The AD prevalence in the global population gradually increases with the increasing lifespan. AD affects more than 10% of people aged 65 and older and approximately one half of people aged 85 and older [1]. Accurate data on AD prevalence in Russia are unavailable. The total affected population of Russia has been estimated at 1.4 million people by extrapolating the data on the Moscow population, where AD affects 4.4% of people aged 60 and older, over the total population with allowances made for gender- and age-related differences in disease incidence [2].

Early-onset familial AD, which affects people younger than 65 years of age, is diagnosed in no more than 5% of AD cases, has an autosomal dominant inheritance, and is due to mutations of the amyloid precursor protein (*APP*) and presenilin (*PSEN1* and *PSEN2*) genes [3–6]. Late-onset AD (at more than 65 years of age) is most likely multifactorial in nature. Allele ε4 of the apolipoprotein E gene (*APOE*) is the main known genetic factor associated with late-onset AD [7, 8]. Several tens of other genes and genome regions whose variation is associated with AD have been revealed in numerous association studies of AD, including recent genome-wide association studies (GWASs). A set of genes identified in more than one GWAS includes *APOJ* (*CLU*), *PICALM*, *CR1*, *GAB2*, *TOMM40*, *APOC1*, *MS4A*, *BIN1*, and *ABCA7* [9–22].

The association of apolipoprotein E variant ε4 with AD has been verified in many populations, including Russian ones [23]. We have similarly replicated the associations of *APOE* and *APOJ* (*CLU*) markers and *APOE–GAB2* genotype combinations in our samples [24, 25].

In spite of the substantial achievements in searching for genetic factors of AD, the set of known genes and markers still fails to totally characterize the hereditary component of late-onset AD. The missing heritability problem is still pressing in AD, like in any other multifactorial disorder. Replicating known associations with new populations and studying the genes associated with related phenotypes provide a means to fill the gap in our knowledge of the hereditary component of multifactorial diseases [26].

While a progressive increase in severity and gradual loss of cognitive functions are characteristic of AD,

Abbreviations: AD, Alzheimer's disease; CVC, cross-validation consistency; FDR, false discovery rate; GWAS, genome-wide association study; MDR, multifactor dimensionality reduction; NSP, single nucleotide polymorphism.

cognitive defects are considered as potential AD endophenotypes. More than one hundred of significant genome regions have been identified in recent GWASs of genetic markers and the variation of cognitive phenotypes in the healthy population [27–31]. In some cases, the same genes and polymorphisms have been associated with both AD and cognitive defects (e.g., *TOMM40* and *APOC1* SNPs).

Data are accumulating that common pathogenetic mechanisms are involved in the pathogenesis of AD and other neurological and mental disorders, in particular, schizophrenia [32]. AD and schizophrenia greatly differ in neuropathology, etiology, age composition of the patient population, and signs, but they still have common pathogenetic and molecular mechanisms. Common characteristics may be found for the cognitive endophenotypes of the two diseases as well. As early as the 1990s, similar features have been observed in regional damage to the brain, biochemical dysfunction, and signs [33]. Highly similar patterns of gene expression in the upper temporal region of the cortex have been revealed for AD and schizophrenia in recent transcriptome studies [34].

Thus, genetic markers reliably associated with cognitive endophenotypes in other neurodegenerative and mental disorders or the individual variation of cognitive alterations in the normal population provide a possible source to search for missing heritability in AD.

In this work, genetic markers identified in GWASs of cognitive parameters in healthy people and schizophrenics were subject to a replicative association analysis with AD in the Russian population, and a bioinformatics analysis was carried out for biological processes and protein interactions related to AD genes.

EXPERIMENTAL

A test group included 110 patients (45% males and 55% females), who had been diagnosed with AD (G30 according to the International Classification of Diseases, 10th revision (ICD-10)) and examined at the Department of Neurology and Neurosurgery (Siberian State Medical University) and Institute of Mental Health (Siberian Branch, Russian Academy of Medical Sciences, Tomsk). The diagnosis was based on the criteria of ICD-10; Diagnostic and Statistical Manual of Mental Disorders, revision IV (DSM-IV); and National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) [35, 36]. The mean age of the patients was 72.15 ± 7.87 years. All of the patients had a standard psychoneurological examination. The diagnosis was verified by neuroimaging (computed tomography and nuclear magnetic resonance imaging of the brain) in some of the patients.

A control group included 285 healthy individuals (41% males and 59% females) without a history of mental or neurological diseases. The mean age of the control subjects was 58.91 ± 8.15 years. The test and control groups did not differ in gender composition, but the mean age of the controls was significantly lower than that of the patients.

To estimate a possible effect of age on allele frequencies, the frequencies were compared for different cohorts of the control group (older or younger of 65 years of age and up to 40, 40–60, and older than 60 years of age). Significant differences in allele frequencies were not observed between the cohorts of the control group, and data on the pooled control group were used to avoid a loss of statistical power in the association analysis.

The association analysis involved 15 single nucleotide polymorphisms (SNPs), which show a significant association with cognitive disorders and schizophrenia according to recent reports [27, 28, 37–41]. The genes and SNPs are characterized in Table 1.

DNA was isolated from venous whole blood by phenol–chloroform extraction. Genotyping was carried out by real-time PCR with TaqMan probes as recommended by Applied Biosystems (United States), using a thermal cycler with real-time PCR detection (Bio-Rad, United States).

The correspondence to Hardy–Weinberg equilibrium was checked and the expected heterozygosity calculated by methods commonly accepted in population biometrics. The allele and genotype frequencies were compared between groups by the maximum likelihood (ML) χ^2 test. The strength of association was inferred from the odds ratio (OR) and its 95% confidence interval (CI). Intergenic interactions were analyzed by multifactor dimensionality reduction (MDR) with the use of MDR software. The biological processes related to the genes under study and the gene networks were analyzed using the bioinformatics resources DAVID (the Database for Annotation, Visualization, and Integrated Discovery), KEGG (Kyoto Encyclopedia of Genes and Genomes), and STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) [42–44].

RESULTS

Allele and Genotype Frequencies and Association of Genetic Polymorphisms with Alzheimer's Disease

Genotype and minor allele frequencies, expected heterozygosities, and the significance levels for the correspondence of the observed genotype distribution to the Hardy–Weinberg proportions in the patient and control samples are summarized in Table 2. A deviation from Hardy–Weinberg equilibrium was observed for two out of the 30 genotype frequency distributions examined, namely, those at *ZNF804A* rs1344706 and *AGBL1* rs16977195 in the control sample. In total, the allele frequencies were similar between the patients and controls and were within the variation ranges

Table 1. Polymorphisms and genes chosen for the association analysis with AD

No.	SNP	Minor allele	Gene/protein product
1	rs1502844	C	*SLCO6A1*/solute carrier organic anion transporter family, member 6A1
2	rs9960767	C	*TCF4*/transcription factor 44
3	rs2312147	T	*VRK2*/vaccinia-related kinase 2
4	rs3131296	T	*NOTCH4*/neurogenic locus, Notch homolog 4
5	rs12807809	C	*NRGN*/neurogranin
6	rs1572299	C	Intergenic region between *TLR4*/Toll-like receptor type 4 and *DBC1*/tumor protein, which is deleted in bladder cancer and is translationally controlled by pseudogene 1
7	rs17594526	T	*TCF4*/transcription factor 4
8	rs1344706	C	*ZNF804A*/zinc finger protein 804A
9	rs16977195	G	*AGBL1*/ATP/GTP-binding protein 1, cytosolic carboxypeptidase
10	rs7341475	A	*RELN*/reelin
11	rs8020441	G	*ZFP64P1*/zinc finger protein 64, pseudogene 1homolog in mice
12	rs2247572	T	*KCNB2*/potential-dependent potassium channel, Shab-related subfamily, member 2
13	rs2616984	G	*CSMD1* (*KIAA1890*)/CUB and Sushi multiple domain
14	rs2229741	T	*NRIP1*/protein 1 interacting with a nuclear receptor
15	rs2252521	T	*CPVL*/vitellogenin-like carboxypeptidase

Table 2. Genotype and allele frequency distributions in AD patients and controls

No.	SNP	MA	Patients					Controls				
			11	12	22	MAF	*p*-value	11	12	22	MAF	*p*-value
1	rs1502844	C	43	47	18	0.384	0.404	120	122	43	0.365	0.197
2	rs9960767	C	99	9	0	0.042	0.651	265	19	0	0.034	0.560
3	rs2312147	T	39	49	20	0.412	0.509	111	126	46	0.385	0.313
4	rs3131296	T	77	27	4	0.162	0.409	224	58	3	0.112	0.725
5	rs12807809	C	75	31	1	0.162	0.554	163	105	17	0.163	0.491
6	rs1572299	C	32	53	22	0.458	0.904	93	125	67	0.454	0.513
7	rs17594526	T	106	2	0	0.009	0.923	276	8	0	0.014	0.810
8	rs1344706	C	43	47	18	0.384	0.404	130	112	42	0.345	**0.032**
9	rs16977195	G	96	11	1	0.060	0.500	241	39	5	0.086	**0.029**
10	rs7341475	A	76	31	1	0.153	0.258	207	73	4	0.143	0.389
11	rs8020441	G	63	42	3	0.222	0.194	174	101	10	0.212	0.314
12	rs2247572	T	74	29	5	0.180	0.336	199	74	11	0.169	0.223
13	rs2616984	G	47	43	17	0.360	0.187	154	105	25	0.273	0.249
14	rs2229741	T	37	49	22	0.430	0.437	72	138	75	0.505	0.595
15	rs2252521	T	69	33	6	0.208	0.444	163	105	17	0.244	0.987

MA, minor allele. Genotypes: 11, homozygotes for the major allele; 12, heterozygotes; 22, homozygotes for the minor allele. MAF, minor allele frequency. The *p*-value characterizes the significance for the correspondence of the observed genotype distribution to the Hardy–Weinberg proportions. Significant deviations from Hardy–Weinberg equilibrium are in bold.

Table 3. Analysis of the association of the genetic polymorphisms with Alzheimer's disease

SNP	Gene	SNP	MA	MAF (AD)	MAF (control)	*OR*	95% CI	*p*-value
1	*SLCO6A1*	rs1502844	C	0.38	0.36	1.09	0.79–1.50	0.616
2	*TCF4*	rs9960767	C	0.04	0.03	1.26	0.56–2.82	0.580
3	*VRK2*	rs2312147	T	0.41	0.39	1.14	0.83–1.57	0.415
4	*NOTCH4*	rs3131296	T	0.16	0.11	1.53	0.98–2.39	**0.060**
5	*NRGN*	rs12807809	C	0.16	0.16	0.99	0.65–1.52	0.970
6	*TLR4/DBC1*	rs1572299	C	0.46	0.45	1.02	0.72–1.35	0.920
7	*TCF4*	rs17594526	T	0.01	0.01	0.65	0.14–3.11	0.856
8	*ZNF804A*	rs1344706	C	0.38	0.35	1.18	0.86–1.64	0.310
9	*AGBL1*	rs16977195	G	0.06	0.09	0.68	0.36–1.28	0.230
10	*RELN*	rs7341475	A	0.15	0.14	1.08	0.70–1.68	0.762
11	*ZFP64P1*	rs8020441	G	0.22	0.21	1.06	0.73–1.55	0.762
12	*KCNB2*	rs2247572	T	0.18	0.17	1.08	0.72–1.63	0.702
13	*CSMD1*	rs2616984	G	0.36	0.27	1.50	1.07–2.09	**0.018**
14	*NRIP1*	rs2229741	T	0.43	0.51	0.74	0.54–1.01	**0.061**
15	*CPVL*	rs2252521	T	0.21	0.24	0.87	0.60–1.28	0.486

OR, odds ratio for the minor allele; 95% CI, 95% confidence interval of OR; *p*-value, the OR significance level.

established for Caucasian populations in the HapMap and 1000 Genomes projects [45, 46].

Genotype frequencies and ORs are summarized in Table 3. One of the 15 SNPs showed a significant association with AD. The minor allele of *CSMD1* rs2616984 was significantly more common in the AD patients compared with the controls (OR = 1.50, 95% CI 1.07–2.09, p-value = 0.018). Differences observed for the *NOTCH4* and *NRIP1* SNPs were nearly significant at $p < 0.05$. The minor allele of *NOTCH4* rs3131296 had even a higher OR as compared with *CSMD1* (OR = 1.53, 95% CI 0.98–2.39, p-value = 0.06), but the difference did not reach statistical significance because of low sample polymorphism. The frequency of the major allele of *NRIP1* rs2229741 in the AD patients was 9% higher than in the controls (OR = 1.35, 95% CI 0.99–1.85, p-value = 0.061). The AD-associated SNPs of *CSMD1* and *NOTCH4* are in gene introns, while *NRIP1* rs2229741 is a synonymous nucleotide substitution in Gly codon 75.

Analysis of Intergenic Interactions

Intergenic interactions between the loci under study in AD were analyzed by MDR, using an exhaustive search algorithm. The MDR analysis revealed one combination of two loci and one combination of three loci with a significant cumulative effect on AD and a high cross-validation consistency (CVC). The model combining the genotypes at two genes (*CSMD1* and *NRIP1*) showed 8 out of 10 noncontradictory cross-validations with a total balanced accuracy of 57% and a specificity of 70%. A combination of genotypes at *NOTCH4*, *CSMD1*, and *NRIP1* showed CVC of 10 out of 10. A balanced accuracy of the model was 62%, and the specificity was 77%.

Complete CVC (10 out of 10) was additionally observed for another model of a cumulative effect of SNPs belonging to different loci, namely, *TLR4*, *CSMD1*, and *NRIP1*. The model had a slightly higher balanced accuracy (66%) a slightly lower specificity (68%) as compared with the model including *NOTCH4*. It is noteworthy that the significant prognostic models of epistatic interactions included the SNPs of the genes (*NOTCH43*, *SMD1*, and *NRIP1*) that showed a significant or nearly significant association with AD in the analysis of individual genes.

For the locus combinations that showed a high predictive accuracy with respect to AD according to MDR data, OR was calculated for carriers of the genotype combinations that occurred more than five (for two-locus combinations) or three (for three-locus combinations) times in our samples. Among the genotype combinations at *CSMD1* and *NRIP1*, the highest risk of AD was observed in the case of a double homozygots for the AD-associated alleles, *CSMD1* GG/*NRIP1* CC (OR = 4.05, 95% CI 1.63–10.03, p-value = 0.0011). In the model combining *NOTCH4*,

CSMD, and *NRIP1*, genotype combination TT/GG/CC could similarly be considered to predispose to AD (OR = 4.47, 95% CI 1.60–12.46, p-value = 0.0045).

Bioinformatics Analysis of the Biological Processes and Protein Interactions Associated with AD Genes

To study the relationships between the genes in question, their interactions with other genes whose involvement in early- or late-onset AD had been confirmed, and the potential biological significance of the observed associations, a functional annotation of the AD genes and an analysis of their protein–protein interactions were carried out using the DAVID, KEGG, and STRING resources [28–30]. Our gene set included the 15 genes whose SNPs were tested for association with AD in this work (*SLCO6A1*, *TCF4*, *VRK2*, *NOTCH4*, *NRGN*, *TLR4*, *DBC1*, *ZNF804A*, *AGBL1*, *RELN*, *AFP64*, *KCNB2*, *CSMD1* (*KIAA1890*), *NRIP1*, and *CPVL*), known genes of early-onset familial AD (*APP*, *PSEN1*, and *PSEN2*), candidate AD genes that had been examined earlier (*EPHA1*, *CD33*, *CD2AP*, *ATP5H*, *EXOC4*, *CTNNA3*, *RNF219*, and *TREM2*), and genes whose association with late-onset AD had been confirmed in more than one GWAS (*APOE*, *APOJ* (*CLU*), *PICALM*, *CR1*, *GAB2*, *TOMM40*, *APOC1*, *MS4A*, *BIN1*, and *ABCA7*).

The most important biological pathways, processes, and molecular functions enriched in the AD genes under study are summarized in Table 4. Only the categories with $p < 0.05$ or a false discovery rate (FDR) < 10 by DAVID clustering were included. The significance level (p-value) evaluates the hypothesis that the given gene set does not occasionally appear in a category in this case. FDR characterizes the proportion of false positive results (the expected percent of cases where the null hypothesis was rejected incorrectly).

DISCUSSION

Functions and Roles of Genes Associated with Alzheimer's Disease

In this work, an association with AD in the Russian population was demonstrated for SNPs or genotype combinations of three genes—*CSMD1*, *NOTCH4*, and *NRIP1*—which had earlier showed a significant association with cognitive endophenotypes in GWASs.

The product of *CSMD1* is a membrane protein that harbors multiple SUB and Sushi domains and is presumably involved in controlling the complement cascade [47]. *CSMD1* is expressed in all tissues with the highest protein level in the brain. Its role in neurodegenerative and neurological processes can be related to the fact that proteins involved in regulating the complement system can regulate the synaptic functions as well [48, 49]. An association with cognitive abilities has been demonstrated for rs2616984 of the gene in GWAS [28]. *CSMD1* rs73660619 has recently been associated with the rate of cognitive function impairment in AD [50]. Other polymorphisms of the gene are associated with schizophrenia [51–53] or susceptibility to familial monoclonal epilepsy [54] or bipolar disorder [55] according to GWASs and replicative studies. We have also confirmed the association of *CSMD1* with schizophrenia in the Russian population (unpublished data).

NOTCH4 codes for a protein of the Notch transmembrane protein family. NOTCH4 acts as a receptor for membrane-binding ligands. Once activated with the ligands Jagged1, Jagged2, and Delta1, NOTCH4 in complex with RBPJ/RBPSUH plays a role in transcriptional activation to regulate the genes involved in cell proliferation, differentiation, and apoptosis. *NOTCH4* has been described in one of the earliest GWASs of schizophrenia [37]. A possible role *NOTCH4* may play in the AD pathogenesis is related to two pathogenetic mechanisms. First, the Notch1–Notch4 proteins interact with presenilins, and distortion of their interaction may contribute to AD manifestation [56, 57]. Second, mutations of Notch3, which is similar to Notch4, are considered to cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), which is a rare hereditary disorder similar in pathogenesis to AD [58, 59]. So far, *NOTCH4* SNPs have not been associated with AD [60, 61].

NRIP1 codes for the RIP140 nuclear protein, which interacts with estrogen receptor 1 (ESR1) and glucocorticoid receptors NR3C1 and NR3C2. *NRIP1* rs2229741 has been associated with cognitive parameters in GWAS [27]. A role RIP140 may play in the pathogenetic mechanisms of AD is possibly related to the glucocorticoid-mediated transcription regulation in nerve tissue. In particular, a downregulation of glucocorticoid receptors interacting with the RIP proteins in the hippocampus has been shown to mediate progressive memory loss in AD [62]. Target repression of gene activity via RIP140 interaction with its ligands has also been described [63, 64].

Thus, the involvement in various molecular mechanisms that determine suppression of cognitive functions in AD can be responsible for the epistatic effect of the genes and gene combinations associated with AD in our work.

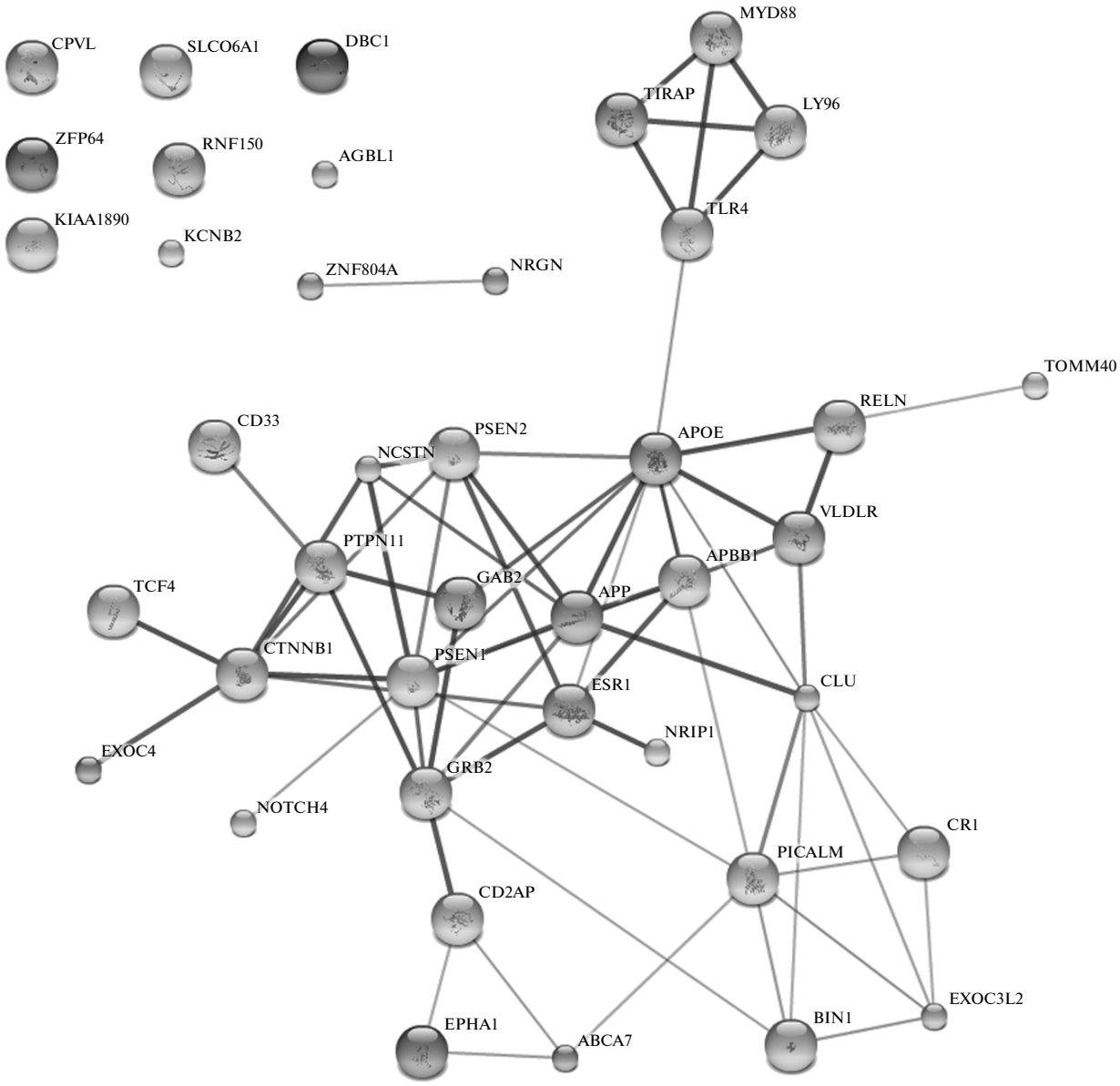
The analysis of biological processes and functions showed that the genes in question play an accessory (modulating or modifying) role in the processes underlying the AD pathogenesis because the genes do not belong to the main known biological categories that include the hereditary AD-causing genes and *APOE*. Among the signaling pathways revealed with the KEGG database, the Notch pathway is worthy of attention. As mentioned above, the Notch pathway involves not only presenilins, but also *NOTCH4*, which was associated with AD in our work.

TLR4, whose individual SPNs were not associated with AD, is still involved in a significant combination of epistatically interacting loci. The gene is widespread

Table 4. Most significant gene ontologies for the Alzheimer's disease-associated genes

Code	Ontology	Total genes	Genes involved	*p*-value	FDR
KEGG pathways					
hsa05010	Alzheimer's disease	5	*APP, PSEN1, APOE, PSEN2, ATP5H*	0.0010	0.86
hsa04330	Notch signaling pathway	3	*PSEN1, PSEN2,* ***NOTCH4***	0.0081	6.47
Biological processes					
GO:0002253	Activation of immune response	5	*CR1, PSEN1, PSEN2, CLU,* ***TLR4***	0.00003	0.06
GO:0050818	Regulation of blood clotting	4	*PSEN1, APOE, PSEN2,* ***TLR4***	0.00008	0.12
GO:0007219	Notch signaling pathway	4	*APP, PSEN1, PSEN2,* ***NOTCH4***	0.00016	0.25
GO:0007176	Regulation of epidermal growth factor activity	3	*APP, PSEN1, PSEN2*	0.00042	0.65
GO:0010469	Regulation of receptor activity	3	*APP, PSEN1, PSEN2*	0.00062	0.95
GO:0051605	Protein maturation via peptide bond cleavage	4	*CR1, PSEN1, PSEN2, CLU*	0.00072	1.11
GO:0016192	Vesicular transport	7	*ABCA7, APP, PICALM, PSEN1, APOE, EXOC4, BIN1*	0.00098	1.50
GO:0006897	Endocytosis	5	*ABCA7, APP, PICALM, APOE, BIN1*	0.00102	1.57
GO:0042325	Regulation of phosphorylation	6	*APP, PSEN1, APOE, PSEN2,* ***RELN, TLR4***	0.00242	3.68
GO:0002366	Leukocyte activation in immune response	4	*PSEN1, PSEN2,* ***TLR4***	0.00249	3.78
GO:0030900	Forebrain development	4	*APP, PSEN1, PSEN2,* ***RELN***	0.00370	5.58
GO:0010876	Lipid localization	4	*ABCA7, APOE, CLU,* ***NRIP1***	0.00406	6.10
GO:0006979	Oxidative stress response	4	*PSEN1, APOE, CLU,* ***TLR4***	0.00458	6.87
GO:0045859	Regulation of protein kinase activity	5	*APP, PSEN1, APOE, PSEN2,* ***RELN***	0.00524	7.82
GO:0051338	Regulation of transferase activity	5	*APP, PSEN1, APOE, PSEN2,* ***RELN***	0.00683	10.07
GO:0010604	Positive regulation of macromolecule metabolic processes	7	*APP, PSEN1, APOE,* ***NOTCH4, TLR4, TCF4, NRIP1***	0.00718	10.56
GO:0016044	Membrane organization	5	*ABCA7, APP, PICALM, APOE, BIN1*	0.00742	10.90
GO:0048167	Regulation of synaptic plasticity	3	*PSEN1, APOE, PSEN2*	0.00769	11.2
Molecular functions					
GO:0045296	Cadherin binding	3	*PSEN1, CD2AP, CTNNA3*	0.00055	0.64
GO:0050839	Binding of cell adhesion molecules	3	*PSEN1, CD2AP, CTNNA3*	0.00174	2.03
GO:0008233	Peptidase activity	6	*APP,* ***AGBL1****, PSEN1, PSEN2,* ***RELN, CPVL***	0.00569	6.77
GO:0046982	Protein heterodimerization activity	4	*APOE,* ***NOTCH4, TCF4****, BIN1*	0.00893	9.98

Pathways and gene ontologies with a p-value < 0.01 or FDR < 10 (DAVID) are included. Codes (identifiers) are given as in KEGG for biological pathways and as in the Gene Ontology Database for biological processes and molecular functions. The loci examined in this work are in bold.



Protein–protein interaction network of the products of AD genes and their partner genes. A circle size corresponds to the protein size. The thickness of a line between two proteins reflects the confidence of their relationship.

in the biological processes related to presenilins, amyloid precursor peptide, and *APOE*. *TLR4* is involved in various regulatory processes, which probably play a role in the AD pathogenesis.

NRIP1 is involved in localizing lipids and regulating macromolecular metabolism. This role agrees with its function as a transcriptional modulator of glucocorticoid-regulated genes. The regulation of macromolecular metabolism involves several other genes of our set, such as *NOTCH4*, *TLR4*, and *TCF4*. The other ontologies that involve the genes under study include the regulation of phosphorylation, development of the forebrain, peptidase activity, and protein heterodimerization.

A network of protein–protein interactions (figure) was obtained using STRING software and confirmed the intergenic interactions revealed in the analysis of biological pathways and processes. *APP*, *PSEN1*, *PSEN2*, and *APOE* occupy a central place in the AD gene network and have the most numerous interactions. The genes examined in our work either occur at the periphery or do not belong to the network. *NRIP1* is connected with other components of the network via interactions with ESR1; and *NOTCH4*, via interactions with *PSEN1*. *CSMD1* (*KIAA1890*) is not a component of the network, although displaying the most significant association with AD in our work. Its role in the disease is probably related to other mechanisms. A

potential role *CSMD1* may play in regulating the synaptic functions still lacks experimental support.

CONCLUSIONS

Several new associations were revealed in our replicative analysis of association with AD for the genetic polymorphisms that had been identified as markers of cognitive functions. The association of *CSMD1* polymorphisms with the disease was replicated in the Russian population, a possible involvement in late-onset AD was demonstrated for *NOTCH4* and *NRIP1*, and AD-associated combinations of interacting genes were identified. The genes under study are not involved in the main processes known to play a role in the AD pathogenesis. Our bioinformatics analysis showed that the genes may play a modulating or modifying role by acting within several regulatory and signaling pathways involved in the AD pathogenesis. Further studies of these pathways and processes may provide a source for filling the gaps in missing heritability of AD and its cognitive endophenotypes.

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SPELL: 1. downregulation