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Original Research Article

Altered Expression of RNA Splicing Proteins in Alzheimer's Disease Patients: Evidence from Two Microarray Studies

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Key Words

Alternative splicing · Microarray · Brain tissue, postmortem · Gene expression · Alzheimer's disease · Hippocampus

Abstract

Background/Aims: Dysregulation of pre-mRNA splicing from an altered expression of RNA splice-regulatory proteins may act as the convergence point underlying aberrant gene expression changes in Alzheimer's disease (AD). ***Methods:*** Two microarray datasets from a control/AD postmortem brain cohort of 31 subjects – 9 controls and 22 AD subjects (National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database) – were used. ***Results:*** Between the two microarray studies, the expression of six splice-regulatory protein genes showed concordant changes in AD. These genes were then correlated with gene expression changes of transcripts reported to be altered in AD. Amyloid beta (A4) precursor protein and tropomyosin receptor kinase B transcripts were found to correlate significantly with the same splice-regulatory proteins in the two studies. ***Conclusion:*** This study highlights a susceptibility network that can potentially link a number of susceptibility genes.

Introduction

Alzheimer's disease (AD) is the most common form of dementia accounting for between 50 and 70% of cases globally [1, 2]. The presence of intracellular neurofibrillary tangles, extracellular neuritic plaques, and brain-wide neurodegeneration are key pathological features which define AD [3]. Other pathological processes associated with AD include al-

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tered levels of neurotransmitter receptor expression [4] as well as dysregulation of cell signaling [5–7], inflammatory response [8, 9], synaptic transmission [10–12], and cholesterol metabolism [13, 14]. At present, a complete understanding of the underlying causes of these pathogenic processes remains elusive. It is predicted that there is a common underlying process that is dysregulated in AD, which serves as a convergence point that links the multitude of dysfunctional signatures.

Alternative splicing is a widespread gene-regulatory process by which exons of primary transcripts (pre-mRNAs) are spliced into different arrangements to produce structurally distinct mRNA variants. This mechanism of gene product diversification plays a critical role in controlling cellular differentiation and development in response to environmental, temporal, or cell type-specific cues [15, 16]. It is estimated that more than 75% of genes in the human genome are alternatively spliced [17]. Dysregulation in alternative splicing has been linked to a number of human diseases including some neurodegenerative diseases (e.g. frontotemporal dementia with parkinsonism) [18]. However, in AD, few studies have investigated the link between alternative splicing dysregulation, aberrant splice-regulatory protein expression, and AD progression. In a recent study, it has been found that generation of the tropomyosin receptor kinase B (TrkB) alternative transcript TrkB-Shc is regulated by the serine/arginine protein Srp20, and that biochemical manipulation of its expression in neuronal cell lines and exposure of cells to amyloidogenic factors could modulate TrkB pre-mRNA splicing and TrkB-Shc expression [19]. In other dementias, alterations in the levels of splice-regulatory protein expression have been shown to affect alternative splicing in frontotemporal dementia with parkinsonism. For instance, in humans, alterations in adult-specific tau exon 10 splicing have been demonstrated to lead to abnormal ratios of tau isoform expression [20–22]. Considering that one splice-regulatory protein is capable of regulating the splicing of multiple pre-mRNAs, it is hypothesized that dysregulation of pre-mRNA splicing from the altered expression of key splice-regulatory proteins in AD may underlie the aberrant changes in gene expression in multiple AD-affected pathways during disease progression.

Materials and Methods

Datasets

The hippocampal CA1 microarray datasets derived from postmortem brain tissue from a total of 31 subjects – 9 controls and 22 AD subjects – of varying AD severity (n = 7 incipient, n = 8 moderate, and n = 7 severe) were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (Affymetrix GeneChip (HG-U133A); accession GDS810 [23] and GSE28146 [24]). Cohort demographics are listed in table 1.

To determine whether there are changes in splice-regulatory protein gene expression during AD progression, expression profiles of all genes involved in RNA splicing were collated from the GDS810 microarray. Genes that were statistically significant by one-way analysis of variance (ANOVA) with a p value <0.05 or showed a trend towards statistical significance with a p value range between 0.05 and 0.1 were then mined from the GSE28146 microarray. Considering that a specific group of genes were to be assessed, all gene expression profiles were included, even those with low expression.

Statistical Analysis

Statistical analyses were conducted using Statistica 7 (StatSoft Inc., 2000, Statistica for Windows). One-way ANOVA was conducted to assess changes in gene expression during AD

Table 1. Cohort demographics

	Gender	Age, years
Control 976	male	85
Control 1003	male	80
Control 1008	female	92
Control 1012	male	80
Control 1015	male	75
Control 1018	female	97
Control 1030	male	95
Control 1039	male	77[a]
Control 1040	male	87
Incipient 715	female	101
Incipient 720	female	95
Incipient 994	female	83
Incipient 1019	male	88
Incipient 1029	female	91
Incipient 1034	male	88
Incipient 1043	female	97
Moderate 826	female	85
Moderate 832	female	89
Moderate 856	female	83
Moderate 965	female	82
Moderate 1020	female	79
Moderate 1025	male	81
Moderate 1031	female	86
Moderate 1037	male	82
Severe 701	male	85
Severe 723	female	65
Severe 807	male	93
Severe 819	female	79
Severe 867	female	94
Severe 872	female	79
Severe 1036	female	93

[a] Control 1039 is excluded in the GSE28146 microarray.

progression. ANOVAs were followed up with the Fisher LSD post hoc analysis to assess significance in gene expression between AD severity groups. Pearson's product moment correlations were used to determine whether any relationship existed between splice-regulatory protein gene expression and the expression of AD susceptibility genes. A p value <0.05 (two-tailed) was considered statistically significant.

Results

In this current report, two publically available microarray datasets from a control/AD hippocampal brain cohort from the NCBI GEO database (Affymetrix GeneChip (HG-U133A); accession GDS810 [23] and GSE28146 [24]) were utilized. In both studies [23, 24], the expression of genes significantly altered in AD was classified into functional categories and biological pathways of the gene ontology consortium (www.geneontology.org). However, a specific category for genes involved in RNA splicing was not described and genes involved in RNA splicing were likely grouped into other gene ontology categories. Thus, to determine whether genes involved in RNA splicing are altered during AD progression, genes whose gene

Table 2. Microarray gene expression changes in the CA1 hippocampus ($p < 0.05$; GDS810 microarray)

Probe	Gene	Description	Control	Incipient	Moderate	Severe	ANOVA F	ANOVA p
206809_s_at	HNRNPA3	heterogeneous nuclear ribonucleoprotein A3	461.2±62.2	305.9±48.3	362.1±53	222.1±38.4	3.63	0.03[a]
208713_at	HNRNPUL1	heterogeneous nuclear ribonucleoprotein U-like 1	610.9±39.4	809.7±72.7	757.5±38.8	836.6±67	3.73	0.02[a]
202903_at	LSM5	U6 snRNA-associated Sm-like protein LSm5	108.8±10.6	167.5±19.9	162.5±7.9	186.5±29.8	3.79	0.02[a]
202697_at	NUDT21	nucleoside diphosphate-linked moiety X	135.4±10.8	188.6±14.2	136±14	141.2±14.4	3.54	0.03[a]
213852_at	RBM8A	RNA-binding motif protein 8A	1,490.1±72.1	1,899.7±104	1,806±80.1	1,887.6±173.1	3.39	0.03[a]
216842_x_at	RBMY1J	RNA-binding motif protein, Y-linked, family 1, member J	144.1±19.4	275.9±42.7	156.5±26.7	232.1±48.3	3.37	0.03[a]
207842_s_at	CASC3	cancer susceptibility candidate 3	1,372.3±115.2	1,646.7±183.4	2,256.4±256.3	2,198.7±212.3	5.12	0.006[b]
203376_at	CDC40	cell division cycle 40 homolog	1,291.1±84.1	1,174.3±91.9	913.5±73.4	1,033.8±67.7	4.46	0.01[b]
201077_s_at	NHP2L1	nonhistone chromosome protein 2-like 1	1,995.8±74.2	1,764±105.5	1,599.1±42.9	1,487.1±107.8	7.39	0.0009[b]
200057_s_at	NONO	non-POU domain-containing octamer binding	3,671.8±98.9	3,872.7±103.9	4,017.1±164.8	4,171.8±110.5	3.07	0.04[b]
202635_s_at	POLR2K	polymerase (RNA) II (DNA-directed) polypeptide K, 7.0 kDa	465.9±61.3	367.5±44.5	253.2±22.6	409.4±46.2	3.85	0.02[b]
208652_at	PPP2CA	serine/threonine protein phosphatase 2A catalytic subunit alpha isoform	3,154.9±298.8	2,558.9±234.3	2,255.6±130.1	1,840.5±278.6	5.15	0.006[b]
221547_at	PRPF18	PRP18 pre-mRNA processing factor 18 homolog	220±15.2	239±11.9	161.4±17.1	258.2±21	6.25	0.002[b]
218053_at	PRPF40A	PRP40 pre-mRNA processing factor 40 homolog A	876±73.5	979.3±63	1,334.4±125.6	1,193.6±79.9	5.54	0.004[b]
211270_x_at	PTBP1	polypyrimidine tract-binding protein 1	565.9±50.9	683.1±78.9	850.1±87.4	870.1±76	4.1	0.02[b]
211271_x_at	PTBP1	polypyrimidine tract-binding protein 1	491.9±36.7	568.4±84.5	772.8±72.8	654.6±57.2	3.91	0.02[b]
212015_x_at	PTBP1	polypyrimidine tract-binding protein 1	362.4±28.4	388.6±67.6	536.2±44.8	448.3±40.2	3.04	0.046[b]
216306_x_at	PTBP1	polypyrimidine tract-binding protein 1	655±76.3	762.9±66.6	1,021.3±100.7	962.4±90.4	4.26	0.01[b]
212262_at	QKI	homolog of mouse quaking QKI	534.1±33.4	673.4±56.8	758.8±46.4	820.6±100.2	4.49	0.01[b]
212636_at	QKI	homolog of mouse quaking QKI	7,246.6±762.9	9,010.5±882.5	11,098.5±793.3	10,982.3±1262.1	4.24	0.01[b]
215089_s_at	RBM10	RNA-binding motif protein 10	261.3±48.8	219.1±47.1	463.6±48.3	314.2±84.7	3.45	0.03[b]
207941_s_at	RBM39	RNA-binding motif protein 39	3,192.2±118.6	3,276.6±263	4,108.2±117.8	3,436.4±238.7	5.32	0.005[b]
219507_at	RSRC1	arginine/serine-rich coiled-coil 1	66.8±12.9	85.2±18.1	123.3±8.45	159.6±30.8	5.1	0.006[b]
221768_at	SFPQ	splicing factor proline/glutamine rich	794.7±40.6	861.6±83.5	1,137.9±126.6	780.4±104.9	3.37	0.03[b]
216364_s_at	AFF2	AF4/FMR2 family, member 2	15.2±1.96	24.1±4.26	23.1±6.56	65.9±23.5	3.92	0.02[c]
203809_s_at	AKT2	RAC-beta serine/threonine-protein kinase	64.4±13.9	75.9±9.3	79.9±11.6	131±20	4.16	0.02[c]
202256_at	CD2BP2	CD2 (cytoplasmic tail)-binding protein 2	365±20.1	381.7±34.1	411.4±28.2	522.8±43.4	4.98	0.007[c]
209056_s_at	CDC5L	cell division cycle 5-like protein	676.3±36.6	623.8±48.4	578.1±25.7	492.2±40.9	4.21	0.01[c]
219539_at	GEMIN6	gem (nuclear organelle)-associated protein 6	143.5±18.9	130.8±14.7	174±15.4	214.5±26.5	3.5	0.03[c]
210588_x_at	HNRNPH3	heterogeneous nuclear ribonucleoprotein H3	486.2±39.8	578.9±62.8	424.5±34.7	689.7±92.5	3.82	0.02[c]
202072_at	HNRNPL	heterogeneous nuclear ribonucleoprotein L	692.3±82.2	746.9±99	694.9±63.6	380±81.3	3.86	0.02[c]
220764_at	LOC100132773	serine/threonine-protein phosphatase 4-regulatory subunit 2-like	50±13.9	21.1±9.33	62.5±15.4	96.8±25.3	3.25	0.04[c]
217415_at	POLR2A	polymerase (RNA) II (DNA-directed) polypeptide A, 220 kDa	76.1±13.3	56.8±14.3	81±14.2	136±26.6	3.55	0.03[c]
202634_at	POLR2K	polymerase (RNA) II (DNA-directed) polypeptide K, 7.0 kDa	850±66.5	836.7±57.5	789.2±34.9	590.1±68.8	3.95	0.02[c]
202494_at	PPIE	peptidylprolyl isomerase E (cyclophilin E)	206.1±16.9	281.4±53.7	262±18.9	360.4±40.7	3.7	0.02[c]
203103_s_at	PRPF19	PRP19/PSO4 pre-mRNA-processing factor 19 homolog	1,384.9±112.7	1,182.1±119.7	1,138.3±89.3	879.1±86.8	4.01	0.02[c]

Table 2 (continued)

Probe	Gene	Description	Control	Incipient	Moderate	Severe	ANOVA F	ANOVA p
218088_s_at	RRAGC	Ras-related GTP-binding C	1,081.5±52.4	1,013.4±82.1	1,218.4±67.1	1,411.2±69.2	6.43	0.002[c]
201070_x_at	SF3B1	splicing factor 3b, subunit 1, 155 kDa	501.4±44.3	517.2±28.9	536.4±53.8	829.1±163.4	3.27	0.04[c]
217608_at	SFRS12IP1	SREK1-interacting protein 1	68.4±6.13	62.8±10.1	86.7±13.1	122.1±12.6	6.02	0.003[c]
218493_at	SNRNP25	small nuclear ribonucleoprotein, 25 kDa (U11/U12)	1,080.4±83	1,070.7±113.3	1,019.8±59.2	714.5±41.7	4.52	0.01[c]
200826_at	SNRPD2	small nuclear ribonucleoprotein D2 polypeptide, 16.5 kDa	2,611.4±94	2,699±193.7	2,648.4±106.6	2,012.6±251.4	3.64	0.03[c]
211439_at	SRSF7	serine/arginine-rich splicing factor 7 (9G8)	137.5±19.8	199.5±43.3	124.1±11.9	235.7±31.4	3.61	0.03[c]
210180_s_at	TRA2B	transformer-2 protein homolog beta	22.2±4.97	6.97±1.06	16.9±6.27	34.6±9.26	3.3	0.04[c]
206067_s_at	WT1	Wilms tumor 1	8.11±1.46	12.1±3.63	13.3±3.26	21.1±3.38	3.41	0.03[c]
214759_at	WTAP	Wilms tumor 1-associated protein	142.9±25.7	174.5±19.6	130±12	216.4±26	2.99	0.049[c]

[a] Incipient $p < 0.05$ (Fisher LSD); [b] moderate $p < 0.05$ (Fisher LSD); [c] severe $p < 0.05$ (Fisher LSD).

products are known to be involved (either directly or indirectly) in RNA splicing were screened.

GDS810 Microarray

The GDS810 microarray published by Blalock et al. [23] assessed gene expression changes in the hippocampal CA1 subfield from a total of 31 subjects – 9 controls and 22 AD subjects – of varying AD severity. A total of 22,286 genes were tested and of these, 499 were involved in RNA splicing. Of the 499 genes, only 45 were found to be altered from the incipient stage of AD to the severe stage (table 2). Specifically, 6 of the 45 genes identified were significantly changed in subjects with incipient AD. Interestingly, hnrnpA3 was the only gene that showed a significant decrease in expression, whereas NUDT21, LSM5, hnrnpUL1, RBM8A, and RBMY1J were significantly increased in expression. In subjects with moderate AD, the expression of 18 genes was found to be significantly altered compared to controls. Of these, 5 genes were significantly decreased, whereas 13 genes were significantly increased. In subjects with severe AD, 21 genes were found to be significantly changed compared to controls. Of these, 6 genes showed a significantly reduced expression, whereas 15 showed a significantly increased expression.

It is worth noting that while 45 genes were found to be significantly altered during AD progression, the expression of 34 genes trended towards statistical significance (one-way ANOVA: $0.05 < p < 0.1$) (table 3). Of these, 11 were decreased in expression and 23 were increased in expression.

GSE28146 Microarray

In the GSE28146 microarray published by Blalock et al. [24], grey matter of the hippocampal CA1 subfield was selectively isolated using laser capture microdissection from the same subjects and analyzed for gene expression changes. Using this dataset, the expression of splice-regulatory proteins that were significantly altered or showed a trend towards statistical significance in AD in the GDS810 microarray was screened to determine whether changes in gene expression were specific to the grey matter. Of the 45 genes that were significantly changed throughout the course of AD in the GDS810 microarray study, only QKI, PTBP1, and SFPQ were significantly altered in the GSE28146 microarray (table 4). Of the 34 genes that

Table 3. Microarray gene expression changes in the CA1 hippocampus (0.05 < p < 0.1; GDS810 microarray)

Probe	Gene	Description	Control	Incipient	Moderate	Severe	ANOVA F	ANOVA p
200041_s_at	BAT1	spliceosome RNA helicase DDX39B	2,839.2±220.5	3,233.4±249	3,948.7±274	3,076.4±412.2	2.9	0.05[a]
209055_s_at	CDC5L	cell division cycle 5-like protein	130.2±19.2	256.1±66.6	126.1±21.6	149.1±35.9	2.53	0.08[a]
215045_at	CELF3	CUGBP, Elav-like family member 3	631.6±98.2	537±59.6	669.9±87	389.9±43.4	2.33	0.1[a]
203947_at	CSTF3	cleavage stimulation factor, 3' pre-RNA, subunit 3, 77 kDa	802±58.7	786.8±65.6	740.2±68.9	1,000±98.5	2.34	0.1[a]
219149_x_at	DBR1	debranching enzyme homolog 1	129.2±11.6	173.5±15.8	171±9.40	172.3±18.2	2.73	0.06[a]
219121_s_at	ESRP1	epithelial splicing-regulatory protein 1	73.9±19.5	40.1±7.51	31.7±3.72	81.4±21.8	2.47	0.08[a]
200959_at	FUS	fused in sarcoma	797.9±56.5	999.8±59.8	821.5±32.5	841.9±73.8	2.5	0.08[a]
215744_at	FUS	fused in sarcoma	127.3±14.9	88.3±21.6	122.8±11.8	160.7±26.1	2.3	0.1[a]
202354_s_at	GTF2F1	general transcription factor IIF, polypeptide 1, 74 kDa	95.5±15.5	144.4±20	75.2±21.9	80±19.5	2.53	0.08[a]
35201_at	HNRNPL	heterogeneous nuclear ribonucleoprotein L	1,963.2±158	2,212±171	2,120.6±118.2	1,689.6±84.3	2.45	0.09[a]
214918_at	HNRNPM	heterogeneous nuclear ribonucleoprotein M	66.1±10.2	72.8±16.7	90.9±21.3	136.3±27	2.66	0.07[a]
219814_at	MBNL3	muscleblind-like 3	33.5±9.70	13.5±3.45	42±16	67.5±20.9	2.39	0.09[a]
212718_at	PAPOLA	poly(A) polymerase alpha	2,442.8±240.9	2,252.2±428.9	2,644.5±251.4	3,686±610.5	2.55	0.08[a]
203378_at	PCF11	cleavage and polyadenylation factor subunit, homolog	491±41.8	533.8±55.3	673.7±50.7	547.9±46.1	2.81	0.06[a]
210183_x_at	PNN	pinin, desmosome-associated protein	4,076.8±556.7	3,036.2±149.6	4,261.8±184.9	3,148±358.9	2.65	0.07[a]
214144_at	POLR2D	polymerase (RNA) II (DNA directed) polypeptide D	161.8±14.8	131.9±7.06	136.3±13.4	182.6±19.5	2.5	0.08[a]
221649_s_at	PPAN	peter pan homolog	173.8±10.5	152.8±13.8	169.7±18	249.6±50.5	2.51	0.08[a]
220553_s_at	PRPF39	PRP39 pre-mRNA processing factor 39 homolog	530±46.5	481.7±40.3	535.7±45.5	381.3±47.9	2.35	0.09[a]
202126_at	PRPF4B	PRP4 pre-mRNA processing factor 4 homolog B	1,002.1±52.7	1,075.9±40.8	1,159.1±62.6	1,198.6±71.7	2.37	0.09[a]
217857_s_at	RBM8A	RNA-binding motif protein 8A	22.1±4.44	42.5±10.8	34.6±11.8	11.5±1.01	2.56	0.08[a]
208307_at	RBMY1J	RNA-binding motif protein, Y-linked, family 1, member J	26.5±5.12	33±14.2	39.3±9.54	78.7±26.8	2.41	0.09[a]
209381_x_at	SF3A2	splicing factor 3a, subunit 2, 66 kDa	172.1±41.3	223.2±58.3	368.3±62.7	515.8±179.2	2.69	0.07[a]
221263_s_at	SF3B5	splicing factor 3b, subunit 5, 10 kDa	1,134.9±70.8	955.7±73.6	914.9±29	1,011.1±72.1	2.46	0.08[a]
213505_s_at	SFRS14	putative splicing factor, arginine/serine-rich 14	832.2±47.1	779.5±51	749.8±67.8	630.4±38.6	2.55	0.08[a]
204978_at	SFRS16	splicing factor, arginine/serine-rich 16	949.1±114.7	1,131.2±161.5	1,134.6±118.2	1,803.3±405.1	2.92	0.05[a]
212438_at	SNRNP27	small nuclear ribonucleoprotein, 27 kDa (U4/U6.U5)	690.8±102.8	451.6±21.4	410.5±31.7	586.9±111.1	2.72	0.06[a]
215722_s_at	SNRPA1	small nuclear ribonucleoprotein polypeptide A'	232±11.7	296.7±33.1	236.5±19.6	205.4±31.1	2.4	0.09[a]
208821_at	SNRPB	small nuclear ribonucleoprotein polypeptides B and B1	337.4±28.8	326.6±15.5	284±26.3	415.6±59.5	2.33	0.1[a]
208610_s_at	SRRM2	serine/arginine repetitive matrix 2	922.4±118.4	1,019.6±110.7	1,177.7±148.3	1,986.8±611	2.53	0.08[a]
206989_s_at	SRSF2IP	SR-related CTD-associated factor 11	449.9±27.2	563.6±42.9	572±41.3	486.7±35.9	2.77	0.06[a]
201129_at	SRSF7	serine/arginine-rich splicing factor 7 (9G8)	1,286.8±76.1	1,001±79.4	1,048±100.7	1,008.9±109.5	2.37	0.09[a]
202553_s_at	SYF2	SYF2 homolog, RNA splicing factor	998±90	1,163.6±71.5	1,307.8±75.3	1,189.4±67.7	2.87	0.06[a]
200020_at	TARDBP	TAR DNA-binding protein (TDP-43)	1,269.4±60.2	1,455.1±135.5	1,201.1±53.4	1,592.9±176.9	2.58	0.08[a]
214814_at	YTHDC1	YTH domain containing 1	72.1±13.8	118.9±14.2	94±14.4	131.5±22.6	2.7	0.07[a]
202126_at	PRPF4B	PRP4 pre-mRNA processing factor 4 homolog B	2,050.7±211.2	1,330.4±124.9	2,149.5±267.1	2,313±353.5	2.8	0.06[b]
200020_at	TARDBP	TAR DNA-binding protein (TDP-43)	4,252.9±372.4	4,204.2±262.5	3,107.2±229.7	4,109.3±488	2.56	0.08[b]

[a] GDS810 microarray. [b] GSE28146 microarray.

Table 4. Microarray gene expression changes in the CA1 hippocampus (grey matter laser capture microdissection; GSE28146 microarray)

Probe	Gene	Control	Incipient	Moderate	Severe	ANOVA F	ANOVA p
212262_at	QKI	534.1±33.4	673.4±56.8	758.8±46.4	820.6±100.2	3.45	0.03[a]
211271_x_at	PTBP1	491.9±36.7	568.4±84.5	772.8±72.8	654.6±57.2	3.48	0.03[b]
221768_at	SFPQ	794.7±40.6	861.6±83.5	1,137.9±126.6	780.4±104.9	3.95	0.02[b]
201129_at	SRSF7	1,286.8±76.1	1,001±79.4	1,048±100.7	1,008.9±109.5	3.79	0.02[b]
204978_at	SFRS16	949.1±114.7	1,131.2±161.5	1,134.6±118.2	1,803.3±405.1	3.5	0.03[b]
214814_at	YTHDC1	72.1±13.8	118.9±14.2	94±14.4	131.5±22.6	5.59	0.004[c]

[a] Incipient $p < 0.05$ (Fisher LSD); [b] moderate $p < 0.05$ (Fisher LSD); [c] severe $p < 0.05$ (Fisher LSD).

Table 5. Correlations: splice-regulatory protein gene expression

	r	p
PTBP1 (GSE28146) PTBP1 (GDS810)	0.36	0.05
QKI (GSE28146) QKI (GDS810)	0.48	0.01[a]
SFPQ (GSE28146) SFPQ (GDS810)	0.18	0.35
SRSF7 (GSE28146) SRSF7 (GDS810)	0.50	0.01[a]
SFRS16 (GSE28146) SFRS16 (GDS810)	–0.15	0.42
YTHDC1 (GSE28146) YTHDC1 (GDS810)	0.11	0.57

[a] $p < 0.05$.

trended towards significance in the GDS810 microarray study, SRSF7, SFRS16, and YTHDC1 were found to be significantly changed in the GSE28146 study.

It was next determined whether the splice-regulatory proteins found to be significantly altered in both the GDS810 and GSE28146 microarray studies correlated in their gene expression pattern over the disease duration. Interestingly, QKI and SRSF7 were the only two genes showing concordance (table 5).

Gene Expression Correlations between Splice-Regulatory Proteins and AD Susceptibility Genes

Next, it was determined whether changes in splice-regulatory protein expression found to be significant in both the GDS810 and GSE28146 microarray studies correlated with gene expression changes of various transcripts reported to be altered in AD. These included progranulin (GRN), microtubule-associated protein tau (MAPT), presenilin-1 (PSEN1), presenilin-2 (PSEN2), presenilin enhancer protein 2 (PSENEN), amyloid beta (A4) precursor protein (APP), apolipoprotein E (APOE), TrkB (NTRK2), and brain-derived neurotrophic factor (BDNF) [25–29]. While the expression of most transcripts correlated with expression levels of the splice-regulatory proteins in their respective studies, only APP and TrkB tran-

Table 6. Correlations: splice-regulatory proteins and AD susceptibility gene transcripts (GDS810)

	r	p
PSEN1, QKI	0.47	0.01
MAPT, SFRS16	–0.44	0.01
PSEN2, QKI	0.53	0.002
PSEN2, SRSF7	–0.42	0.02
PSEN2, SRSF7	–0.45	0.01
BDNF, SFPQ	–0.41	0.03
NTRK2, SFRS16	0.61	0.0003[a]
NTRK2, YTHDC1	0.55	0.002
PSEN1, SFRS16	–0.39	0.03
APP, QKI	0.49	0.01
APP, SRSF7	–0.46	0.01
APP, SFRS16	0.87	0.0000000003
APP, YTHDC1	0.64	0.0001[a]
GRN, PTBP1	0.39	0.03
NTRK2, SFRS16	–0.65	0.0001[a]
APP, SFRS16	–0.54	0.002
APP, YTHDC1	–0.38	0.04[a]
GRN, QKI	0.40	0.03
PSENEN, SFRS16	–0.55	0.002
NTRK2, SRSF7	–0.43	0.02[a]
NTRK2, SFPQ	0.36	0.05[a]
NTRK2, SFRS16	–0.44	0.01[a]

[a] $p < 0.05$ in GDS810 and GSE28146 microarrays.

scripts correlated significantly with the same splice-regulatory proteins in the two studies (tables 6, 7). Two APP transcripts showed significant positive correlations with YTHDC1, whereas one transcript showed a negative correlation. The TrkB transcripts showing significant correlations with splice-regulatory protein expression were those encoding the C-terminal truncated TrkB isoform TrkB-TK–. TrkB-TK– transcripts correlated negatively with SRSF7 and SFRS16 but positively with SFPQ expression throughout AD progression.

Discussion

In the two microarray datasets utilized in this study, most changes in gene expression were found to occur during the moderate to severe stages of AD, periods coinciding with gross pathological brain changes and cognitive decline [30], whereas fewer changes occurred during the incipient stage of AD. Interestingly, of the 45 splice-regulatory proteins whose gene expression was found to be significantly altered in the GDS810 microarray study, only

Table 7. Correlations: splice-regulatory proteins and AD susceptibility gene transcripts (GSE28146)

	r	p
GRN, SFPQ	–0.45	0.01
APOE, PTBP1	0.37	0.05
PSEN1, SFPQ	0.48	0.01
MAPT, SFPQ	–0.63	0.0002
NTRK2, QKI	–0.47	0.01
PSEN1, SFPQ	0.37	0.05
APP, YTHDC1	0.49	0.01[a]
GRN, YTHDC1	0.44	0.01
NTRK2, SRSF7	–0.44	0.02[a]
NTRK2, QKI	0.44	0.01
NTRK2, SFRS16	–0.41	0.03[a]
NTRK2, SFPQ	0.39	0.03[a]
NTRK2, SRSF7	–0.52	0.003[a]

[a] $p < 0.05$ in GDS810 and GSE28146 microarrays.

6 were significant in the GSE28146 study; moreover, these significant changes in gene expression occurred during the incipient stages of AD. This finding suggests that changes in splice-regulatory protein expression may occur in the grey matter early in the disease process, prior to gross pathological brain changes. Indeed, when the GDS810 and GSE28146 microarrays were assessed to determine whether those splicing proteins found to be significantly altered in both microarray studies correlated in their gene expression pattern over the disease duration, QKI and SRSF7 were the only two genes showing concordance. The lack of concordance in splice-regulatory protein expression between the two microarray studies suggests that the majority of gene expression changes in the GDS810 study were likely influenced by changes in the white matter, which was largely excluded by the laser capture microdissection in the GSE28146 study.

Relationship between Splice-Regulatory Proteins and AD Susceptibility Genes

When it was assessed whether the expression of splice-regulatory proteins found to be significantly altered in AD correlated with the expression levels of AD susceptibility genes, significant correlations in APP and TrkB-TK– transcripts throughout AD progression in both the GDS810 and GSE28146 microarray studies suggest that changes in gene expression may be primarily influenced by changes in the grey matter, which is consistent with the cellular expression of APP (expressed primarily in neurons and glia and to a lesser extent in endothelial cells) and TrkB-TK– (expressed principally by astrocytes and to a lesser extent by neurons) [31–33]. The APP transcripts showing a significant correlation with YTHDC1 were targeted by pan probes, whereas the TrkB transcripts showing significant correlations with SRSF7, SFRS16, and SPQF were targeted by probes specific for the transcript variant encoding TrkB-TK–. These findings suggest that YTHDC1 may be involved in regulating constitutive splicing of the APP pre-mRNA rather than regulating alternative splicing of specific exons.

The TrkB-TK– transcript variant encodes a TrkB protein isoform truncated at the C terminus and in comparison to the full-length receptor, and thus cannot partake in classical receptor tyrosine kinase signaling [34–36]. This is due to alternative exon splicing of the pre-mRNA where exon 16 is incorporated into the final transcript. Exon 16 encodes a translation stop codon and polyadenylation sequence; thus, transcripts are differentially regulated posttranscriptionally [34, 36]. The finding that TrkB-TK– transcript levels correlated with the same splice-regulatory proteins significantly altered in both microarray studies implicates these splice-regulatory proteins as potential regulators of TrkB-TK– expression. In particular, SRSF7 has previously been demonstrated to be a regulator of tau alternative splicing [20]. Abnormal ratios of tau isoforms 3R and 4R (imperfect repeats of approximately 32 amino acids in the microtubule-binding domain) lead to tau aggregation and neurofibrillary tangle formation. The generation of tau isoforms 3R and 4R is determined by alternative mRNA splicing of exon 10 [20–22], and point mutations in the splice-regulatory region affecting SRSF7 binding have been shown to modulate inclusion/exclusion of tau exon 10 [20]. In both the GDS810 and GSE28146 microarray studies, the probes targeted to MAPT transcripts (gene encoding tau) were not specific for exon 10 detection, thus no significant correlation between changes in SRSF7 and MAPT expression was found. However, MAPT expression is likely to be regulated by additional mechanisms. Considering that SRSF7 was one of only two splice-regulatory proteins to be significantly and positively correlated between the two microarray studies and is significantly correlated with TrkB-TK– transcript levels in the two studies, the findings reported here implicate SRSF7 as a potential regulator of TrkB pre-mRNA splicing in the production of TrkB-TK– alternative transcripts in the grey matter. The lack of concordance between SFRS16 and SPQF gene expression changes between the GDS810 and GSE28146 microarray studies suggests that these two splice-regulatory proteins may function in regulating TrkB-TK– transcript levels in the white matter.

Conclusion

Dysregulation in splice-regulatory protein gene expression can adversely affect alternative splicing and gene expression of a number of cellular processes. The findings reported in this study offer mechanistic insight into how aberrant changes in alternative transcript expression may occur in AD and highlight a susceptibility network – splice-regulatory proteins – which can potentially link a number of susceptibility genes/pathways.

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Disclosure Statement

There are no conflicts of interest and financial disclosures.

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