Gene Expression Profiling in Alzheimer's Disease Brain Microvessels

Article *in* Journal of Alzheimer's disease: JAD · April 2012

DOI: 10.3233/JAD-2012-120454 · Source: PubMed

4 authors, including:

Shu Wang
Texas Tech University
61 PUBLICATIONS **1,541** CITATIONS

SEE PROFILE

Uzma Qaisar
University of the Punjab
32 PUBLICATIONS **190** CITATIONS

SEE PROFILE

Paula Grammas
Texas Tech University Health Sciences Center
148 PUBLICATIONS **5,486** CITATIONS

SEE PROFILE

Journal of Alzheimer's Disease 30 (2012) 1–13
DOI 10.3233/JAD-2012-120454
IOS Press

Gene Expression Profiling in Alzheimer's Disease Brain Microvessels

Shu Wang[a,b,1], Uzma Qaisar[c,1], Xiangling Yin[a] and Paula Grammas[a,*]
[a]*Garrison Institute on Aging, Texas Tech University Health Sciences Center, Lubbock, TX, USA*
[b]*Department of Nutrition, Texas Tech University, Lubbock, TX, USA*
[c]*Department of Immunology and Microbiology, Texas Tech University Health Sciences Center, Lubbock, TX, USA*

Accepted 5 April 2012

Abstract. The enigma that is Alzheimer's disease (AD) continues to present daunting challenges for effective therapeutic intervention. The lack of disease-modifying therapies may, in part, be attributable to the narrow research focus employed to understand this complex disease. Most studies into disease pathogenesis are based on *a priori* assumptions about the role of AD lesion-associated proteins such as amyloid-β and tau. However, the complex disease processes at work may not be amenable to single-target therapeutic approaches. Genome-wide expression studies provide an unbiased approach for investigating the pathogenesis of complex diseases like AD. A growing literature suggests a role for cerebrovascular contributions to the pathogenesis of AD. The objective of the current study is to examine human brain microvessels isolated from AD patients and controls by microarray analysis. Differentially expressed genes with more than 2-fold change are used for further data analysis. Gene ontology analysis and pathway analysis algorithms within GeneSpringGX are employed to understand the regulatory networks of differentially expressed genes. Twelve matched pairs of AD and control brain microvessel samples are hybridized to Agilent Human 4 × 44 K arrays in replication. We document that more than 2,000 genes are differentially altered in AD microvessels and that a large number of these genes map to pathways associated with immune and inflammatory response, signal transduction, and nervous system development and function categories. These data may help elucidate heretofore unknown molecular alterations in the AD cerebromicrovasculature.

Keywords: Alzheimer's disease, brain, gene expression profile, microarray, microvessel

INTRODUCTION

Alzheimer's disease (AD) is an age-associated dementing disorder that currently affects over 5 million Americans [1]. Despite intense research efforts, disease-modifying therapies remain elusive, highlighting our still incomplete understanding of disease pathogenesis. Classification of AD as a "non-vascular" dementia has hampered a rigorous exploration of the role blood vessel abnormalities and/or vascular-derived factors could play in the pathogenesis of this disease. Nevertheless, an increasing body of literature implicates vascular factors and brain blood vessel function in the development of AD. For example, population-based studies find that atherosclerosis is positively associated with the risk of developing dementia [2]. Cardiovascular risk factors such as hypertension, the ε4 allele of apolipoprotein E, elevated homocysteine levels, hyperlipidemia, metabolic syndrome, obesity, and diabetes are also risk factors for AD [3–12]. A role for the cerebrovasculature in the evolution of AD lesions is suggested by several studies that document an intimate relationship between the microvasculature and AD lesions [13–17]. These studies show that in the AD brain the distribution of amyloid deposits is spatially associated with the global pattern of small blood vessel damage [15, 16]. Serial brain sections examined by electron microscopy reveal that amyloid fibrils forming senile plaques have

[1]These authors contributed equally to this work.

*Correspondence to: Paula Grammas, PhD, Garrison Institute on Aging, Texas Tech University Health Sciences Center, 3601 4th Street Stop 9424, Lubbock, TX 79430, USA. Tel.: +1 806 743 3612; Fax: +1 806 743 3636; E-mail: paula.grammas@ttuhsc.edu.

ISSN 1387-2877/12/$27.50

a close relationship to brain capillaries [14]. Also, microvascular pathology displays regional and laminar patterns which parallel patterns of neuronal loss in AD [16]. Thus, cerebral microvessels may be central to a destructive cycle of events in the AD brain. In this regard, brain microvessels isolated from AD patients, but not controls, release a large number of factors that can directly affect neuronal viability, including nitric oxide, cytokines and chemokines, proteases, and neurotoxins (reviewed in [18]).

To date, human and animal studies that are based on *a priori* assumptions about the role of specific AD lesion-associated proteins, in particular amyloid-β (Aβ), in the development of AD are narrowly focused [19, 20]. In contrast, unbiased genomic approaches can identify novel genes and proteins that may be relevant in AD but have not been previously characterized. Unraveling the mechanisms underlying AD pathology and neuronal loss is also difficult because of the complexity of the networks that drive AD disease processes. Another advantage of the gene expression profiling approach is that this method, through massive parallel analysis of most genes expressed in a tissue, provides the investigative power needed to address the complexity of neurodegenerative disease processes [21].

A study investigating genome-wide gene alterations in the temporal cortex of a well-characterized cohort of AD patients finds consistent patterns of changes in the AD transcriptome, documenting significant altered expression in genes associated with synaptic dysfunction, perturbed neurotransmission and activation of neuroinflammation [22]. Recently, Logic Mining, a large scale expression data analysis model employed in an animal model of AD, identified specific genes as potential biomarkers for early and late-stage neurodegeneration [23]. These microarray studies in AD patient samples and animal models have examined whole brain or region-specific tissue, but microvascular gene expression in blood vessels isolated from AD brains has not been explored.

The objective of the current study is to use an unbiased gene expression profiling approach to identify abnormal gene expression in the AD brain microvasculature. Human brain microvessels isolated from AD patients and controls are examined by microarray analysis. Differentially expressed genes with fold change more than two are used for further data analysis. Gene ontology (GO) analysis and pathway analysis algorithms within GeneSpringGX are employed to understand the regulatory networks of differentially expressed genes. These data may help elucidate heretofore unknown molecular alterations in the AD cerebromicrovasculature.

MATERIALS AND METHODS

Human brain microvessel isolation

Right cerebral hemispheres from control and AD patients were stored at −80°C and used for the experiments described herein. Left cerebral hemispheres were histologically processed for diagnostic and morphometric studies for the clinical diagnosis of primary degenerative AD dementia. Each case was examined for neuritic plaques and neurofibrillary tangles as recommended by National Institutes of Health Neuropathology Panel and each case fulfilled the rigorous morphometric criteria of AD [24, 25]. Control samples from age- and gender-matched patients without evidence of neuropathology and similar postmortem intervals were also collected (Table 1). Microvessels were isolated from pooled temporal, parietal, and frontal cortices, as we have previously described [26]. Briefly, brain microvessels were filtered through a 210 μm sieve and collected on a 53 μm sieve, resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and dimethyl sulfoxide and stored frozen in liquid nitrogen until use. This procedure yields approximately 6 to 10 mg microvessel protein from 15 g human cortex. A separate microvessel preparation was isolated from each human brain. The microvessel preparation has been previously characterized as largely capillary (>85%) and relatively free of nonvascular contaminants.

Table 1
Human tissues used for microvessel isolation. Right cerebral hemispheres were obtained from AD as well as control patients that were matched for age, gender, and postmortem interval (PMI)

	Control			AD		
Array	Gender	Age	PMI	Gender	Age	PMI
1	M	66	22	M	75	20
2	F	57	12	F	78	28
3	F	82	22	F	87	16
4	M	87	14	M	80	19
5	M	64	52	M	79	39
6	M	89	7	M	84	8
7	F	89	14	F	81	16
8	F	89	7	F	89	9
9	F	77	13	F	81	22
10	M	85	21	M	84	21
11	M	78	8	M	80	13
12	M	85	20	M	80	19
	Mean ± SD	**79 ± 11**	**18 ± 12**	**14**	**82 ± 4**	**19 ± 8**

Labeling of RNA and microarray hybridization

Microvessel RNA was extracted by using Qiagen RNeasy mini plus kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Quality and quantity of RNA was checked by measuring absorbance at wavelengths of 260 nm and 280 nm on Nanodrop spectrophotometer (Nanodrop Technologies, Inc., Wilmington, DE). Samples with 260/280 ratio above 1.8 were analyzed on Agilent BioAnalyzer (Agilent RNA 6000 nano kit) for assessment of RNA integrity number (RIN). Good quality RNA with RIN >7 was reverse transcribed into cDNA, linearly amplified and labeled by cy3 or cy5 fluorescent dyes using an RNA amplification and labeling kit (Agilent Technologies, Palo Alto, CA) according to manufacturer's protocol. Concentration (ng/μl) and dye label (pmols/μl) of labeled RNA were measured by Nanodrop. Total yield and specific activity (pmols of dye per μg of RNA) were determined and the samples with yield >825 nng and specific activity >8 pmols/μg of RNA were selected for hybridization. The hybridization mix for each array contained RNA from AD and normal microvessel samples matched by age, gender, and postmortem intervals and labeled with different colored dye along with Agilent hybridization buffer and blocking agent. Twenty-four total independent hybridizations were performed with 12 pairs of biological samples and 2 technical dye-swap replicates. Labeled RNA was hybridized on Agilent human 4×44 K oligo chips according to manufacturer's protocol for two color microarrays. Chips were washed stringently to remove nonspecifically bound probes and scanned using Agilent microarray scanner (Agilent Technologies, Palo Alto, CA).

Microarray data analysis

Microarray data was filtered by various quality control criteria to eliminate any systematic or procedural bias of dye coupling and hybridization. Quality of microarray data was determined by spatial distribution of hybridized probes, MA plots (representing ratio of red to green intensities plotted against average intensities) and spike in standard curves which determine the hybridization intensities of replicated probes randomly distributed all over the array. Only those arrays which passed quality controls were included in data analysis. Feature extraction software V.9.5.3 (Agilent Technologies, Palo Alto, CA, http://www.chem.agilent.com) was used to place the design grid, calculate the intensities of signal and background, and flag spots. Spots with intensities below the mean background intensity were flagged as not detected. Saturated and non-uniform spots were flagged compromised, while remaining spots were flagged detected. Compromised spots were excluded for further analysis. Background subtracted signal intensities were normalized within array by locally weighted scatter plot smoothing (Lowess) method to eliminate the effect of special distribution of background signals. The processed signal intensities were imported to GeneSpringGX commercial software (Agilent Technologies, Inc., http://www.chem.agilent.com) for further normalization and processing of data. Between arrays normalization was performed to eliminate variation between individual slide hybridizations by quantile algorithm within GeneSpringGX.

Statistical method of *t*-test algorithm along with asymptotic *p*-value computation was used to find the differentially expressed genes. Multiple test correction of Benjamini Hochberg with False Discovery Rate (FDR) of <0.05 was implied to minimize false positives. Statistically significant differentially expressed genes filtered on fold difference and genes with a fold change more than 2 (as recommended by Agilent) in at least one of the conditions studied were used for further data analysis. The differentially expressed genes were subjected to GO analysis and pathway analysis within GeneSpringGX to find if any of the GO functional categories or pathways is significantly enriched with these genes. GO categories with a weight-corrected *p*-value of >0.1 were considered significant. Biological pathways significantly affected by AD with the corrected *p*-value cut off of 0.05 were considered significant. Gene networks among the entities of an enriched GO category of differentially expressed genes were analyzed by pathway analysis tool within GeneSpringGX.

Validation of microarray data by real-time PCR

An aliquot of the total RNA that was previously extracted from human brain microvessels was used for validation of microarray data using real-time PCR. Single strand cDNA was synthesized from 500 ng of total RNA using Oligo-dT primers and SuperScript™ Π reverse transcriptase (Invitrogen Corp., Calsbad, CA) according to manufacturer's instructions. cDNA levels of genes were measured using Taqman® gene expression assays and Taqman® PreAmp master mix kit (Applied Biosystems, Foster City, CA) on ABI 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA). The real-time PCR reaction condition was 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C

for 1 min, 1 cycle of dissociation stage. Comparative Ct (cycle threshold) method was used as a quantitation approach. The Ct is defined as the number of cycles required for the fluorescent signal to pass the threshold. mRNA fold changes of randomly selected genes were calculated using the $2^{-[\text{delta}][\text{delta}]Ct}$ method. The calculation of $[\text{delta}][\text{delta}]C_t$ was the following: $[\text{delta}][\text{delta}]C_t = [\text{delta}]C_{t,AD} - [\text{delta}]C_{t,control}$. $[\text{delta}]C_{T,AD}$ was the C_t value for any AD sample normalized to the endogenous housekeeping gene β-actin. $[\text{delta}]C_{t,control}$ was the C_t value for the calibrator also normalized to the endogenous housekeeping gene β-actin.

RESULTS

Volcano plot of microvessel microarray data

The purity of microvessel preparations was assessed by phase-contrast microscopy. A representative microvessel preparation isolated from a human control brain is shown in Fig. 1. Twelve matched pairs of AD and control brain microvessel samples were hybridized to Agilent Human 4 × 44 K arrays in replication. Out of 24 independent microarray experiments, 22 passed all the quality controls suggested by GeneSpringGX and were included in statistical analysis. All the 41,093 probes were flagged in the detected or non-detected categories in at least one array and hence no probe was thrown out of the analysis. 2,865 genes (6.97%) were statistically significantly differentially regulated at a FDR <5% and had *p*-value <0.05. The data are presented in a volcano plot (Fig. 2). The volcano plot is used to identify changes in large datasets and is generated by plotting significance versus fold-change.

417 genes (14%) of 2,865 differentially expressed genes showed more than 2 fold change. 237 genes were upregulated in AD as compared to control brain microvessels with maximum fold change of 4.3 while 180 genes were downregulated with the highest fold change of 3.9 (Fig. 2).

Gene ontology analysis of functional categories up- or downregulated in AD microvessels

GO accessions were searched for all the genes on the chips from the GO database on the Agilent server within GeneSpringGX using the "annotation update" function. GO has been used extensively in recent years as a way to mine large data sets obtained from genome-scale experiments. Accessions were assigned

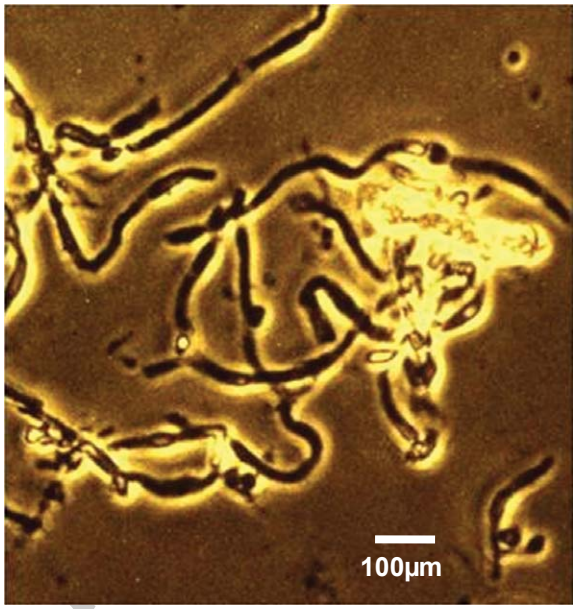

Fig. 1. Phase-contrast micrograph of isolated microvessel segments from control human brain (X600).

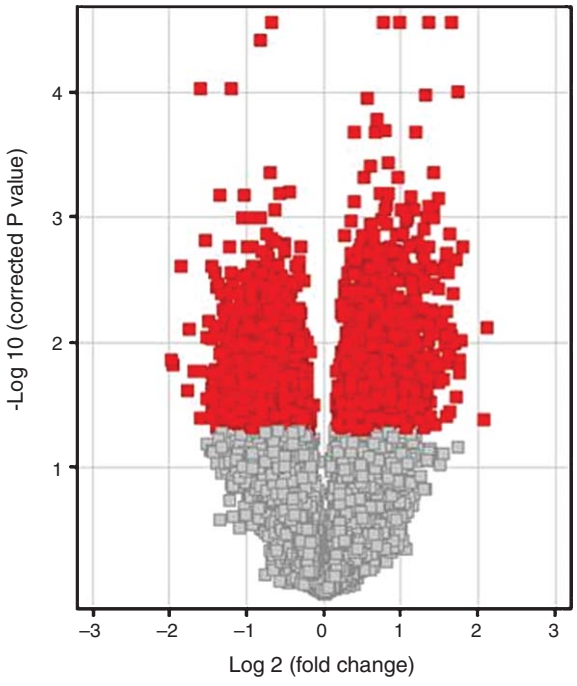

Fig. 2. A volcano plot showing genes differentially expressed in brain microvessels between control and AD subjects. Each square represented a gene on the microarray while the position denotes the expression (x-axis) and probability of differential expression (y-axis). Red squares represented 2865 statistically significantly differentially expressed genes. The data-points less than −1 and more than 1 on y-axis are genes having fold change less than 0.5 (Log $2^{0.5} = -1$) and more than 2 (Log $2^2 = 1$), respectively.

Table 2
Functional categories upregulated in AD brain microvessels. Statistically significantly upregulated genes >2 fold were subjected to gene ontology (GO) analysis within GeneSpringGX to classify these genes by biological process, molecular function and cellular component. To determine if any of the GO categories is significantly enriched with these genes, statistical test was performed and *p*-value assigned to each category. The functional categories above *p*-value cut off of e-5 are shown

GO term	*p*-value
Molecular function	
Signal transducer activity	2.21E-16
Molecular transducer activity	2.21E-16
Receptor activity	8.80E-17
Transmembrane receptor activity	1.06E-10
Cellular component	
Cell periphery	1.85E-14
Plasma membrane	7.82E-14
Intrinsic to membrane	2.34E-09
Integral to membrane	4.65E-09
Membrane part	1.55E-08
Membrane	3.72E-08
Integral to plasma membrane	8.32E-08
Intrinsic to plasma membrane	1.32E-07
Plasma membrane part	4.20E-07
Biological process	
Immune system process	*8.20E-17*
Defense response	*5.45E-13*
Immune response	*5.42E-12*
Response to wounding	*3.65E-11*
Response to stimulus	*9.67E-11*
Regulation of immune system process	*7.36E-10*
Response to stress	*9.05E-10*
Signal transduction	*3.82E-09*
Biological regulation	*4.48E-09*
Inflammatory response	*5.74E-09*
Signaling	1.11E-08
Signal transmission	1.58E-08
Signaling process	1.60E-08
Cell surface receptor linked signaling pathway	2.14E-08
Positive regulation of immune system process	*2.57E-08*
Regulation of immune response	*4.42E-08*
Regulation of biological process	6.61E-08
Activation of immune response	*1.06E-07*
Positive regulation of immune response	*3.48E-07*
Immune response-activating signal transduction	*5.66E-07*
Regulation of cellular process	6.04E-07
Response to external stimulus	*6.13E-07*
Immune response-regulating signaling pathway	*6.75E-07*
Leukocyte migration	*8.58E-07*
Cell adhesion	1.25E-06
Biological adhesion	1.25E-06
Regulation of response to stimulus	*1.59E-06*
Immune response-activating cell surface receptor signaling pathway	*1.64E-06*

to 26,335 genes (64.1%), while the genes without any GO accession were excluded from enrichment analysis. Enrichment analysis of the GO categories was performed on significantly up and down regulated genes to find out the functional category of genes most affected by AD in the brain microvessels. All functional categories (terms) descend from one of three roots: molecular function, cellular component, or biological process.

GO analysis of upregulated genes resulted in 105 significant upregulated GO terms. Data with *p*-values less than 1E-05 are shown in Table 2. In the molecular function group, the largest and most significantly upregulated categories were signal transducer activity (51 genes), molecular transducer activity (51 genes), and regulated receptor activity (47 genes). The functional category in the cellular component group that contained the largest number of significantly

upregulated genes was membrane-associated genes (106 genes). In the functional categories in the biological process group, the most significantly upregulated category was immune system process. The largest category was the biological regulation, comprising 70 genes, which represented 51% of the total upregulated genes in AD brain microvessels. 35 out of 70 genes were involved in immune system process, 24 were involved in immune response, and 16 were involved in inflammatory response. Other prominent upregulated categories among biological process categories were response to stimulus (52 genes), defense response (29 genes), response to wounding (20 genes), chemotaxis (12 genes), leukocyte migration (10 genes), and regulation of immune system process (9 genes). Overall, half of significantly upregulated categories were related to immune and inflammatory responses; these are italicized in Table 2.

GO analysis was also performed on 180 downregulated genes in AD and found that 39 GO terms were significantly downregulated. Data with *p*-value less than 1E-05 are shown in Table 3. Within molecular function group, almost all downregulated categories were related to glutamate receptor activity. Among cellular component group, the most downregulated genes were involved in synapse and its function. The significantly downregulated categories within biological processes group were neurological developmental and functional pathways. Overall, almost all of significantly downregulated categories were related to nervous and neurological system development and function (Table 3).

Validation of microarray identified genes by real-time PCR analysis

A large number of genes involved in immune-inflammatory-related responses were significantly altered. About 98% of those genes were significantly upregulated in AD compared to control brain microvessels. In this study, we documented that the expression of cytokines, chemokines, and their receptors was upregulated in microvessels isolated from AD brains compared to those from control brains. We observed the increased expression of interleukin 1 receptor, type II (IL1R2), chemokine (C-C motif) ligand 2 (CCL2) also known as monocyte chemoattractant protein-1 (MCP-1), chemokine (C-C motif) receptor 1 (CCR1), chemokine (C-C motif) receptor 5 (CCR5), and decreased expression of chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1) (CXCL12) in microvessels isolated from AD brains compared to those from control brains. CCR5 and CCL2 were validated using real-time PCR (Table 4). Also, we documented that genes involved in chemotaxis and inflammation were significantly upregulated in AD brain microvessels by microarray analysis. Validation of these data by real-time PCR confirmed the upregulation of CCR5 and CCL2 integrin alpha X (ITGAX) (Table 4).

Representative genes from functional categories that showed significant downregulation by microarray and GO analysis, such as signal transduction and neurological system development and function, were also validated by real-time PCR. Dysregulation of the

Table 3
Functional categories downregulated in AD brain microvessels. Statistically significantly downregulated genes >2 fold were subjected to gene ontology (GO) analysis within GeneSpringGX to classify these genes by biological process, molecular function and cellular component. To determine if any of the GO categories is significantly enriched with these genes, statistical test was performed and *p*-value assigned to each category. The functional categories above *p*-value cut off of e-5 are shown

GO term	*p*-value
Molecular function	
Glutamate receptor activity	1.69E-06
N-methyl-D-aspartate selective glutamate receptor activity	7.61E-06
Ionotropic glutamate receptor activity	7.82E-06
Extracellular-glutamate-gated ion channel activity	9.86E-06
Cellular component	
Synapse	8.98E-07
Synapse part	1.08E-06
Neuron projection	1.45E-06
Biological process	
Regulation of neuronal synaptic plasticity	1.40E-06
Glutamate signaling pathway	2.03E-06
Regulation of long-term neuronal synaptic plasticity	3.55E-06
Regulation of transmission of nerve impulse	4.60E-06
Regulation of neurological system process	9.41E-06

Table 4
Validation of microarray identified genes by real-time PCR analysis. In order to validate microarray data, the expression of 5 randomly selected genes is measured using real-time PCR. Fold changes and *p*-values are compared

Gene symbol	GenBank	Description	Microarray fold change	Microarray corrected *p* value	Real-time PCR fold change	Real-time PCR *p* value
CCR5	NM_000579	Chemokine (C-C motif) receptor 5	2.21	0.0028	6.04	<0.05
CCL2	NM_002982	Chemokine (C-C motif) ligand 2	2.21	0.0213	3.59	<0.05
ITGAX	NM_000887	Integrin, alpha X	2.47	0.0001	5.24	<0.05
RAB3B	ENST00000371655	Member RAS oncogene family	0.36	0.0033	0.27	<0.05
IGF1	NM_000618	Insulin-like growth factor 1	0.46	0.0440	0.51	<0.05

intracellular signaling cascade has been implicated in the pathogenesis of AD. Some signaling cascades showed numerous genes with significant expression changes. RAB3B, a member of the RAS oncogene family, was significantly downregulated in AD compared to control brain microvessels. This result was validated using real-time PCR (Table 4). Also, the expression of significantly regulated genes involved in nervous and neurological system development and function was decreased in AD compared to control brain microvessels. Expression of the neurotrophic protein insulin-like growth factor 1(IGF-1, somatomedin C) was significantly downregulated in AD compared to control brain microvessels in this study. The IGF-1 data were confirmed by real-time PCR (Table 4).

Pathway analysis of immune/inflammatory genes differentially expressed in AD microvessels

'Find significant pathway' function within GeneSpringGX was used to identify significantly ($p < 0.05$) altered gene networks in AD brain microvessels. Pathway analysis, a graphical representation of interactions among gene products in a biological system, is shown in Fig. 3. Most genes significantly dysregulated in AD overlap among immune system, defense, chemotaxis, and inflammatory response networks. Thirteen genes differentially expressed in AD microvessels have direct network interactions with each other (Fig. 3). For example, both interleukin 8 receptor A (IL8RA) and IL 8 receptor B (IL8RB) are upregulated in AD microvessels compared to levels in microvessels from control brains. GeneSpring analysis showed that arachidonate 5-lipoxygenase (ALOX5), also elevated in AD, can modify glutamate alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor subunit 1 (GRIA1), which in turn interacts with IL8RB. IL8RB also regulated the activity of CCR1 by protein modification. IL8RA regulates the expression of CCR5 and chemokine (C-X-C motif) ligand 4 (CXCR4). Taken together, pathway analysis showed the complex interplay and regulation of immune/inflammatory genes differentially expressed in AD microvessels (Fig. 3).

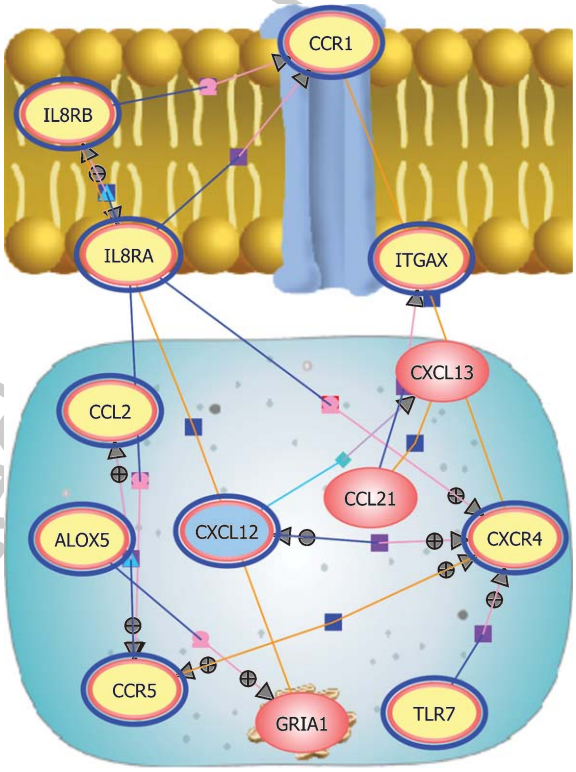

Fig. 3. Regulatory network of the defense response genes differentially expressed in AD brain microvessel. Network is graphically represented by nodes (genes/proteins) and internodes (relationship between proteins) and placement of each gene in the cellular view represents localization in the cell. The color of gene represents regulation in AD as compared to control, red color represents upregulation and blue color represents downregulation while pink color represents not differentially expressed. The genes encircled with blue hallow are inflammatory response genes. Internodes are marked with different color squares describing the nature of relationship between nodes, purple (regulation), red (expression), blue (binding), pink (protein modification) and turquoise (metabolism). The inter node endings are marked with (+) or (−) signs indicating positive or negative interaction between nodes, respectively.

DISCUSSION

Unraveling the mechanisms underlying impaired brain function in AD has been difficult, likely due to the complexity of the networks that drive disease pathology. As a consequence, the enigma that is AD continues to present daunting challenges for effective therapeutic intervention. The lack of disease-modifying therapies may, in part, be attributable to the narrow research focus employed to understand this complex disease. Most studies into disease pathogenesis are based on *a priori* assumptions about the role of AD lesion-associated proteins, in particular Aβ [19, 20]. However, the complex disease processes at work may not be amenable to single-target therapeutic approaches.

Genome-wide expression studies, which are not influenced by deductive assumptions, provide an unbiased approach for investigating the pathogenesis of complex diseases like AD [27–34]. This approach allows comprehensive overviews of the interplay among genes within the context of relevant gene networks and pathways. Transcriptome analyses have been performed using transgenic animal models of AD [23, 35, 36]. A recent study using the AD 11 transgenic mouse, a model which expresses nerve growth factor neutralizing antibody, finds differential expression of mRNAs related to inflammation and immune response, neurotrophic response, synaptic neurotransmission, and signaling pathways [23]. Although data from animal studies are useful, genome-wide expression studies using human postmortem brain tissue are necessary because the direct relevance of transgenic mouse models to human AD remains unclear. Use of RNA isolated from the whole brain to identify transcriptomic changes is problematic because it is derived from heterogenous cell populations. Recently, laser capture microscopy of specific cells populations, such as astrocytes, has documented dysregulated genes in AD that are associated with cell signaling, the cytoskeleton, and cell junctions [37]. Based on our previous work [26, 38–45] and a growing literature suggesting a role for cerebrovascular contributions to the pathogenesis of AD [46–50], we examined isolated brain microvessels from AD and control brains using microarray analysis. We document that more than 2,000 genes are differentially altered in AD microvessels and that a large number of these genes map to pathways associated with the immune and inflammatory response, signal transduction, and nervous system development and function.

Chronic inflammation is associated with a broad spectrum of neurodegenerative diseases of aging including AD [51]. Numerous studies show the presence of markers of inflammation in the AD brain [52–59]. Elevated cytokines and chemokines as well as the accumulation of activated microglia are found in or near the pathologic lesions of AD [54, 58]. A consistent pattern of change in the AD brain transcriptome associated with neuroinflammation has been documented. A microarray study using cortical extracts from AD and control patients shows that among the gene categories significantly upregulated in AD samples are inflammatory/immune response genes [22]. Similarly, a microarray study using an animal model of AD, finds inflammatory and immune response genes are differentially expressed [23]. The results presented herein agree with these published studies and reveal that expression of several chemokines, cytokines, and their receptors is upregulated in microvessels isolated from AD brains compared to controls. However, in contrast to published studies that employ cortical homogenates and likely reflect the contributions of neurons as well as non-neuronal cells including astrocytes and endothelial cells, the current study is the first to document differential expression of inflammatory/immune response genes in the cerebromicrovasculature in AD.

In AD, there is a robust elevation in inflammatory mediators in the cerebral microcirculation. AD brain endothelial cells express high levels of inflammatory adhesion molecules such as intercellular adhesion molecule-1 and release significantly higher levels of a number of inflammatory factors including nitric oxide, thrombin, tumor necrosis factor-α, transforming growth factor-β, interleukin (IL) IL-1β, IL-6, IL-8, and matrix metalloproteinases [18, 38, 42, 60].

In this study, microarray analysis reveals that expression of several chemokines, cytokines, and their receptors is upregulated in microvessels isolated from AD brains compared to controls. Increased expression of CCR5, CCR1, and CCL2 are detected. Changes in expression of CCR5 and CCL2 are also validated using real-time PCR. CCR5 has been shown to be associated with an increased inflammatory response and decreased cognitive decline [61, 62]. CCR5−/− mice are protected against Aβ-induced impairment of learning and memory functions [62]. CCR1 is expressed specifically in Aβ plaques and it is undetectable in normal or control brains [63]. CCL2, also known as MCP-1, is upregulated in the AD brain. These data are consistent with our previously published study which documents increased expression of MCP-1 protein in AD-derived microvessels compared to controls [39]. It has also been shown that exposure of cultured human brain endothelial cells to $A\beta_{1\text{-}40}$ causes increased

expression of MCP-1 [64]. The cerebral microvasculature may drive destructive events in the AD brain where inflammation precedes Aβ deposition and Aβ in turn further promotes release of inflammatory mediators [43].

Most of the genes differentially regulated in AD microvessels overlap in immune system, defense, chemotaxis, and inflammatory response pathways. Both IL8RA and IL8RB are upregulated in AD microvessels compared to control-derived vessels. IL8RB can regulate the activity of CCR1 via receptor phosphorylation [65]. IL8RA regulates the expression of CCR5 and CXCR4 [66]. Upregulation of IL8RA, IL8RB, CCR5, and CXCR4 is found associated with AD pathological changes [67]. Increased ALOX5 immunoreactivity has also been observed in AD brains [68]. ALOX5 catalyzes the conversion of arachidonic acid to proinflammatory leukotrienes [69]. ALOX5 protein levels are positively associated with the formation of Aβ plaques and neurofibrillary tangles [68, 70]. Here we show upregulation of ALOX5 in AD microvessels. GeneSpring analysis indicates that ALOX5 can modify GRIA1, which in turn can bind and interact with IL8RB. More studies are required to determine the functional interactions in the ALOX5-GRIA1-IL8RB pathway. Taken together, these data support the idea that the cerebral microcirculation is an active participant in the neuroinflammatory processes ongoing in the AD brain.

Another network/pathway which contains a large number of genes differentially expressed in AD mircrovessels is signal transduction. Dysregulation of intracellular signaling cascade has been widely implicated in the pathogenesis of AD [71]. Both upregulation and downregulation of Ras/Rap signaling and their downstream mitogen-activated protein kinases (ERK, JNK, and p38MAPK) are linked to AD progression [72]. Here we document MAP kinase interacting serine/threonine kinase 2 (MKNK2) and mitogen-activated protein kinase kinase kinase 8 (MAP3K8) are significantly upregulated in AD compared to control brain microvessels. The current data are congruent with our previous report showing an increase in the phosphorylated forms of p38MAPK and ERK in AD microvessels compared to levels in control-derived brain microvessels [45]. The results of the current study are also in agreement with published microarray studies, in both human and animal models, that document significant alterations in the expression of genes related to signal transduction pathways in AD [22, 23].

In contrast to the MAPKinases, RAB3B, a member of the RAS oncogene family, was significantly downregulated in AD compared to control brain microvessels. RAB3 is thought to be involved in presynaptic vesicle trafficking and to contribute to learning and memory processes [73, 74]. Interestingly, the GTP-binding protein family which includes Rab3 affects formation of Weibel-Palade bodies, vesicular structures found in endothelial cells [75]. In addition to its role in signal transduction, RAB3B expression is also relevant for the functional category, Nervous System Development and Function. In this regard, a large number of genes differentially expressed in AD microvessels map to the Nervous System Development and Function category. Other studies have indicated consistent patterns of changes in the AD transcriptome that are associated with synaptic dysfunction and perturbed neurotransmission [22]. Overall, the expression of significantly regulated genes involved in nervous system development and function is decreased in AD microvessels compared to expression levels in control brain microvessels.

Synapse-related genes including growth associated protein 43 (GAP43) and synaptotagmin V (SYT5) are significantly downregulated in AD compared to control brain microvessels. SYT5 is a presynaptic vesicle protein and GAP43 is a synaptic membrane protein. Increased loss of both of them has been found in AD compared to control brains, and is associated with cognitive impairments in AD [76]. Synapse loss and dysfunction precede the formation of plaques and neurofibrillary tangles [77]. The regulation and signaling of glutamate receptors may also be involved in AD progression. Some categories related to ionotropic glutamate receptors are significantly downregulated in AD brain microvessels compared to controls. The ionotropic glutamate receptor channels are involved in long-term potentiation and play an important role in memory and learning through increasing efficiency of synaptic transmission [78]. The channels are heteromers composed of glutamate receptor, ionotropic, N-methyl D-aspartate 2A (GRIN2A), GRIN2B, GRIN2C, and GRIN2D. In this study, GRIN2A and GRIN2B are significantly downregulated in AD compared to control brain microvessels.

The presence of a large number of "neuronal"-associated genes in the microvessel preparation could reflect the presence of neuronal contamination. However, our experience with this procedure suggests that while astrocytic end feet may be a minor contaminant, neuronal contamination (based on lack of neuronal markers) is not significant. The presence of "neuronal"-associated proteins in the current study

highlights important functional connections between the vascular and nervous systems. In this regard, the presence of "neuronal" proteins such as glutamate, NMDA receptors, and synapse-related proteins in brain microvessels has been documented [79–81]. Also, a growing literature documents that, in the brain, the vessels and nerves share common signals and pathways and are actively engaged in neurovascular cross-talk [82–84].

The cerebral microvasculature is important for neuronal viability and survival. Brain endothelial cells are an important source of growth factors with neuroprotective properties including IGF-1, brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) [82]. Indeed, studies comparing brain-derived endothelial cells to systemic-derived endothelial cells demonstrate that expression of neurotrophic factors is largely a feature of brain-derived not peripheral endothelial cells [85]. IGF-1 is a potent neurotrophic factor that is known to decline with age [86]. In the current study, IGF-1 is significantly downregulated in AD microvessels compared to control brain microvessels. IGF-1 is thought to mediate exercise-induced production of growth factors such as BDNF, enhanced neurogenesis, and improved cognition [87]. IGF-1 may regulate Aβ levels by increased clearance via the choroid plexus. Thus, decreased vascular expression in AD may contribute to AD pathology via both effects on neuronal viability and Aβ levels.

In this study, GO analysis and pathway analysis algorithms within GeneSpringGX are used to understand the regulatory networks of genes differentially expressed in AD microvessels. These data, by focusing on interrelated gene networks instead of individual genes, could provide insight into the complex changes that occur in the vasculature in AD. However, there are caveats to bear in mind in interpreting and extrapolating these data. First, it is unclear whether changes in the AD transcriptome contributes to brain dysfunction or results from ongoing disease pathology. Also, in the current study we examine gene expression in end-stage AD tissue; essentially providing a snap-shot of genetic abnormalities that are present at that disease stage. Recent work suggests that disease stage affects gene expression. Simpson et al. [37] compare astrocytes based on Braak stages and show that dysregulation of genes associated with actin cytoskeleton, proliferation, and apoptosis occurs at low Braak stages whereas altered signaling pathways, including MAPK, are associated with high levels of AD pathology. The current results also show differential gene expression in signal transduction in end-stage AD; suggesting that the evolution of genetic changes may be similar in astrocytes and endothelial cells.

A common limitation in all studies that employ human material is that many variables, including patient medical history and medication usage, are hard to control. Samples are standardized and matched for age, gender, and postmortem time; but other variables remain unknown. Finally, mRNA analysis underestimates the contribution of post-transcriptional modifications and thus provides only a partial view of the molecular changes in the AD brain. Combining mRNA studies with protein expression analysis may provide a more global picture of the biological processes associated with AD dementia.

Development of new therapeutic approaches to AD depends on new thinking about brain function and dysfunction. The intimate relationship between blood vessels and nerves which use similar signals to differentiate, grow, and navigate toward their targets as well as common genetic pathways should be explored and exploited [82]. Endothelial cells influence neurogenesis [82–84]. Similarities in patterning and proximity between vascular and nervous system reflects coordinated development based on responsiveness to similar growth factors [88]. The interactions between the two systems involving common growth factors suggest these two systems have evolved in an interconnected way. An understanding of vascular gene network abnormalities in AD could highlight candidates for further investigation relevant for neurons as well as other cell types in the AD brain.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health (AG15964, AG020569 and AG028367). Human Tissue was provided by the Garrison Institute on Aging Brain Bank at Texas Tech University Health Sciences Center. Dr. Grammas is the recipient of the Shirley and Mildred Garrison Chair in Aging. The authors gratefully acknowledge the secretarial assistance of Terri Stahl.

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=1252).

REFERENCES

[1] Alzheimer's Association (2012) Alzheimer's facts and figures, http://www.alz.org/alzheimers_disease_facts_and_figures.asp.

[2] Ruitenberg A, den Heijer T, Bakker SL, van Swieten JC, Koudstaal PJ, Hofman A, Breteler MM (2005) Cerebral hypoperfusion and clinical onset of dementia: The Rotterdam Study. *Ann Neurol* **57**, 789-794.

[3] Strittmatter JW, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* **90**, 1977-1981.

[4] Hofman A, Ott A, Breteler MM, Bots ML, Slooter AJ, van Harskamp F, van Duijn CN, Van Broeckhoven C, Grobbee DE (1997) Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* **349**, 151-154.

[5] Miller JW (1999) Homocysteine and Alzheimer's disease. *Nutr Rev* **57**, 126-129.

[6] Shi J, Perry G, Smith MA, Friedland RP (2000) Vascular abnormalities: The insidious pathogenesis of Alzheimer's disease. *Neurobiol Aging* **21**, 357-361.

[7] Sparks DL, Martin TA, Gross DR, Hunsaker JC III (2000) Link between heart disease, cholesterol and Alzheimer's disease: A review. *Microsc Res Tech* **50**, 287-290.

[8] Marx J (2001) Alzheimer's disease. Bad for the heart, bad for the mind? Science **294**, 508-509.

[9] Rhodin JA, Thomas T (2001) A vascular connection to Alzheimer's disease. *Microcirculation* **8**, 207-220.

[10] de la Torre JC (2002) Alzheimer disease as a vascular disorder: Nosological evidence. *Stroke* **3**, 1152-1162.

[11] Pansari K, Gupta A, Thomas P (2002) Alzheimer's disease and vascular factors: Facts and theories. *Int J Clin Pract* **56**, 197-203.

[12] Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW, Wolff PA (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* **346**, 476-483.

[13] Miyakawa T, Shimoj IA, Kuramoto R, Higuchi Y (1982) The relationship between senile plaques and cerebral blood vessels in Alzheimer's disease and senile dementia. Morphological mechanism of senile plaque production. *Virchows Arch B Cell Pathol Incl Mol Pathol* **40**, 121-129.

[14] Miyakawa T (1997) Electron microscopy of amyloid fibrils and microvessels. *Ann NY Acad Sci* **826**, 25-34.

[15] Fischer VW, Siddiqi A, Yusufaly Y (1990) Altered angioarchitecture in selected areas of brains with Alzheimer's disease. *Acta Neuropathol* **79**, 672-679.

[16] Buée L, Hof PR, Bouras C, Delacourte A, Perl DP, Morrison JH, Fillit HM (1994) Pathological alterations of the cerebral microvasculature in Alzheimer's disease and related dementing disorders. *Acta Neuropathol* **87**, 469-480.

[17] Marchesi VT (2011) Alzheimer's dementia begins as a disease of small blood vessels, damaged by oxidative-induced inflammation and dysregulated amyloid metabolism: Implications for early detection and therapy. *FASEB J* **25**, 5-13.

[18] Grammas P (2010) A damaged microcirculation contributes to neuronal cell death in Alzheimer's disease. *Neurobiol Aging* **21**, 199-205.

[19] Pimplikar SW (2009) Reassessing the amyloid cascade hypothesis of Alzheimer's disease. *Int J Biochem Cell Biol* **41**, 1261-1268.

[20] Karran E, Mercken M, DeStrooper B (2011) The amyloid cascade hypothesis for Alzheimer's disease: An appraisal for the development of therapeutics. *Nat Rev Drug Discov* **10**, 698-712.

[21] Blalock EM, Chen KC, Stromberg AJ, Norris CM, Kadish I, Kraner SD, Porter NM, Landfield PW (2005) Harnessing the power of gene microarrays for the study of brain aging and Alzheimer's disease: Statistical reliability and functional correlation. *Ageing Res Rev* **4**, 481-512.

[22] Tan MG, Chua WT, Esiri MM, Smith AD, Vinters HV, Lai MK (2010) Genome wide profiling of altered gene expression in the neocortex of Alzheimer's disease. *J Neurosci Res* **88**, 1157-1169.

[23] Arisi I, D'Onofrio M, Brandi R, Felsani A, Capsoni S, Drovandi G, Felici G, Weitschek E, Bertolazzi P, Cattaneo A (2011) Gene expression biomarkers in the brain of a mouse model for Alzheimer's disease: Mining of microarray data by Logic classification and feature selection. *J Alzheimers Dis* **24**, 721-738.

[24] Ball MJ, Griffin-Brooks S, MacGregor JA, Ojalvo-Rose E, Fewster PH (1998) Neuropathological definition of Alzheimer disease: Multivariate analyses in the morphometric distinction between Alzheimer dementia and normal aging. *Alzheimer Dis Assoc Disord* **2**, 29-37.

[25] Khachaturian ZS (1985) Diagnosis of Alzheimer's disease. *Arch Neurol* **42**, 1097-1105.

[26] Grammas P, Moore P, Weigel PH (1999) Microvessels from Alzheimer's disease brains kill neurons *in vitro*. *Am J Pathol* **154**, 337-342.

[27] Ginsberg SD, Hemby SE, Lee VM, Eberwine JH, Trojanowski JH (2000) Expression profile of transcripts in Alzheimer's disease tangle-bearing CA1 neurons. *Ann Neurol* **48**, 77-87.

[28] Loring JF, Wen X, Lee JM, Seihamer J, Somogyi R (2001) A gene expression profile of Alzheimer's disease. *DNA Cell Biol* **20**, 683-695.

[29] Pasinetti GM (2001) Use of cDNA microarray in the search for molecular markers involved in the onset of Alzheimer's disease dementia. *J Neurosci Res* **65**, 471-476.

[30] Colangelo V, Schurr J, Ball MJ, Pelaez RP, Bazan NG, Lukiw WJ (2002) Gene expression profiling of 12633 genes in Alzheimer hipppocampal CA1: Transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. *J Neurosci Res* **70**, 462-473.

[31] Yao PJ, Zhu M, Pyun EI, Brooks AI, Therianos S, Meyers VE, Coleman PD (2003) Defects in expression of genes related to synaptic vesicle trafficking in frontal cortex of Alzheimer's disease. *Neurobiol Dis* **12**, 97-109.

[32] Blalock EM, Geddes JW, Chen KC, Porter NM, Markesbery WR, Landfield PW (2004) Incipient Alzheimer's disease: Microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc Natl Acad Sci U S A* **101**, 2173-2178.

[33] Liang WS, Dunckley T, Beach TG, Grover A, Mastroeni D, Ramsey K, Caselli RJ, Kukull WA, McKeel D, Morris JC, Hulette CM, Schmechel D, Reiman EM, Rogers J, Stephan DA (2008) Altered neuronal gene expression in brain regions differentially affected by Alzheimer's disease: A reference data set. *Physiol Genomics* **33**, 240-256.

[34] Blalock EM, Buechel HM, Popovic J, Geddes JW, Landfield PW (2011) Microarray analyses of laser-captured hippocampus reveal distinct gray and white matter signature associated with incipient Alzheimer's disease. *J Chem Neuroanat* **42**, 118-126.

[35] George AJ, Gordon L, Beissbarth T, Koukoulas I, Holsinger RM, Perreau V, Cappai R, Tan SS, Masters CL, Scott HS, Li QX (2010) A serial analysis of gene expression profile of Alzheimer's disease Tg2576 mouse model. *Neurotox Res* **17**, 360-379.

[36] Tseveleki V, Rubio R, Vamvakas SS, White J, Taoufik E, Petit E, Quackenbush J, Probert L (2010) Comparative gene

expression analysis in mouse models for multiple sclerosis, Alzheimer's disease and stroke for identifying commonly regulated disease-specific gene changes. *Genomics* **96**, 82-91.

[37] Simpson JE, Ince PG, Shaw PJ, Heath PR, Raman R, Garwood CJ, Gelsthorpe C, Baxter L, Forster G, Matthews FE, Brayne C, Wharton SB (2011) MRC Cognitive Function and Ageing Neuropathology Study Group, Microarray analysis of the astrocyte transcriptome in the aging brain: Relationship to Alzheimer's pathology and *APOE* genotype. *Neurobiol Aging* **32**, 1795-1807.

[38] Dorheim MA, Tracey WR, Pollock JS, Grammas P (1994) Nitric oxide is elevated in Alzheimer's brain microvessels. *Biochem Biophys Res Comm* **205**, 659-665.

[39] Grammas P, Ovase R (2001) Inflammatory factors are elevated in brain microvessels in Alzheimer's disease. *Neurobiol Aging* **22**, 837-842.

[40] Grammas P, Ovase R (2002) Cerebrovascular TGF-β contributes to inflammation in the Alzheimer's brain. *Am J Pathol* **160**, 1583-1587.

[41] Grammas P, Samany PG, Thirumangalakudi L (2006) Thrombin and inflammatory proteins are elevated in Alzheimer's disease microvessels: Implications for disease pathogenesis. *J Alzheimers Dis* **9**, 51-58.

[42] Thirumangalakudi L, Samany PG, Owoso A, Wiskar B, Grammas P (2006) Angiogenic proteins are expressed by brain blood vessels in Alzheimer's disease. *J Alzheimers Dis* **10**, 111-118.

[43] Grammas P (2011) Neurovascular dysfunction, inflammation and endothelial activation: Implications for the pathogenesis of Alzheimer's disease. *J Neuroinflammation* **8**, 26.

[44] Grammas P, Martinez J, Miller B (2011) Cerebral microvascular endothelium and the pathogenesis of neurodegenerative diseases. *Expert Rev Mol Med* **13**, e19.

[45] Grammas P, Sanchez A, Tripathy D, Luo E, Martinez J (2011) Vascular signaling abnormalities in Alzheimer disease. *Cleve Clin J Med* **78**, S50-S53.

[46] Iadecola C (2004) Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci* **5**, 347-360.

[47] Zlokovic BV (2005) Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci* **28**, 202-208.

[48] Bell RD, Zlokovic BV (2009) Neurovascular mechanisms and blood–brain barrier disorder in Alzheimer's disease. *Acta Neuropathol* **118**, 103-113.

[49] Altman R, Rutledge JC (2010) The vascular contribution to Alzheimer's disease. *Clin Sci (Lond)* **119**, 407-421.

[50] de la Torre JC (2010) The vascular hypothesis of Alzheimer's disease: Bench to bedside and beyond. *Neurodegener Dis* **7**, 116-121.

[51] Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH (2010) Mechanisms underlying inflammation in neurodegeneration. *Cell* **140**, 918-934.

[52] Cooper NR, Bradt BM, O'Barr S, Yu JX (2000) Focal inflammation in the brain: Role in Alzheimer's disease? *Immunol Res* **21**, 159-165.

[53] Neuroinflammation Working Group (2000) Inflammation and Alzheimer's disease. *Neurobiol Aging* **21**, 383-421.

[54] Tarkowski E (2002) Cytokines in dementia. *Curr Drug Targets Inflamm Allergy* **1**, 193-200.

[55] McGeer EG, McGeer PL (2003) Inflammatory processes in Alzheimer's disease. *Prog Neuropsycholpharmacol Biol Psychiatry* **27**, 741-749.

[56] McGeer EG, McGeer PL (2004) Inflammation and the degenerative diseases of aging. *Ann NY Acad Sci* **1035**, 104-116.

[57] Ho GJ, Drego R, Hakimian E, Masliah E (2005) Mechanisms of cell signaling and inflammation in Alzheimer's disease. *Curr Drug Targets Inflamm Allergy* **4**, 247-256.

[58] Wyss-Coray T (2006) Inflammation in Alzheimer disease: Driving force, bystander or beneficial response? *Nat Med* **12**, 1005-1015.

[59] Lee YJ, Han SB, Nam SY, Oh KW, Hong JT (2010) Inflammation and Alzheimer's disease. *Arch Pharm Res* **33**, 1539-155.

[60] Yin X, Wright J, Wall T, Grammas P (2010) Brain endothelial cells synthesize neurotoxic thrombin in Alzheimer's disease. *Am J Pathol* **176**, 1600-1606.

[61] Balistreri CR, Caruso C, Grimaldi MP, Listì F, Vasto S, Orlando V, Campagna AM, Lio D, Candore G (2007) CCR5 receptor: Biologic and gentic implications in age-related diseases. *Ann N Y Acad Sci* **1100**, 162-172.

[62] Passos GF, Figueiredo CP, Prediger RD, Pandolfo P, Duarte FS, Medeiros R, Calixto JB (2009) Role of macrophage inflammatory protein-1alpha/CC chemokine receptor 5 signaling pathway in the neuroinflammatory response and cognitive deficits induced by beta-amyloid peptide. *Am J Pathol* **175**, 1586-1597.

[63] Savarin-Vuaillat C, Ransohoff RM (2007) Chemokines and chemokine receptors in neurological disease: Raise, retain, or reduce? *Neurotherapeutics* **4**, 590-601.

[64] Vukic V, Callaghan D, Walker D, Lue LF, Liu QY, Couraud PO, Romero IA, Weksler B, Stanimirovic DB, Zhang W (2009) Expression of inflammatory genes induced by beta-amyloid peptides in human brain endothelial cells and in Alzheimer's brain is mediated by the JNK-AP1 signaling pathway. *Neurobiol Dis* **34**, 95-106.

[65] Richardson RM, Pridgen BC, Haribabu B, Snyderman R (2000) Regulation of the human chemokine CCR1. Cross-regulation by CXCR1 and CXCR2. *J Biol Chem* **275**, 9201-9208.

[66] Davidson B, Dong HP, Holth A, Berner A, Risberg B (2007) Chemokine receptors are infrequently expressed in malignant and benign mesothelial cells. *Am J Clin Pathol* **127**, 752-759.

[67] Xia MQ, Hyman BT (1999) Chemokines/chemokine receptors in the central nervous system and Alzheimer's disease. *J Neurovirol* **5**, 32-41.

[68] Ikonomovic MD, Abrahamson EE, Uz T, Manev H, Dekosky ST (2008) Increased 5-lipoxygenase immunoreactivity in the hippocampous of patients with Alzheimer's disease. *J Histochem Cytochem* **56**, 1065-1073.

[69] Firuzi O, Zhuo J, Chinnici CM, Wisniewski T, Pratico D (2008) 5-Lipoxygenase gene disruption reduces amyloid-beta pathology in mouse model of Alzheimer's disease. *FASEB J* **22**, 1169-1178.

[70] Chu J, Pratico D (2011) 5-Lipoxygenase as an endogenous modulator of amyloid beta formation *in vivo*. *Ann Neurol* **69**, 34-46.

[71] Crews L, Masliah E (2010) Molecular mechanisms of neurodegeneration in Alzheimer's disease. *Hum Mol Genet* **19**, R12-R20.

[72] Stornetta RL, Zhu JJ (2011) Ras and rap signaling in synaptic plasticity and mental disorders. *Neuroscientist* **17**, 54-78.

[73] Schlüter OM, Basu J, Südhof TC, Rosenmund C (2006) Rab3 superprimes synaptic vesicles for release: Implications for short-term synaptic plasticity. *J Neurosci* **26**, 1239-1246.

[74] Tsetsenis T, Younts TJ, Chiu CQ, Kaeser PS, Castillo PE, Südhof TC (2011) Rab3 protein is required for long term-depression of hippocampal inhibitory synapses for normal reversal learning. *Proc Natl Acad Sci U S A* **108**, 14300-14305.

[75] Knop M, Aareskjold E, Bode G, Gerke V (2004) Rab3D and annexin A2 play a role in regulated secretion of vWF, but not tPA, from endothelial cells. *EMBO J* **23**, 2982-2992.

[76] Reddy PH, Mani G, Park BS, Jacques J, Murdoch G, Whetsell W Jr, Kaye J, Manczak M (2005) Differential loss of synaptic proteins in Alzheimers disease: Implications for synaptic dysfunction. *J Alzheimers Dis* **7**, 103-117.

[77] Proctor DT, Coulson EJ, Dodd PR (2011) Post-synaptic scaffolding protein interactions with glutamate receptors in synaptic dysfunction and Alzheimers disease. *Prog Neurobiol* **93**, 509-521.

[78] Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg PH (1992) Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science* **256**, 1217-1221.

[79] Solhonne B, Gros C, Pollard H, Schwartz JC (1987) Major localization of aminopeptidase M in rat brain microvessels. *Neuroscience* **22**, 225-232.

[80] Parfenova H, Fedinec A, Leffler CW (2003) Ionotropic glutamate receptors in cerebral microvascular endothelium are functionally linked to heme oxygenase. *J Cereb Blood Flow Metab* **23**, 190-197.

[81] St'astný F, Lisý V, Mares V, Lisá V, Balcar VJ, Santamaría A (2004) Quinolinic acid induces NMDA receptor-mediated lipid peroxidation in rat brain microvessels. *Redox Rep* **9**, 229-233.

[82] Carmeliet P (2003) Blood vessels and nerves: Common signals, pathways and diseases. *Nat Rev Genet* **4**, 710-720.

[83] Arese M, Serini G, Bussolino F (2011) Nervous vascular parallels: Axon guidance and beyond. *Int J Dev Biol* **55**, 439-445.

[84] Quaegebeur A, Lange C, Carmeliet P (2011) The neurovascular link in health and disease: Molecular mechanisms and therapeutic implications. *Neuron* **71**, 406-424.

[85] Kallmann BA, Wagner S, Hummel V, Buttmann M, Bayas A, Tonn JC, Rieckmann P (2002) Characteristic gene expression profile of primary human cerebral endothelial cells. *FASEB J* **16**, 589-591.

[86] Apel PJ, Ma J, Callahan M, Northam CN, Alton TB, Sontag WE, Li Z (2010) Effect of locally delivered IGF-1 on nerve regeneration during aging: An experimental study in rats. *Muscle Nerve* **41**, 335-341.

[87] Foster PP, Rosenblatt KP, Kuljiš RO (2011) Exercise-induced cognitive plasticity, implications for mild cognitive impairment and Alzheimer's disease. *Front Neurol* **2**, 28.

[88] Ward NL, LaManna JC (2004) The neurovascular unit and its growth factors: Coordinated response in the vascular and nervous systems. *Neurol Res* **26**, 870-883.