Published in final edited form as:

Physiol Genomics. 2008 April 22; 33(2): 240–256. doi:10.1152/physiolgenomics.00242.2007.

Altered neuronal gene expression in brain regions differentially affected by Alzheimer's disease: a reference data set

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Abstract

Alzheimer's Disease (AD) is the most widespread form of dementia during the later stages of life. If improved therapeutics are not developed, the prevalence of AD will drastically increase in the coming years as the world's population ages. By identifying differences in neuronal gene expression profiles between healthy elderly persons and individuals diagnosed with AD, we may be able to better understand the molecular mechanisms that drive AD pathogenesis, including the formation of amyloid plaques and neurofibrillary tangles. In this study, we expression profiled histopathologically normal cortical neurons collected with laser capture microdissection (LCM) from six anatomically and functionally discrete postmortem brain regions in 34 AD-afflicted individuals, using Affymetrix Human Genome U133 Plus 2.0 microarrays. These regions include the entorhinal cortex, hippocampus, middle temporal gyrus, posterior cingulate cortex, superior frontal gyrus, and primary visual cortex. This study is predicated on previous parallel research on the postmortem brains of the same six regions in 14 healthy elderly individuals, for which LCM neurons were similarly processed for expression analysis. We identified significant regional differential expression in AD brains compared with control brains including expression changes of genes previously implicated in AD pathogenesis, particularly with regards to tangle and plaque formation. Pinpointing the expression of factors that may play a role in AD pathogenesis provides a foundation for future identification of new targets for improved AD therapeutics. We provide this carefully phenotyped, laser capture microdissected intraindividual brain region expression data set to the community as a public resource.

Keywords

expression profiling; neuron; Affymetrix microarrays; laser capture microdissection

Alzheimer's Disease (AD) is currently the most common cause of cognitive impairment in the elderly. Today, it is estimated that ~5.1 million Americans have AD, and, based on the demographics of aging, this number is expected to grow each year. Furthermore, additional estimates project that by 2050 11.3 to 16 million individuals will be affected by this devastating disease (48). Developing proper treatment that can delay AD onset by even 5 years may halve the number of affected individuals after 50 years (15).

At a cellular level, AD is characterized by several histopathological markers including extracellular β -amyloid plaques, neurofibrillary tangles (NFTs) within neurons (136), and the loss of synaptic connections manifested as brain atrophy. Because these markers can only be evaluated in the postmortem brain, many physicians have turned to clinical analyses and neuroimaging to diagnose "probable AD" (101,108). Available imaging techniques include positron emission tomography (PET), which measures the cerebral glucose metabolic rate in the brain to identify patterns of reduced glucose use characteristic of AD (31,94,109,120–122), as well as magnetic resonance imaging (MRI), which allows for identification of brain atrophy correlated with AD (49,67,94,101,122).

Ongoing research continues to provide clues into AD pathogenesis, but it is necessary to also obtain a global view of neuronal processes to establish a complete picture of AD. Powerful techniques that may be used to address this issue are laser capture microdissection (LCM) and gene expression profiling analysis. LCM allows us to select specific cells of interest for analysis in order to maintain a high level of tissue homogeneity. Selected cells can then be utilized for gene expression profiling, which allows the simultaneous monitoring of the steady-state expression of all characterized human genes on oligonucleotide arrays so that we can visualize the entire cellular environment across separate samples. Previous work has also shown that this methodology of using LCM and expression profiling is a powerful approach for identifying dysregulation of gene expression in tangle-bearing and non-tangle-bearing neurons in neurologically healthy and AD-affected brains (35).

To understand the gene dysregulation driving the development and pathogenesis of AD, the baseline gene expression across six different regions of postmortem brains of neurologically normal elderly individuals has already been defined, so a comparative reference to identify changes in gene expression is currently available (74). Areas were selected on the basis of previous research that showed that different areas of the brain display characteristic differences in AD-affected individuals, and also to provide overarching coverage of the different functional zones of the human brain. For this study, we focused our analysis on healthy non-tangle-bearing neurons to support a direct comparison with healthy non-tangle-bearing neurons from neurologically healthy elderly individuals of the control cohort. This focus will be useful for gaining insight into molecular and functional mechanisms that may play a role in AD pathogenesis or that may be enacting neuroprotective pathways to prevent toxicity in healthy neurons.

MATERIALS AND METHODS

Tissue collection

Brain samples were collected at three Alzheimer's Disease Centers (Washington University, Duke University, and Sun Health Research Institute) from clinically classified late-onset AD-afflicted individuals (15 men and 18 women) with a mean age at death of 79.9 ± 6.9 yr.

Individuals were matched as closely as possible for mean age of death, mean educational level, and proportion of persons with underrepresented racial backgrounds. Subjects in this group had a Braak stage ranging from III to VI (14) with a Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuritic plaque density of moderate or frequent (86). Neuropathological data and available Mini Mental State Examination (MMSE) data for each individual are listed in the supplemental material in the online version of this article.¹ Samples were collected [mean postmortem interval (PMI) of 2.5 h] from six brain regions that are either histopathologically or metabolically relevant to AD: entorhinal cortex [EC; Brodmann area (BA) 28 and 34], superior frontal gyrus (SFG; BA 10 and 11), hippocampus (HIP), primary visual cortex (VCX; BA 17), middle temporal gyrus (MTG; BA 21 and 37), and posterior cingulate cortex (PC; BA 23 and 31). After dissection, samples were frozen, sectioned (8 μ m), and fixed on glass slides.

Brain sections were stained with a combination of Thioflavin-S (Sigma, Dallas, TX) and 1% neutral red (Fisher Scientific, Chicago, IL), and pyramidal neurons were identified by their characteristic size, shape, and location within the region of interest, while tangles were identified by the bright green fluorescence of Thioflavin-S staining. In EC, the large stellate neurons lacking Thioflavin-S staining were collected from layer II and pyramidal cells lacking Thioflavin-S staining were collected from CA1 of HIP. The CA1 region was selected for study because this area is the earliest and most heavily affected region in HIP in terms of tangle formation and this region has already been expression profiled in neurologically healthy elderly individuals. In all other regions, cortical layer III pyramidal neurons lacking Thioflavin-S staining were collected (for all collected neurons, cell bodies were extracted). Layer III pyramidal neurons and layer II stellate neurons in EC were selected because these layer III neurons are selectively vulnerable to tangle formation and layer II stellate neurons are among the first neurons to be affected by tangle formation (layer V pyramidal neurons are also vulnerable but are less numerous and more difficult for array analysis). Furthermore, layer III pyramidal neurons are the major corticocortical projection neurons and are thus responsible for much of the communication between cortical regions and therefore involved in higher mental functions. For each individual, approximately 500 histopathologically normal pyramidal neurons were collected from EC, HIP, MTG, PC, SFG, and VCX with LCM and the Arcturus Veritas Automated Laser Capture Microdissection System (Mountain View, CA). Cells were collected onto Arcturus CapSure Macro LCM Caps and extracted according to the manufacturer's protocol. Total RNA was isolated from the cell lysate with the Arcturus PicoPure RNA Isolation Kit with DNase I treatment using Qiagen's RNase-free DNase Set (Valencia, CA). The same classes of neurons from each of the six regions were similarly collected from healthy elderly control subjects (74).

Expression profiling

Expression profiling was performed as previously described (74). Isolated total RNA from each sample of ~500 neurons was double-round amplified, cleaned, and biotin labeled with Affymetrix's GeneChip Two-Cycle Target Labeling kit (Santa Clara, CA) with a T7 promoter and Ambion's MEGAscript T7 High Yield Transcription kit (Austin, TX) per the manufacturer's protocol. Amplified and labeled cRNA was quantitated on a spectrophotometer and run on a 1% Tris-acetate-EDTA (TAE) gel to check for an evenly distributed range of transcript sizes. Twenty micrograms of cRNA was fragmented to ~35–200 bp by alkaline treatment (200 mM Tris-acetate, pH 8.2, 500 mM KOAc, 150 mM MgOAc) and run on a 1% TAE gel to verify fragmentation. Separate hybridization cocktails were made with 15 μ g of fragmented cRNA from each sample per Affymetrix's protocol.

¹The online version of this article contains supplemental material.

Microarray analysis

Two hundred microliters of each cocktail was separately hybridized to an Affymetrix Human Genome U133 Plus 2.0 Array for 16 h at 45°C in a Hybridization Oven 640. The Affymetrix Human Genome Arrays measure the expression of >47,000 transcripts and variants, including 38,500 characterized human genes. Hybridization cocktails for nine EC samples (normal neurons from AD brains) previously collected following the same profiling methodology used in this project (35) were reanalyzed on the Affymetrix Human Genome U133 Plus 2.0 Array, and a tenth EC sample was also separately processed for this sample group. Arrays were washed on Affymetrix's upgraded GeneChip Fluidics Station 450 with a primary streptavidin phycoerythrin (SAPE) stain, subsequent biotinylated antibody stain, and secondary SAPE stain. Arrays were scanned on Affymetrix's GeneChip Scanner 3000 7G with AutoLoader. Scanned images obtained by Affymetrix GeneChip Operating Software (GCOS) v1.2 were used to extract raw signal intensity values per probe set on the array and calculate detection calls (absent, marginal, or present). Assignment of detection calls was based on probe pair intensities for which one probe was a perfect match of the reference sequence and the other was a mismatch probe for which the thirteenth base (of the 25 oligonucleotide reference sequence) was changed. All raw chip data were scaled in GCOS to 150 to normalize signal intensities for interarray comparisons. Reports generated by GCOS were reviewed for quality control: we looked for at least 20% present calls, a maximum 3'-to-5' GAPDH ratio of 30, and a scaling factor under 10. Twenty arrays that failed to pass these standards were not included in further analyses.

Pyramidal cell quality control

To ensure neuronal cell purity in the samples, expression of glial fibrillary acidic protein (GFAP), an astrocyte cell marker, was evaluated. Samples that had GFAP expression >1 SD from the mean were removed from statistical analyses.

Statistical analysis

Data for samples from neurologically healthy elderly controls were generated in a previous study (74). Microarray data files of the normal samples are available on the Gene Expression Omnibus (GEO) site at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5281> (project accession no. GSE5281). Overall regional analyses consisted of 13 control subjects and 10 AD cases for EC, 13 control subjects and 10 AD cases for HIP, 12 control subjects and 16 AD cases for MTG, 13 control subjects and 9 AD cases for PC, 11 control subjects and 23 AD cases for SFG, and 12 control subjects and 19 AD cases for VCX.

Direct comparisons between brains of neurologically healthy and AD-afflicted brains were performed in all brain regions to analyze expression differences. For each analysis, genes that did not demonstrate at least ~10% present calls for each region-specific comparison were removed with Genespring GX 7.3 Expression Analysis software (Agilent Technologies, Palo Alto, CA). A two-tailed unpaired *t*-test, assuming unequal variances [with multiple testing corrections using the Benjamini and Hochberg false discovery rate (FDR)], was applied to each comparison in Excel to locate genes that were statistically significant in differentiating expression between the two regions of healthy and AD brains: for each analysis genes that had a maximum *P* value of 0.01 were collected, and those genes whose average AD signal and average control signal were both below a threshold of 150 were removed. On the basis of this analysis, 4,030 genes fell through from the EC analysis, 5,315 genes from the HIP analysis, 7,687 from the MTG analysis, 4,886 from the PC analysis, 1,178 from the SFG analysis, and 2,058 from the VCX analysis. Because of the size of these gene lists and in order to evaluate the relevance of expression changes, statistically significant factors (*P* < 0.01, corrected) that have been previously implicated or suggested as having a role in AD were considered (see Fig. 7). Genes from each of the regional lists were also input into GeneGo for pathway analysis.

To consider regional susceptibilities to pathologies, EC and HIP genes were compared, MTG and PC genes were compared, and SFG and VCX genes were compared. Overlapping genes were analyzed by using the MetaCore pathway analysis software v4.0.0 (GeneGo, Encinitas, CA) to identify top processes in each comparison. All processes for these comparisons are posted in the supplemental material in the online version of this article.

From these regional lists, to generate heat maps approximately the top 40 genes that showed the highest fold changes between AD and normal brains were identified. Fold change values were calculated by calculating the ratio between the average scaled expression signal (for all samples) for a gene from the AD sample region and the average scaled expression signal for the same gene from the normal samples. For EC, a minimum (increased or decreased) fold change of 9.5, for HIP a 5.8-fold change, for MTG an 8.2-fold change, for PC a 5.8-fold change, for SFG a 5.0-fold change, and for VCX a 2.4-fold change was applied. Heat maps for each brain region were created with GeneCluster v2.0, with no gene or sample clustering applied (111).

RT-PCR validation of neuron-specific candidate genes

Total RNA was isolated from cortical gray matter from unprofiled MTG (control subjects $n = 9$, AD cases $n = 6$) and PC (control subjects $n = 8$, AD cases $n = 8$) frozen tissue with the RNeasy Mini kit (GE 25-0500-71) using the manufacturer's protocol, modified by increasing the initial volume of buffer RA1 to 500 μ l to prevent subsequent column blockage. RNA quality was assessed on an Agilent 2100 Bioanalyzer with RNA Nano chips (Agilent 5067-1511). RIN numbers of 6.5 (range 6.5–8.9) and above were considered sufficient for this analysis. cDNA was generated with the Superscript First Strand Synthesis kit (Invitrogen 12371-019) using 1 μ g of total RNA in a 40- μ l reaction. Quantitative RT-PCR (qRT-PCR) was performed with Taqman primer/probe sets (Applied Biosystems) to amplify the following neuron-specific gene transcripts: Synaptophysin (SYP, Hs00300531_m1), Synaptoporin (SYNPR, Hs00376149_m1), MAP2 (Hs00258900_m1), MAP1B (Hs00195485_m1), M1 Cholinergic Receptor (CHRM1, Hs00265195_m1), and AMPA1 Receptor (GRIA1, Hs00181348_m1), with β -Gluconuridase (GUSB, 4333767F) as a normalization control. GUSB was selected for two reasons: it was the only available control gene transcript that did not show significant expression changes between AD and control in the microarray analysis and has also been successfully employed for this purpose previously (4,64). qRT-PCR reactions were performed in 30- μ l reactions with Taqman Gene Expression Master Mix (Applied Biosystems 4369016) according to the manufacturer's protocol on a Bio-Rad iCycler IQ qPCR system. Threshold values were calculated with the maximum curvature approach. Threshold cycle (C_t) values were used to calculate fold changes with the $2^{-\Delta\Delta C_t}$ method (77). Significance of observed changes was assessed with the Student t -test.

Data posting

MIAME-compliant microarray data files are located on the GEO site (project accession no. GSE5281). Fold change and P value data for each of the six regions are available in the supplemental material in the online version of this article. Posted lists show region-specific P values and fold changes and also expression signals for genes that have at least ~10% present calls across regional samples with a maximum P value of 0.01 with multiple testing corrections applied (no fold change thresholds have been applied on these lists).

RESULTS AND DISCUSSION

Brain regions evaluated in this study were selected based on global functional coverage of the human brain and region-specific susceptibilities to AD pathogenesis. These six regions included EC, HIP, MTG, PC, SFG, and VCX. Functional areas are represented with regions

from the limbic (HIP), paralimbic (EC and PC), heteromodal (SFG and some portions of MTG), unimodal (MTG), and primary sensory (VCX) zones (87). Each of the six regions has also been shown previously to display characteristic pathological and metabolic differences in the brains of individuals afflicted with AD. EC and HIP are areas that have been found to be susceptible to early NFT formation (8,10,13,29,33,41,43,53), MTG and PC have been found to show metabolic changes relative to AD brains (6,57,89,90,121) and elevated susceptibilities to amyloid plaque formation (14,91,131), SFG has been found to show metabolic changes relative to normal aging (2,24,56,78,92), and VCX has been found to be relatively spared from any form of age-related or disease-related neurodegeneration (88). Analysis based on these common regional susceptibilities is described in MATERIALS AND METHODS, and analysis results are provided in the supplemental material. Because of the large size of statistically significant genes demonstrating differential expression in each of the regional analyses, we focused on discussion of factors that have been previously implicated as having a role in AD, particularly with regards to characteristic tangle and plaque pathologies. While this analytical approach focuses in on a subset of genes, the entire expression data set has been made available to scientists to serve as a resource for future research.

Region-specific comparisons and analysis

To pinpoint differences in expression between the same brain regions from the two groups of study, AD data and normal data were individually compared for each area [the same classes of neurons in control brains were collected previously (74)]. Such comparisons clearly demonstrate regional expression differences manifested in the AD brain. Statistically significant genes ($P < 0.01$, corrected for multiple testing) with the greatest fold changes are shown in Fig. 1 through Fig. 6. Those statistically significant genes that were identified when VCX was compared between AD-affected brains and healthy control brains showed significantly smaller fold changes compared with other profiled regions. Because VCX shows the least amount of AD-related changes and is relatively spared from AD pathologies, we expect to see few expression differences relative to healthy VCX, and this is borne out in the data.

To evaluate what processes may be affected by expression changes, pathway analysis was performed on statistically significant genes ($P < 0.01$, corrected) from each regional analysis. The top five processes for each region are listed in Table 1 (all processes are listed in the supplemental material). In general, potentially affected mechanisms across all regions include cellular physiological processes, transport, metabolism, and cellular localization. In SFG, a region demonstrating metabolic deficits with regard to aging, a number of unique processes fell through—these processes include biopolymer/protein modification, regulation of neurotransmitter secretion, and regulation of phosphorylation. Similar to other regions, top processes in VCX also include cell physiological process, cellular localization, and cellular transport; this overlap suggests that these processes may be key in determining whether a region is neuroprotected and is able to stave off disease pathologies.

NFT formation in the AD brain

One of the major pathological markers of AD is the presence of intracellular NFTs in the postmortem brain. In the earlier stages of AD NFTs, which are primarily comprised of hyperphosphorylated tau organized into paired helical fragments (PHFs), are found in EC and HIP (Braak stages I–IV), but with the progression of AD NFT distribution spreads to the association neocortex and primary cortical areas (Braak stages V and VI) (14). Because nontangle-bearing cortical neurons were evaluated, differential gene expressions identified in AD-affected brains may provide clues about events that precede NFT formation or potential neuroprotective pathways enacted to inhibit such aggregations. A summary of the results is

shown in Fig. 7, which provides an overview of statistically significant genes ($P < 0.01$) that show altered expression and may have roles in NFT pathways.

Under normal healthy conditions, tau proteins bind and stabilize microtubules, which serve major functions in cell division and intracellular transport and also provide structural intracellular support. Aberrant hyperphosphorylation of tau leads to the dissociation of tau from microtubules to aggregate into NFTs and results in subsequent breakdown of microtubules. Statistically significant differential expression was identified for numerous proteins involved in the NFT formation pathway. First and foremost, altered expression was found for microtubule-associated protein tau (MAPT): MAPT showed decreased expression in EC, HIP, MTG, and PC. Such changes in MAPT expression suggest a change in neuronal demand for tau; decreased expression may indicate cellular attempts to inhibit formation of NFTs by lowering availability of tau. Across these same regions, predominantly decreased expression was also identified for isoforms of the alpha and beta tubulin proteins (TUBA1, TUBA2, TUBA3, TUBA6, TUBB, TUBB2, TUBB3, and TUBB4, TUBB6), which normally associate into heterodimers to serve as the building blocks of microtubules. Again, such downregulated differential expression may insinuate lowered neuronal demand for tubulin heterodimers, potentially due to their increased availability resulting from microtubule breakdown. These findings may demonstrate neuronal responses to potential toxicity or events that lie upstream of eventual NFT formation.

NFT formation in the AD brain: chaperones and kinases

Studies on mechanisms that may control tau aggregation have pinpointed a chaperone system that appears to aid in ubiquitination and subsequent degradation of aberrant tau (106,119). This system is composed of heat shock protein (Hsp)70 and carboxy terminus of heat shock cognate (Hsc)70-interacting protein (CHIP), a cochaperone that inhibits Hsc70 activity (3) and acts as a ubiquitin E3 ligase to target proteins for degradation in the ubiquitin/proteasomal pathway (95,96,119). Findings have varied with regards to CHIP's role in NFT formation, but studies show that CHIP may play a role in reducing formation of tau aggregates (106,113). Furthermore, molecular chaperones such as Hsp70 can stabilize tau to support its binding to microtubules (32) and reduce levels of insoluble tau (106). In this study, we identified statistically significant downregulated expression of STUB1/CHIP in EC, HIP, and PC as well as downregulated expression of different Hsp70 proteins (HSC70, HSPA1A, HSPA2, HSPA4, HSPA5, HSPA8, HSPA9B, HSPA12A, HSPA12B, and HSPA14) in the same regions. In a recent animal study using tau^{P301L} transgenic mice, Karsten et al. (59) also identified puromycinsensitive aminopeptidase (PSA/Npepps) as a neuroprotective factor against tau-related degeneration that acts potentially by cleaving tau. In HIP, MTG, and PC, statistically significant decreased expression of NPEPPS was found compared with an upregulation of this gene in the transgenic mice from the Karsten et al. study. Because these changes were found in non-tangle-bearing neurons of AD brains, this finding, along with those changes identified for the chaperone system, may suggest a weakening of a system that may help regulate tau aggregation.

In EC, HIP, MTG, and PC, statistically significant downregulated expression was also identified for cyclin-dependent kinase 5 (CDK5); Cdk5 is normally regulated by p35, which can be truncated by calpain to form p25 in response to exposure to A β peptides (65,70). Different subunits of calpain (CAPN1, CAPN2, CAPN3, CAPNS1) showed altered expression in this study; upregulated and downregulated expression were identified in EC, HIP, MTG, and PC. In addition, elevated levels of p25 have been found in the brains of AD-affected individuals (103) and activation of cdk5 by p25 has been found to lead to abnormal hyperphosphorylation of tau (28,103,132). Furthermore, cdk5 phosphorylation of tau potentiates additional tau phosphorylation by glycogen synthetase kinase-3 β (GSK3 β) (117).

Interestingly, CDK5R1, which codes for p35, also showed upregulated expression in HIP. Because of cdk5's role in tau phosphorylation, studies have also shown that use of a cdk5-inhibitory peptide (CIP) directs a decrease in neuronal tau hyperphosphorylation and death (61,142,143). These findings, along with the identified decreased expression of CDK5 in multiple regions, may demonstrate the action of neuroprotective efforts enacted by healthy neurons to prevent downstream or further NFT formation.

In addition to CDK5, other genes coding for proteins that have been found to phosphorylate tau also showed statistically significant altered expression. These include cAMP-dependent protein kinase (PKA; Ref. 58), calcium/calmodulin-dependent protein kinase II (CaMKII; Ref. 138), and, as mentioned above, GSK3 β (39,104,105,140). Numerous genes coding for both regulatory and catalytic subunits of PKA displayed statistically significant increased and decreased expression in AD brains across all regions (these genes include PRKACA, PRKACB, PRKAR1A, PRKAR1B, PRKAR2A, and PRKAR2B). In addition to the action of cdk5, initial phosphorylation of tau by PKA also promotes downstream tau phosphorylation by GSK3 β to outline a potential pathway for NFT formation through tau hyperphosphorylation (75,76). Multiple genes coding for different CaMKII subunits also displayed statistically significant upregulated and downregulated expression across all regions (these genes include CAMK2A, CAMK2B, CAMK2D, and CAMK2G). CaMKII has also specifically been found to be associated with PHFs in AD brains (138). Although the altered expression identified in AD brains for both PKA and CaMKII shows expression changes in different directions, the statistical significance of the differences provides evidence of neuronal changes specific to the AD brain. Finally, decreased expression of GSK3 β was identified in HIP (which had an alternate probe demonstrating increased expression), MTG, and PC. Past research has identified increased levels of GSK3 β in the tissue of AD brains as well as its association with NFTs in AD brains (54,104,105,140). Again, GSK3 β activity has also been found to be associated with tau hyperphosphorylation (5,40,44) through potential regulation by cdk5 (107). Thus its decreased expression in this study's analysis suggests that the healthy profiled neurons may be diverting pathogenic NFT formation pathways at a local level.

NFT formation in the AD brain: regional susceptibilities

On the basis of increased susceptibilities to tangle formation in EC and HIP, genes from both regional analyses were also separately considered apart from the other regions profiled (a subset of Fig. 7 focusing on NFT formation with regard to tangle-susceptible regions is located in the supplemental material): 922 genes demonstrating statistically significant expression changes in both regional comparisons of AD cases vs. control subjects (EC and HIP) were identified. To pinpoint processes that may play a role in the pathological susceptibilities of these two regions, gene ontology analysis was performed. The top 10 processes from this analysis are shown in supplemental Fig. S1. Particularly interesting processes include synaptic transmission and synaptic vesicle transport, which suggest that there are dramatic synaptic changes in AD brains in EC and HIP and glycolysis because AD has been suggested to be related to energy metabolic deficits (31,94,109,120–122). For those genes that only demonstrated significant expression changes in both EC and HIP and did not demonstrate such changes in more pathologically spared regions (SFG and VCX), the top 10 processes specific to EC and HIP are also shown in supplemental Fig. S1. Also shown are SFG- and VCX-specific processes, which include mechanisms involved in cell migration, morphogenesis, organization, and biogenesis—these processes may have roles in neuroprotection due to the sparing of these regions in AD brains.

β -Amyloid plaque formation in the AD brain

A second histopathological hallmark of AD brains is the widespread distribution of extracellular β -amyloid plaques in the frontal, temporal, and, to a lesser extent, occipital

isocortexes as measured by CERAD staging (14,91,93). These neuritic plaques contain primarily insoluble 40- and 42-amino acid-long A β proteins, in addition to other proteins. Identification of altered expression of factors implicated as having a role in plaque formation (Fig. 7) may provide insight into neuronal responses to already existing plaques or generation of plaques.

β -Amyloid plaque formation in the AD brain: links to NFT pathology

Although the relationship between NFT and plaque pathologies has not been entirely elucidated, the roles played by cdk5 and GSK3 β may establish a putative connection (outlined in Fig. 7). As previously described, cdk5, which can phosphorylate tau, has also been found to be activated by A β in HIP neurons (1). Likewise, research has shown that GSK3 β may also be activated by A β peptides to result in tau phosphorylation (20,129). Regulation of GSK3 β activity is governed by phosphorylation of multiple sites on GSK3 β by enzymes that show statistically significant altered expression across different brain regions. The first phosphorylation site is serine 9, which can be phosphorylated by multiple kinases including protein kinase B (Akt) (27,118), protein kinase A (PKA) (38,71), protein kinase C (PKC) (46), p70 S6 kinase (128), and p90Rsk (128) (47) and which can be dephosphorylated by protein phosphatase 2A (PP2A) (128). Multiple forms of AKT, including AKT1, AKT2, and AKT3, the gamma form of AKT, showed differential expression across different regions. AKT1 was downregulated in EC but upregulated in VCX, AKT2 was modestly upregulated in PC, and AKT3 showed decreased expression in EC, MTG, PC, and VCX but also increased expression in HIP, SFG, and MTG (for additional AKT3 probe sets). In addition to those previously mentioned, other subunits of PKA (PRKAA1, PRKAB1, PRKAB2, PRKAG2) also showed statistically significant increased and decreased expression in all regions profiled. Different isoforms of PKC (PRKCA, PRKCB1, PRKCE, PRKCG, PRKCH, PRKCI, PRKCZ) also showed primarily downregulated expression changes across all regions except for SFG, while RPS6KA2 (p90Rsk) showed increased expression only in MTG. Finally, multiple subunits of PP2A as well as PP2A activators (PPP2CA, PPP2CB, PPP2R1A, PPP2R1B, PPP2R2A, PPP2R2B, PPP2R2C, PPP2R2D, PPP2R4, PPP2R5A, PPP2R5B, PPP2R5C, PPP2R5E) displayed both increased and decreased expression across all regions. A second phosphorylation site that also appears to regulate GSK3 β activity is tyrosine 216, which may be phosphorylated by several tyrosine kinases including Fyn (69), which was separately identified as a putative factor in effecting AD-related cognitive deficits (23), and Zak1 (62). Fyn showed significant increased expression in MTG and VCX but also decreased expression in PC. Significant increased expression was also found for Zak1 in EC and MTG. Additional upstream factors that may regulate kinase activity and resulting GSK3 β phosphorylation at serine 9 include apoptosis (133), oxidative stress (11,22,84), and signaling by Wnt (25), insulin (27), and epidermal growth factor (Egf) (36,114). A number of receptors that have roles in these signaling pathways also displayed statistically significant altered expression. Increased expression across EC, MTG, and VCX was identified for multiple “frizzled” genes (FZD4, FZD7, and FZD8), which encode receptors for Wnt proteins, while downregulated expression of FZD3 was identified in PC. Furthermore, numerous Wnt-encoding genes (WNT2B, WNT3, WNT6, and WNT10B) showed both upregulated and downregulated expression in EC, HIP, and MTG. Substantial increased expression in EC, MTG, SFG, and VCX was also identified for Egf receptor (EGFR). With regard to phosphorylation regulation by apoptotic pathways, a number of related factors also showed altered expression. Genes from the caspase (cysteine-aspartic acid protease) family also showed statistically significant increased expression: CASP3 showed a modest increase specific to PC, while CASP7 showed an increase specific to MTG and VCX.

GSK3 β activity appears to also be regulated by the phosphoinositide 3-kinase (PI3K)/Akt cell survival pathway. This regulation begins with activation of the PI3K/Akt pathway when PI3K

helps to promote phosphorylation of Akt at multiple sites (18). Phosphorylation of Akt activates it so that it can phosphorylate multiple targets including GSK3 β at its serine 9 residue to inactivate GSK3 β (27,60). Additional studies have found that inhibition of both PRKC (protein kinase C) and PI3K allows for GSK3 β activation and subsequent tau phosphorylation (72, 139). In this study, regulatory and catalytic subunits of PIK3 showed upregulated and downregulated expression across all regions profiled (PIK3C2A, PIK3C2B, PIK3CB, PIK3CD, PIK3R1, PIK3R2, PIK3R3, and PIK3R4). Such changes in expression suggest that there may be differential regulation of GSK3 β specific to different areas of the brain to potentially influence downstream NFT and plaque formation.

β -Amyloid plaque formation in the AD brain: amyloid precursor protein pathways

Well-established factors implicated in plaque generation that displayed significant altered expression in AD brains include amyloid precursor protein (APP), beta secretase 1 (BACE1), components of the gamma secretase complex including presenilin 1 (PSEN1), presenilin enhancer 2 homolog (*Caenorhabditis elegans*) (PEN2), transmembrane emp24-like trafficking protein 10 (yeast) (TMP21/TMED10), and anterior pharynx defective 1 homolog A (*C. elegans*) (APH1A). APP, which is processed by beta secretase and the gamma secretase complex to yield insoluble A β peptides, demonstrated marked increased expression in HIP, MTG, PC, and VCX. Interestingly, BACE1, which encodes a transmembrane aspartyl protease, displayed decreased expression across EC, HIP, and MTG, while its homolog BACE2 displayed significant increased expression in EC, MTG, PC, and VCX. Although BACE2 function is still unclear, studies show that BACE1 is correlated with increased processing of APP at the beta site and resulting A β generation, while BACE2 is not (127). However, BACE2 overexpression reduced A β production (127), so that its increased expression in this study may represent efforts to prevent further A β generation and plaque formation. PSEN1 showed mild increased expression in MTG and modest decreased expression in HIP, and in PC multiple PSEN1 probes displayed expression changes in both directions. Finally, PEN2 showed decreased expression in EC and PC, TMP21 showed both increased expression in HIP, MTG, and PC (which also had additional probes showing decreased expression), and APH1A displayed increased expression in HIP and MTG.

These factors all play a role in influencing the enzymatic activity of the gamma secretase complex. Mutations in PSEN1 have been confirmed to be associated with the early-onset, or familial, form of AD (along with PSEN2 and APP) and have been found to lead to generation of the 42-amino acid-long A β peptide to result in amyloid plaque formation (9,19,68,137). More importantly, the majority of familial AD cases are correlated with mutations in PSEN1 (123). Studies that have evaluated inhibitors against the active site of PSEN1 and that have found a decrease in the amount of insoluble A β peptides in response to PSEN1 inhibition provide evidence of the catalytic role of PSEN1 as a part of the gamma secretase complex (37,73,116). Furthermore, any deficiencies of PSEN1 inhibit APP processing and A β secretion from neurons (30,100). Because of the histopathological analysis of amyloid plaques in the postmortem brains of AD patients evaluated in this study, the modest PSEN1 expression increase that has been identified in MTG and PC correlates with APP processing and A β transport into the extracellular space for formation of amyloid plaques. PEN2 has also been found to be essential for normal functioning of the gamma secretase complex (42,124). Interestingly, PEN2, along with the APH1 and nicastrin proteins, was implicated for increasing levels of A β (85). Thus the decreased PEN2 expression found in this study may demonstrate neuroprotective efforts to hinder the growth of A β levels in late stages of the AD brain. However, APH1A showed statistically significant increased expression in HIP and MTG, parallel to an increase in PSEN1 expression in MTG. APH1A, also shown to be required for proper gamma secretase functioning (42), is also found to regulate and be regulated by PEN2, PSEN1, and nicastrin, another component of gamma secretases (66,80). Finally, the most

recently discovered element required for gamma secretase functioning is TMP21 (21), a protein that has normal functions in vesicle trafficking (7). Along with the identification of TMP21 as a component of the gamma secretase complex, the same study found that overexpression of TMP21 did not affect the level of gamma secretase components and also did not affect the generation of A β . However, suppression of TMP21 with short interfering RNA (siRNA) resulted in an increase in the generation of insoluble A β peptides (21). In light of this finding, the primarily upregulated expression of TMP21 seen in this study suggests that this alteration may not influence A β levels.

β -Amyloid plaque formation in the AD brain: additional factors

Additional potential amyloid plaque-related factors that displayed statistically significant altered expression in this study include apolipoprotein E (APOE), α 2-macroglobulin (A2M), lipoprotein-related receptor protein (LRP), Golgi-associated, gamma adaptin ear containing, ARF binding protein 1 (GGA1), protein (peptidylprolyl *cis/trans* isomerase) NIMA-interacting 1 (PIN1), sortilin-related receptor 1 (SORL1), and peroxiredoxin II (PRDX2). APOE, whose e4 allele is associated with late-onset AD (26,115,125,130) and whose protein has been found to localize to amyloid plaques (98), showed increased expression in MTG and decreased expression in PC. Normally, apoE acts as a cholesterol transporter by binding to low-density lipoprotein (LDL) receptors (16,17,82,83) to support lipoprotein degradation (81). One study (55) correlated apoE with reduced cleavage by gamma secretase to lower A β levels. In light of this finding, the increased expression identified in this study may represent efforts to reduce A β levels. A2M, a protease inhibitor, showed increased expression only in MTG, while different isoforms of LRP, an endocytosis receptor for which A2M is a ligand, showed increased expression in EC (LRP4), HIP (LRP3/12), MTG (LRP1B/4/6/10), PC (LRP3), SFG (LRP4), and VCX (LRP1/3) and also decreased expression in EC (LRP10/16) and SFG (LRP12). Past research has found that A2M binds the A β peptide (34,52) and also localizes to amyloid plaques along with LRP (110). Of particular interest is A2M's ability to bind and direct A β toward degradation via the clearance receptor activity of LRP (99), which also binds ApoE-containing lipoproteins (51) and endocytoses normally secreted APP (63). Thus simultaneous increase in expression of both A2M and LRP in MTG suggests that there may be increased activity utilizing a potential A β clearance mechanism in this region.

GGA1, a coat protein involved in mediating protein trafficking through the trans-Golgi network (TGN), displayed marked increased expression in PC. GGA1, which also appears to influence intracellular transport of BACE1 (134), may influence processing of APP through such protein trafficking (135). Furthermore, overexpression of GGA1 has been found to lead to decreased levels of BACE1-directed APP cleavage (135), so that the increased expression identified in this study may demonstrate neuronal efforts to inhibit A β generation.

PIN1, a peptidyl-prolyl isomerase recently found to bind tau at the phosphorylated threonine 231 residue and to play a role in AD pathogenesis (79), displayed statistically significant increased expression in PC along with decreased expression in EC and HIP to parallel a separate study reporting downregulated Pin1 expression in the AD-affected HIP (126). Pin1, which catalyzes transformation of serine/threonine-proline residues between *cis* and *trans* conformations, appears to influence APP processing possibly by binding to APP's threonine 668-proline motif and changing the conformation of the intracellular domain of APP (102). Because Pin1 knockouts exhibit elevated A β secretion (102), the decreased expression identified in this study may suggest that the global expression analysis evaluated in EC and HIP of the profiled AD brains may portray an already instigated pathogenic pathway that contributes to extracellular amyloid plaque formation but has not manifested in NFT formation.

SORL1 displayed widespread decreased expression in HIP, MTG, and PC. SORL1, which was recently identified for having a genetic association with AD (112), has been found to

demonstrate decreased levels in AD brains and limited correlative increases in A β generation (12,97,144). Interestingly, SORL1 appears to directly bind APP to mediate its sorting into recycling pathways (112). Thus its statistically significant downregulated expression identified in multiple regions of AD brains provides evidence of regional differences with regard to protein recycling, or sorting, in AD pathogenesis.

Primarily decreased expression ($P < 0.01$, corrected) of PRDX2 in EC, HIP, MTG, PC, and SFG was also found. Yao et al. (141) found increased levels of Prdx2 in AD affected brains with an A β and amyloid binding alcohol dehydrogenase (ABAD) transgenic mouse. Yao et al. suggest that this increase is neuroprotective from toxicity resulting from A β peptides. Because the postmortem brains evaluated in this study are all CERAD staged at moderate/frequent levels of plaques (to suggest elevated levels of A β in the brains), these findings show that the nontangle-bearing neurons profiled may have lost or are lacking protection from A β and may thus be at an earlier time point in neurodegeneration before tangle formation.

β -Amyloid plaque formation in the AD brain: regional susceptibilities

Because of increased susceptibilities to plaque formation in MTG and PC, genes from both regional analyses of AD cases vs. control subjects were considered (parallel to the EC/HIP analyses, a subset of Fig. 7 focusing on plaque formation with regard to plaque susceptible regions is also located in the supplemental material): 2,586 genes demonstrating statistically significant expression changes in both regional comparisons were found. To understand what pathways may play a role in the pathological susceptibilities of these two regions, gene ontology analysis was performed with GeneGo MetaCore. The top 10 processes from GeneGo MetaCore analysis are shown in supplemental Fig. S1. Processes include microtubule-based processes and movement, axon cargo transport, and transport, all of which may be potentially related to tangle formation, or specifically protection from this pathology. For those genes that only demonstrated significant expression changes in both MTG and PC and did not show such changes in more pathologically spared regions (SFG and VCX), the top 10 processes specific to MTG and PC are also shown in supplemental Fig. S1 (along with SFG- and VCX-specific processes).

Additional AD-relevant pathways

In evaluating the pathways surrounding protein aggregates that characterize AD, it is also important to consider the ubiquitin-proteasomal pathway, which serves as the major mechanism for disposal of proteins. The ubiquitin-proteasomal pathway involves the action of three enzymes, ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3), which covalently marks and directs a protein to the proteasome for degradation. Across all profiled regions, widespread altered expression was identified for numerous proteasomal subunits (including PSMA1–7; PSMB1–7, 10; PSMC1–6; PSMD1, 4, 6–14; PSME1,3, 4; PSMF1) as well as ubiquitin [ubiquitin B (UBB) and ubiquitin C (UBC)], an ubiquitin-activating enzyme (UBE1DC1), ubiquitin-conjugating enzymes (UBE2A, 2B, 2D1, 2D2, 2D3, 2E1, 2E2, 2G1, 2H, 2I, 2J1, 2L3, 2L6, 2N, 2Q, 2R2, 2V1, 2V2), ubiquitin ligases (UBE3A, 3B, 3C), and ubiquitin-specific peptidases (USP1, 2, 4, 6–12, 14, 16, 19, 22, 25, 30–34, 36–38, 40, 42, 46–48, 53, 54). Additional ubiquitin-related factors that displayed differential expression include ubiquilin 1 (UBQLN1) and ubiquitin hydrolase (UCHL1). UBQLN1, which showed decreased expression in MTG and PC, has been found to mediate APP trafficking as well as secretion of the A β peptide (50). UCHL1, which also showed significant decreased expression in HIP, MTG, PC, and SFG, appears to play a role in protection against damaging of synaptic functions as a result of A β toxicity (45).

Finally, because SFG and VCX are areas shown to be affected in later stages of the disease or are more pathologically spared, pathway analysis was also performed on genes that showed

statistically significant changes in both regional analyses (the top 10 processes are shown in supplemental Fig. S1). Because of the disease characteristics of SFG and VCX, the sparing of these regions may be associated with a number of the top processes including changes in cell organization, biogenesis, morphogenesis, and intracellular signaling cascades.

RT-PCR validation of selected genes

We performed RT-PCR on additional unprofiled fresh frozen brain sections (healthy elderly control subjects as well as pathologically confirmed AD cases) to validate gene expression changes. Genes for validation were selected based on established relevance to AD as well as demonstration of statistically significant expression changes in regions for which tissue was available. Expression of synaptophysin (SYP), synaptoporin (SYNPR), microtubule-associated protein 2 (MAP2), microtubule-associated protein 1B (MAP1B), M1 cholinergic receptor (CHRM1), and GRIA1 (AMPA1 receptor) was evaluated in MTG and PC [using GUSB (β -glucuronidase) as a normalization control]. Results from RT-PCR analysis are shown in Table 2 and Fig. 8.

Data from the MTG demonstrated downregulation of all six selected genes in AD brains to parallel the expression changes ($P < 0.05$, corrected) identified from microarray analysis. RT-PCR results also achieved significance for SYP ($P = 3.93\text{E-}02$), CHRM1 ($P = 5.47\text{E-}02$), GRIA1 ($P = 5.44\text{E-}02$), MAP2 ($P = 2.58\text{E-}02$), and MAP1B ($P = 2.94\text{E-}02$), while SYNPR demonstrated a trend toward significance ($P = 1.01\text{E-}01$). In PC, while significant downregulated expression changes ($P < 0.05$, corrected) were only identified with microarrays for SYNPR, CHRM1, GRIA1, and MAP1B, RT-PCR validation also demonstrated significant parallel changes for CHRM1 ($P = 3.73\text{E-}02$) and MAP2 ($P = 6.32\text{E-}02$). Furthermore, while RT-PCR showed nonsignificant changes for SYNPR, GRIA1, and MAP1B, these genes showed downregulated expression in the array data comparing AD brains to control brains. Overall, these RT-PCR data provide independent validation of expression changes identified through array analysis of six AD relevant genes in MTG and PC.

Summary

In this study, we present an expression profile reference detailing levels of steady-state expression of all human genes and transcripts in six discrete regions of the AD brain. Along with the identification of altered expression of factors previously implicated in AD pathogenesis including those coded by APOE, BACE1, STUB1 (CHIP), FYN, GGA1, and SORL1, we have also pinpointed statistically significant genes that demonstrate the greatest expression changes in each region and that have roles in pathways surrounding formation of the characteristic pathological markers of AD. Such differences indicate that there are unique regional activities in AD brains and demonstrate that neurodegenerative mechanisms and/or neuroprotective efforts may be enacted at different levels and through different avenues within each region. Furthermore, expression changes identified across the differentially susceptible regions may represent different time points during neurodegeneration. While future downstream functional studies will be needed to elucidate the roles of these factors, this expression reference will serve as an important resource for future research aimed at delineating AD pathogenesis and developing improved therapeutics to treat this devastating disease. Finally, we provide to the research community our expression data from carefully phenotyped, low-PMI, intraindividual regional neurons as a public resource.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Roger Higdon (National Alzheimer's Coordinating Center) for help in acquiring samples, Elizabeth Salomon (Translational Genomics) for help with GEO data posting, Lucia Sue (Sun Health Research Institute) for help with collecting neuropathological data, and Nick Lehman (Translational Genomics) for help with supplemental data posting. We also thank the National Institute on Aging's Alzheimer's Disease Centers program and the National Alzheimer's Coordinating Center for help in obtaining samples for analysis.

GRANTS

This project was funded by grants from the State of Arizona and the NIH Neuroscience Microarray Consortium, the National Institute on Aging (NIA) (Grants K01-AG-024079 to T. Dunckley, 1-RO1-AG-023193 to D. A. Stephan, AG-07367 to J. Rogers, P30-AG-19610 to E. M. Reiman, P50-AG-05681 to J. C. Morris, P01-AG-03991 to J. C. Morris, and AG-05128 for the Duke University Alzheimer's Disease Center), the National Alzheimer's Coordinating Center (U01AG016976), and the Arizona Alzheimer's Research Center (to E. M. Reiman) under a collaborative agreement from NIA.

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Fig. 1. Heat map of differentially expressed genes in the entorhinal cortex (EC). A heat map for EC was created to display those statistically significant ($P < 0.01$ with multiple testing corrections applied) genes with the greatest region-specific fold changes. Normalized expression signals are represented on a log scale for which colder colors correspond to lower levels of expression and warmer colors correspond to higher levels of expression. Heat maps were generated with Genecluster 2. AD, Alzheimer's disease.

Fig. 2.

Heat map of differentially expressed genes in the hippocampus (HIP). A heat map for HIP was created to display those statistically significant ($P < 0.01$ with multiple testing corrections applied) genes with the greatest region-specific fold changes.

Fig. 3. Heat map of differentially expressed genes in the middle temporal gyrus (MTG). A heat map for MTG was created to display those statistically significant ($P < 0.01$ with multiple testing corrections applied) genes with the greatest region-specific fold changes.

Fig. 4.
Heat map of differentially expressed genes in the posterior cingulate cortex (PC). A heat map for PC was created to display those statistically significant ($P < 0.01$ with multiple testing corrections applied) genes with the greatest region-specific fold changes.

Fig. 5.

Heat map of differentially expressed genes in the superior frontal gyrus (SFG). A heat map for SFG was created to display those statistically significant ($P < 0.01$ with multiple testing corrections applied) genes with the greatest region-specific fold changes.

Fig. 6.

Heat map of differentially expressed genes in the primary visual cortex (VCX). A heat map for VCX was created to display those statistically significant ($P < 0.01$ with multiple testing corrections applied) genes with the greatest region-specific fold changes.

Fig. 7.

AD pathogenesis: relationships in neurofibrillary tangle (NFT) and plaque formation. This figure shows a summary of the relationships of factors encoded by statistically significant genes ($\alpha = 0.01$, corrected) and which displayed altered expression across profiled regions. Factors shown were selected (through literature searches) based on previous research implicating/showing a role of respective factors in pathways surrounding formation of tangle and plaque pathologies. The statistically significant genes shown in this figure were only identified in EC, HIP, MTG, and PC analyses. Increased and decreased expression are identified with up and down arrows, respectively. Arrows with 2 heads indicate that multiple probes, isoforms, or subunits demonstrating different directions in expression changes were identified (separate fold changes for all factors are located in the supplemental material for this article). Each brain region is represented with a color code. For those factors that are encoded by multiple subunits (e.g., multiple genes), an average of the fold changes was calculated to evaluate the expression

change. UBE, ubiquitin factors (ubiquitin-activating and -conjugating enzymes, ubiquitin ligases); PSM, proteasomal subunits; PHF, paired helical fragment.

Fig. 8. RT-PCR validation of selected genes. RT-PCR was used to independently validate unprofiled control subjects and AD cases in MTG (control: $n = 9$, AD cases: $n = 6$) and PC (control: $n = 8$, AD cases: $n = 8$). Genes for validation were selected based on relevance to AD pathophysiology and demonstration of significant expression changes from array analysis. Normalized folds are shown on the y-axis, and genes are listed on the x-axis. Quantitative results are listed in Table 2.

Table 1

Top processes in each regional comparison

Brain Region	Process	No. of Genes from Input List	Total No. of Genes
EC	Cellular physiological process	1,459	6,652
EC	Transport	400	1,513
EC	Cellular process	1,615	7,676
EC	Transmission of nerve impulse	143	435
EC	Synaptic transmission	130	390
HIP	Cellular physiological process	1,838	6,652
HIP	Intracellular transport	240	531
HIP	Establishment of cellular localization	244	549
HIP	Cellular localization	244	558
HIP	Metabolism	1,314	4,511
MTG	Cellular physiological process	2,458	6,652
MTG	Cell organization and biogenesis	714	1,571
MTG	Intracellular transport	282	531
MTG	Establishment of cellular localization	289	549
MTG	Cellular localization	291	558
PC	Cellular physiological process	1,744	6,652
PC	Macromolecule metabolism	798	2,652
PC	Protein metabolism	604	1,909
PC	Intracellular transport	213	531
PC	Establishment of cellular localization	218	549
SFG	Biopolymer modification	97	1,007
SFG	Protein modification	93	966
SFG	Neurotransmitter secretion	17	85
SFG	Regulation of neurotransmitter levels	21	121
SFG	Regulation of phosphorylation	16	79
VCX	Cellular physiological process	815	6,652
VCX	Cell organization and biogenesis	246	1,571
VCX	Cellular localization	109	558
VCX	Establishment of cellular localization	107	549
VCX	Intracellular transport	104	531

The top 5 processes for each regional GeneGo analysis are listed. The number of genes from each regional analysis input into GeneGo that falls in the specific process is shown. The total number of genes in the specified process is also shown. EC, entorhinal cortex; HIP, hippocampus; MTG, middle temporal gyrus; PC, posterior cingulate cortex; SFG, superior frontal gyrus; VCX primary visual cortex.

Table 2

RT-PCR validation of selected genes

RT-PCR Data						
Normalized Fold				Array Data		
Probe Set ID	Gene Name	Control (n = 9)	AD (n = 6)	P Value	Fold	Corrected P Value
MTG						
213200_at	SYP	1.00	-2.33	3.93E-02	-4.43	1.99E-04
230303_at	SYNPR	1.00	-2.13	1.01E-01	-1.82	2.24E-02
231783_at	CHRM1	1.00	-2.27	5.47E-02	-3.90	9.03E-04
209793_at	GRIA1	1.00	-1.96	5.44E-02	-2.57	2.86E-06
210015_s_at	MAP2	1.00	-2.33	2.58E-02	-2.22	1.12E-02
212233_at	MAP1B	1.00	-2.13	2.94E-02	-4.27	2.21E-07
PC						
213200_at	SYP	1.00	-1.54	6.17E-02	1.26	1.33E+00
230303_at	SYNPR	1.00	-1.30	2.66E-01	-2.33	2.36E-02
231783_at	CHRM1	1.00	-1.61	3.73E-02	-2.67	7.90E-04
209793_at	GRIA1	1.00	-1.33	1.35E-01	-1.87	1.80E-05
225540_at	MAP2	1.00	-1.56	6.32E-02	-1.13	1.06E+00
226084_at	MAP1B	1.00	-1.22	4.32E-01	1.53	4.03E-02

Normalized fold changes for RT-PCR validation data in MTG and PC of unprofiled control subjects and AD cases are shown; n = 9 (control) and 6 (AD) for MTG and 8 (control) and 8 (AD) for PC. The respective microarray data are also shown for each gene.