Uncovering Molecular Biomarkers That Correlate Cognitive Decline with the Changes of Hippocampus’ Gene Expression Profiles in Alzheimer’s Disease

Martín Gómez Ravetti¹,²,³, Osvaldo A. Rosso¹,²,⁴, Regina Berretta¹,², Pablo Moscato¹,²,³

¹ Centre for Bioinformatics, Biomarker Discovery and Information-Based Medicine, The University of Newcastle, Callaghan, New South Wales, Australia, ² Hunter Medical Research Institute, Information Based Medicine Program, John Hunter Hospital, New Lambton Heights, New South Wales, Australia, ³ Australian Research Council Centre of Excellence in Bioinformatics, Callaghan, New South Wales, Australia, ⁴ Instituto de Cálculo, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina

Abstract

Background: Alzheimer’s disease (AD) is characterized by a neurodegenerative progression that alters cognition. On a phenotypical level, cognition is evaluated by means of the MiniMental State Examination (MMSE) and the post-mortem examination of Neurofibrillary Tangle count (NFT) helps to confirm an AD diagnostic. The MMSE evaluates different aspects of cognition including orientation, short-term memory (retention and recall), attention and language. As there is a normal cognitive decline with aging, and death is the final state on which NFT can be counted, the identification of brain gene expression biomarkers from these phenotypical measures has been elusive.

Methodology/Principal Findings: We have reanalysed a microarray dataset contributed in 2004 by Blalock et al. of 31 samples corresponding to hippocampus gene expression from 22 AD subjects of varying degree of severity and 9 controls. Instead of only relying on correlations of gene expression with the associated MMSE and NFT measures, and by using modern bioinformatics methods based on information theory and combinatorial optimization, we uncovered a 1,372-probe gene expression signature that presents a high-consensus with established markers of progression in AD. The signature reveals alterations in calcium, insulin, phosphatidylinositol and wnt-signalling. Among the most correlated gene probes with AD severity we found those linked to synaptic function, neurofilament bundle assembly and neuronal plasticity.

Conclusions/Significance: A transcription factors analysis of 1,372-probe signature reveals significant associations with the EGR/Krox family of proteins, MAZ, and E2F1. The gene homologous of EGR1, zif268, Egr-1 or Zenk, together with other members of the EGR family, are consolidating a key role in the neuronal plasticity in the brain. These results indicate a degree of commonality between putative genes involved in AD and prion-induced neurodegenerative processes that warrants further investigation.

Introduction

Gomez Ravetti and Moscato have recently shown that the abundance of five proteins, within a panel that also measured other 115 cytokines and growth factors, can be used to predict the development of clinical Alzheimer’s Disease (AD) [1]. The biomarker molecular signature is composed of IL-1α, TNF-α, IL-3, EGF and G-CSF and has the same level of specificity and sensitivity as the original 18-protein signature proposed by Ray et al. [2] in late 2007, who introduced this important dataset in the literature. In the original work, Ray et al. had employed the abundance of 120 signalling proteins in plasma to obtain their 18-protein signature set. They used a training set of 83 samples to identify patients that progressed to AD in two to six years. The proposed 5-protein signature has an average of 96% accuracy in predicting clinical AD but it is still linked to the joint measurement of 120 protein abundances.

In this paper, we are revisiting the quest of finding biomarkers of AD. However, this time we aim at finding biomarkers in hippocampus tissue samples which would complement the results of the previous studies on plasma biomarkers. This study will now give a different perspective on the progression of the disease, keeping a systems biology and functional genomics approach. Towards this end, we have chosen to rely on an informative experimental design and dataset contributed by Blalock et al. [3]. We believe that their dataset may help us to locate, either directly or indirectly, other biomarkers of interest that could eventually be detectable in plasma.

Blalock et al. analysed samples from 35 patients with four different levels of AD severity: control, incipient, moderate and...
severe; for this paper we used only 31 samples for which information is available online. The label assigned to each sample (its “level of severity”) was decided after considering two important scores, those provided by the MiniMental State Examination (MMSE) and the Neurofibrillary Tangle count (NFT). The MMSE score is based on a questionnaire that aims at measuring the level of cognitive impairment of a patient. The questions are aimed at evaluating different aspects of cognition, such as orientation, short-term memory (retention and recall), attention and language. A normal score can range from 24 to 30, mild cognitive impairment on the interval 20 to 23, moderate AD between 10 to 19, and the rest (from 0 to 9) are all considered severe AD cases.

As previously mentioned, Blalock et al. [3] also used the NFT score to assign a severity label to each sample. The NFT score is a well established method for the neuropathological diagnosis of AD [4]. The score is usually based on the average counts of neurofibrillary tangles considering different regions of the brain. A NFT score is a recognised indicator of AD, nevertheless, it is not completely effective as there is evidence that NFTs were also identified in healthy aging brains [5,6,7,8].

The analysis by Blalock et al. [3] focused on the identification of AD-related genes (ADG) and incipient ADG (IADG) using a methodology based on the correlation of the genes with NFT and MMSE scores. In turn, they identified putative biological processes and signalling pathways that are significantly present in those gene lists. Our analysis takes a different direction. While still based on the same dataset, we are attempting to map the progression of the disease, finding biomarkers linked to disease severity, by identifying the genes associated with the divergence of the gene expression profile of a sample with the gene expression average profile of the rest (from 0 to 9) are all considered severe AD cases.

Figure 1. This plot illustrates that the third step of our methodology, the use of the Jensen-Shannon divergence, does not appear to give an interesting separation of the samples in the absence of a previous feature selection step. For this graph, all 22,215 genes were considered in the calculation of the average profile of the samples in the “Control” and “Severe AD” classes. The square root of the Jensen-Shannon divergences to the “Control” and “Severe AD” average profile are computed, respectively giving, for each sample, its x and y coordinates in this plot. Observe that most of the “Control” samples have values lower than 0.12, with two exceptions. This result is expected, as the probability distribution function of the “Control” class was used. However, most of the samples from AD patients (having either “Incipient AD”, “Moderate” or “Severe” labels), show a divergence with the Control average expression profile. Figure 2 shows the important contribution provided by the feature selection step.

doi:10.1371/journal.pone.0010153.g001

Figure 2. This plot illustrates that after application of the feature selection steps, followed by the computation of the gene expression profile’s average profile of the samples in the “Control” and “Severe AD” classes (now on a set of 1,372 probes), the samples are now more clearly separated. Here, all “Control” samples have the square root of the Jensen-Shannon divergences to the average gene expression of the “Control” samples (x-coordinate) smaller than 0.12 (almost all severe AD have x-coordinates greater than 0.15). In addition to that, most samples labelled “Severe AD” are located on the same region. Both results are expected. However, it is interesting that in this (x,y)-plot most samples that are labelled “Incipient AD” and “Moderate AD” seem to “bridge” between the regions that have most of the “Control” samples and the region that have most of the “Severe AD” group. This result is interesting as no samples from “Incipient AD” nor “Moderate AD” have been used in the first three steps of our methodology. In essence, the work is a “test set” indicating that it is reasonable to expect that some genes in the genetic signature of 1,372 probes have information about a putative “progression” trend of the disease, from the “Control” to the “Severe AD” profile. In what follows, correlations across all the samples with these divergences are used as a method to try to identify those gene profiles that are most correlated with the progression from “Control” to “Severe AD”.

doi:10.1371/journal.pone.0010153.g002

Figure 3. This plot shows the MMSE scores as a function of the square root of the Jensen-Shannon divergences to the average gene expression of the “Control” samples. Incipient AD samples, although having a lower value for their MMSE score, still do not show a dramatic change in their x-coordinates compared to the ‘Control’ samples. ‘Moderate AD’ samples appear to be more scattered, with some of them already having a significant divergence from the ‘Control’ average profile.

doi:10.1371/journal.pone.0010153.g003
“Control” group. Analogously, we are interested in identifying the genes that seem to best correlate with the “convergence” to the average profile of the “AD Severe” group of samples. The difference between Blalock et al.’s [3] methodological approach to data analysis and ours is very important. We aim to uncover genes that correlate with the divergence of the gene expression profiles, instead of relying only on correlations with the NFT and MMSE values.

Our objective is to uncover genes which are highly correlated to the progression of the disease. With this objective in mind, we will concentrate the first part of our analysis on the two most extremely separated classes, the sets of samples that have been labelled as “Control” and those labelled “AD Severe”. This important initial decision was made based on the fact that the four classes are, in some sense, arbitrarily defined as specific thresholds for the MMSE and NFT scores that were decided ad hoc. Therefore, we decided to first focus on the transitional patterns that can be identified from a “normally aging” to an “AD-severe” gene expression profile in hippocampus. With this approach, we also avoid selecting genes that diverge from the normal-aged profile by causes other than AD, as we expect that the severity scale in AD has a higher probability of being correct in the “Severe AD” cases (since they have high values of NFT and low MMSE scores, clearly a joint combination highly appreciated as a disease hallmark). This approach has an additional advantage. Using this particular dataset and with focus on the effects of incorrect diagnoses, two publications indentify four possible misdiagnoses between control and incipient AD [9,10]. In our case, the samples that have been labelled either “Incipient AD” or “Moderate AD” play the role of a “Test set”, as they are not used to select probes for establishing a molecular signature, thus avoiding misdiagnoses problems.

Results

The results have been obtained using four steps in tandem: 1) abundance quantization of gene expression values and filtering of probes (this step is supervised by using the samples labelled either “Control” or “Severe AD”); 2) a feature selection algorithm to refine the probe selection based on numerical solution of a combinatorial optimization problem (the \((\alpha,\beta)\)-k-Feature Set methodology); 3) a correlation analysis (that requires the computation of Jensen-Shannon divergences). Finally, a fourth step involves the pathway and Gene Ontology analysis of the results.

The first two steps only used the samples labelled either “Control” or “Severe AD”. The third step requires several procedures and uses all of the samples. We first compute an

![Figure 4. Correlation of the expression profiles of 1,372 probes (across samples) with the sqrtJSD of the samples of two reference groups (“Control” and “Severe AD”, represented by the average expression profile in the group). The 50 probes in red are those most distant from the origin of this system of coordinates. Those probes have expression-value variations that are correlated with the divergences of the average “Control” profile and at the same time with the “Severe AD”.](https://doi.org/10.1371/journal.pone.0010153.g004)
### Table 1.
For each sample, we have calculated the sample’s Jensen-Shannon divergence with the average Control gene expression profile.

| Gene symbol | Probe | Spearman rank correlation |
|-------------|-------|---------------------------|
| CSF1        | 211839_s_at | 0.79388                  |
| MCL1        | 214057_at | 0.75484                   |
| PSMC3IP     | 205956_x_at | 0.74816                  |
| ZHX3        | 217367_s_at | 0.74416                  |
| C10orf76    | 55662_at | 0.74093                   |
| FCAR        | 211307_s_at | 0.72002                  |
| TUBD1       | 210389_x_at | 0.71835                  |
| AW974666    | 222365_at | 0.71835                   |
| LRP10       | 201412_at | 0.71079                   |
| SERTAD2     | 202656_s_at | 0.70679                  |
| ITGB5       | 201125_s_at | 0.7059                   |
| CDC2L6      | 212899_at | 0.70412                   |
| RNF19A      | 220483_s_at | 0.70367                  |
| TTN         | 208195_at | 0.70278                   |
| DHFR        | 202534_x_at | 0.69844                  |
| FYCO1       | 216204_s_at | 0.69655                  |
| HBEFG       | 38037_at | 0.69388                   |
| ZBTB20      | 205383_s_at | 0.69121                  |
| KCNK5       | 219615_s_at | 0.69121                  |
| KLHL20      | 204177_s_at | 0.68988                  |
| DLG5        | 201681_s_at | 0.68899                  |
| CHD2        | 203461_at | 0.68621                   |
| TUG1        | 222244_s_at | 0.68721                  |
| ZNF500      | 213641_at | 0.68454                   |
| NS5524      | 222332_at | 0.68276                   |
| KIR2DL5A    | 211410_s_at | 0.68165                  |
| CYBRD1      | 217889_s_at | 0.67964                  |
| DLG1        | 217208_s_at | 0.67831                  |
| IL15        | 205992_s_at | 0.67731                  |
| RND2        | 214393_s_at | 0.67508                  |
| TN51        | 221748_s_at | 0.67253                  |
| CTBP2       | 210835_s_at | 0.6703                   |
| AL050204    | 213929_s_at | 0.66852                  |
| YES1        | 202933_s_at | 0.66763                  |
| MYB1L       | 213906_s_at | 0.66719                  |
| G6PC         | 48048_s_at | 0.66363                   |
| FOXO1       | 202724_s_at | 0.66318                  |
| UPF1        | 211168_s_at | 0.66096                  |
| STAG3L1     | 221191_at | 0.66007                   |
| SLC12A7     | 210806_s_at | 0.65784                  |
| CYP3A44     | 205999_x_at | 0.65695                  |
| KRCC1       | 218303_x_at | 0.65562                  |
| PS3AP1      | 220402_at | 0.65462                   |
| TLE3        | 212769_at | 0.6535                    |
| ZNF669      | 220215_s_at | 0.65206                  |
| CFLAR        | 214486_x_at | 0.65206                  |
| PAK4        | 203154_s_at | 0.65028                  |

### Table 1. Cont.

| Gene symbol | Probe   | Spearman rank correlation |
|-------------|---------|---------------------------|
| M78162      | 217536_x_at | 0.6485                  |
| MPP11       | 203876_s_at | 0.6485                  |
| RGS7        | 206290_s_at | 0.67475                  |
| ASTN1       | 213197_at | 0.67653                   |
| TMSB10      | 217733_s_at | 0.67653                  |
| SUPT4H1     | 201484_at | 0.67731                   |
| COX6B1      | 201441_at | 0.67742                   |
| WASF1       | 204165_s_at | 0.67742                  |
| RALYL       | 213967_at | 0.67786                   |
| BB57        | 219688_at | 0.67875                   |
| SEC31A      | 200945_s_at | 0.68009                  |
| DDX1        | 201241_at | 0.68009                   |
| RP11-336K24.9 | 218291_at | 0.68098                  |
| GABBR2      | 209990_s_at | 0.68231                  |
| SLC25A12    | 203340_s_at | 0.68454                  |
| ATP5C1      | 205711_s_at | 0.68587                  |
| NEFL        | 221805_at | 0.68632                   |
| NDUFA8      | 201226_at | 0.68854                   |
| OPA1        | 212214_s_at | 0.69255                  |
| KPN2A       | 201088_at | 0.69522                   |
| PPIA        | 211765_s_at | 0.69566                  |
| CYP26B1     | 219825_s_at | 0.69566                  |
| COX7AP2     | 217249_s_at | 0.69878                  |
| VSNL1       | 203798_s_at | 0.69878                  |
| ATP6V1D     | 208898_at | 0.70145                   |
| ATP5C1      | 213366_s_at | 0.70234                  |
| NRXN1       | 209915_s_at | 0.7059                   |
| PCSK2       | 204870_s_at | 0.70901                  |
| AI708767    | 211978_s_at | 0.71034                  |
| UGCGL2      | 218801_at | 0.71257                   |
| KIAA0528    | 212943_at | 0.71392                   |
| SERPIN1     | 205352_s_at | 0.71657                  |
| LZT51       | 219042_at | 0.71835                   |
| NEFM        | 205113_s_at | 0.71835                  |
| FRY         | 204072_s_at | 0.71924                  |
| CSPG5       | 205344_at | 0.72291                   |
| COX6A1      | 200925_at | 0.72777                   |
| COX4I1      | 202698_s_at | 0.73037                  |
| KIAA0368    | 212428_at | 0.73126                   |
| MYT1L       | 210016_at | 0.73304                   |
| PP3CA       | 202457_s_at | 0.74194                  |
| LOC100131599 | 213222_at | 0.74549                  |
| CACNG3      | 206384_at | 0.75484                   |
| PPP3R1      | 204506_s_at | 0.75573                  |
| MAN1A1      | 221760_at | 0.75929                   |
| NETO2       | 218888_s_at | 0.76819                  |
| LPHN1       | 219145_at | 0.76852                   |
| CAPRIN2     | 218456_s_at | 0.76997                  |
| CAMK1G      | 215161_at | 0.77041                   |
| LDB2        | 206481_s_at | 0.7802                   |
average gene expression profile for the classes “Control” and “Severe AD”. This step is followed by the computation of the square root of the Jensen-Shannon divergence [11] of the gene expression profile of each sample with the average profiles of the classes “Control” and “Severe AD”. Finally, we perform a correlation analysis of each gene expression profile (now across all samples) with the results of the square root of the Jensen-Shannon divergence (we do it twice, one for the “Control” and the other for the “Severe AD” case). With this information, and using state-of-the-art pathway analysis and text mining tools, as a result of our final analysis step, we provide a comprehensive list of results of the differentially regulated genes, patterns of up (down)-regulation and the pathways that seem to be implicated in the progression of AD. We refer to the Methods section for a completely reproducible and in-depth explanation of our methodology.

Probe selection and Jensen-Shannon divergence computations based on class information

We start our analysis with a baseline comparison, which we have chosen to include for illustrative purposes. Figure 1 provides an example of the importance of performing an initial probe/gene selection step. The example serves as an argument for the necessity of the first two steps of our method. We have normalized each individual gene expression profile, and we have computed the average gene expression profile for classes “Control” and “Severe AD” (following the same procedure we will use in the third step of our method, but in this case using all probes in the array).

We have used the square root of the Jensen-Shannon divergence of a pair of samples (a pair of gene expression profiles) as our measure of “dissimilarity” between them. The square root of the Jensen-Shannon divergence quantifies the difference between two probability distribution functions (PDFs) and it is a metric (we refer the reader to the Methods section for a mathematical definition and a discussion of its properties). Figure 1 plots the divergence of each sample with the average expression profile of the classes ‘Control’ and ‘Severe AD’; \( \sqrt{\text{JS}(P, P_C)} \) denotes the square root of the Jensen-Shannon divergence between sample \( P \) and the average profile on the ‘Control’ class \( (P_C) \). Analogously, \( \sqrt{\text{JS}(P, P_S)} \) denotes the square root of the Jensen-Shannon divergence between sample \( P \) and the average profile on the ‘Severe AD’ class \( (P_S) \). The advantage of using the probe/gene selection steps, which reduces the number of genes to the most informative ones, will be evident when we later compare Figure 1 with Figure 2. However, Figure 1 already shows some interesting patterns. For instance, we can observe that a high percentage of the samples from AD patients (having either ‘Incipient AD’, ‘Moderate’ or ‘Severe’ labels) show \( \text{JS}(P, P_C) \) values greater than 0.115, which indicates measurable divergence with the Control average gene expression profile.

Figure 2 presents the same procedure, but only after the feature selection step has significantly reduced the number of probes from 22,213 to 1,372. We refer to the Methods section for details. In Figure 2, an arguably more coherent arrangement can be observed. As expected, the group of control samples (in green) have lower values of \( \sqrt{\text{JS}(P, P_C)} \) and higher values of \( \sqrt{\text{JS}(P, P_S)} \). Obviously, the opposite behaviour is observed for the samples belonging to the severe cases. What cannot be expected, however, is a layout of the samples that could provide evidence of a continuous “progression” of the disease. The Figure shows that the samples of ‘Incipient AD’ are close to the control group and the ‘Moderate AD’ samples are closer to them and also link to severe AD. A priori, since those samples had not been used for probe selection, they could have been in any position in the \( \sqrt{\text{JS}(P, P_C)}, \sqrt{\text{JS}(P, P_S)} \) plane.

Finally, Figure 3 presents the results of the MMSE score as a function of the \( \sqrt{\text{JS}(P, P_C)} \), showing an inverse correlation between them. A similar situation happens between MMSE and \( \sqrt{\text{JS}(P, P_S)} \), but in this case low MMSE scores correspond to low values of \( \sqrt{\text{JS}(P, P_P)} \), giving a positive correlation. It is this interplay between positive and negative correlations that has enabled us to find interesting biomarkers. In the next subsection, we explain how these correlations were used to identify probes that “diverge from” their values in the “Control” group and “converge to” the values in the “Severe AD” group.

Gene correlation analysis

The third step employs a correlation analysis to select the group of probes that are the most strongly correlated. Intuitively, the idea is fairly straightforward as illustrated in the following “Gedankenexperiment” [a thought experiment]. Assume, for argument’s sake, that the MMSE of each patient \( P \) is not actually phenotypical information assigned to each sample. Instead, assume that the MMSE values are the microarray probe expression of some gene. In this “thought experiment”, let \( \text{MMSE}(P) \) be the expression of this hypothetical gene probe on sample \( P \), and \( \text{fDataset} \) be the set of values it has for each sample. The correlation of the sample-ordered set of values \( \{\text{MMSE}(P)\} \) with the set of sample-ordered values \( \{\sqrt{\text{JS}(P, P_P)}\} \) is negative, indicating that, in general, this hypothetical MMSE probe reduces its values as the whole gene expression profile of sample \( P \) diverges from the average “Control” profile (Figure 3). Analogously, there exists a positive correlation of the set of values \( \{\text{MMSE}(P)\} \) with the values of the set \( \{\sqrt{\text{JS}(P, P_S)}\} \). This indicates that the values of MMSE tend to be reduced as the profile of sample \( P \) “converges to” the average profile of samples in the “Severe AD” group. We have computed these correlations for all probes in the signature, which are given in the supplementary material (File S2 sheet ‘correlation Analysis’) and are the basis for our analysis.

We also refer the reader to Figure 4, which presents the computed correlations. Tables 1 and 2 present the one hundred most correlated probes (in absolute values). In the supplementary material (File S2 sheet ‘correlation Analysis’), the correlation of each of the 1,372 probes that were selected by our method is given (and annotated, including Affymetrix and Stanford’s Source outputs) to facilitate further analyses.

As the objective is to detect the probes correlated with the progression of AD, we will select those probes with high absolute correlations values with both groups, an indication of a
### Table 2. List of the 100 probes with the highest Spearman correlation (absolute value, computed over all samples) between the expression of the probe and the square root of the Jensen-Shannon divergence of all samples with the average Severe AD gene expression profile.

| Gene symbol | Probe     | Spearman rank correlation |
|-------------|-----------|----------------------------|
| 1           | NEFM      | 0.84472                    |
| 2           | NRG1      | 0.83003                    |
| 3           | VSNL1     | 0.80156                    |
| 4           | NEFL      | 0.79889                    |
| 5           | SLC25A12  | 0.79666                    |
| 6           | BCL11A    | 0.79266                    |
| 7           | RALYL     | 0.78776                    |
| 8           | SERPIN1   | 0.78242                    |
| 9           | ATP2B2    | 0.78154                    |
| 10          | LDB2      | 0.7802                     |
| 11          | ENSA      | 0.77931                    |
| 12          | NDUFV2    | 0.77753                    |
| 13          | KIAA0319  | 0.76418                    |
| 14          | ATP5C1    | 0.7584                     |
| 15          | TAGLN3    | 0.75617                    |
| 16          | SV2B      | 0.75484                    |
| 17          | DOPEY1    | 0.75439                    |
| 18          | FAR2      | 0.75395                    |
| 19          | SNRK      | 0.7535                     |
| 20          | TRIM36    | 0.74994                    |
| 21          | NRXN1     | 0.74772                    |
| 22          | PKP4      | 0.74461                    |
| 23          | CALM3     | 0.74149                    |
| 24          | PIP4K2C   | 0.73971                    |
| 25          | CRYM      | 0.73437                    |
| 26          | SCFD1     | 0.73037                    |
| 27          | COX6A1    | 0.72992                    |
| 28          | OPA1      | 0.7277                     |
| 29          | ATP5C1    | 0.72414                    |
| 30          | LETMD1    | 0.71969                    |
| 31          | PPP2R2B   | 0.71657                    |
| 32          | UQCRQ     | 0.71301                    |
| 33          | FKBP3     | 0.71268                    |
| 34          | PTHX1     | 0.71123                    |
| 35          | CACNG3    | 0.71079                    |
| 36          | TMSB10    | 0.70812                    |
| 37          | KIAA1467  | 0.70812                    |
| 38          | INA       | 0.7059                     |
| 39          | ARF5      | 0.70455                    |
| 40          | CD200     | 0.70456                    |
| 41          | CAMK1G    | 0.70367                    |
| 42          | TUBG2     | 0.70234                    |
| 43          | LDHA      | 0.70189                    |
| 44          | LOC100131599 | 0.70056            |
| 45          | DMTT1     | 0.697                      |
| 46          | RGS4      | 0.69655                    |

### Table 2. Cont.

| Gene symbol | Probe     | Spearman rank correlation |
|-------------|-----------|----------------------------|
| 47          | CAMK2     | 0.69611                    |
| 48          | BE371738  | 0.69477                    |
| 49          | PPP2CA    | 0.69388                    |
| 50          | SRD5A1    | 0.69388                    |
| 51          | DMN       | 0.68409                    |
| 52          | AW794666  | 0.68721                    |
| 53          | SLC33A1   | 0.68899                    |
| 54          | SYNC1      | 0.68954                    |
| 55          | ITGB5     | 0.69299                    |
| 56          | CNOT6     | 0.69655                    |
| 57          | DYNLT1    | 0.697                      |
| 58          | ZMYND8    | 0.697                      |
| 59          | TBL1X      | 0.69768                    |
| 60          | RND2      | 0.70378                    |
| 61          | LRP10     | 0.70545                    |
| 62          | GMPR      | 0.70678                    |
| 63          | LTF       | 0.70812                    |
| 64          | CSNK1A1   | 0.70812                    |
| 65          | NBPF12    | 0.70901                    |
| 66          | ZFP36L2    | 0.70945                    |
| 67          | AV712577  | 0.71212                    |
| 68          | FDFT1     | 0.71257                    |
| 69          | ADARB2    | 0.71301                    |
| 70          | CPT2      | 0.7139                     |
| 71          | ADD3      | 0.71524                    |
| 72          | 37681     | 0.71613                    |
| 73          | ITGB8     | 0.71924                    |
| 74          | RBM19     | 0.71969                    |
| 75          | HIST1H1C  | 0.72058                    |
| 76          | NM_018612 | 0.73037                    |
| 77          | CD68      | 0.73259                    |
| 78          | GTF2A1L    | 0.73348                    |
| 79          | FAM114A1  | 0.73571                    |
| 80          | FOXO1     | 0.73749                    |
| 81          | C6orf145  | 0.73882                    |
| 82          | KRC1      | 0.74149                    |
| 83          | TGBF3R2   | 0.74372                    |
| 84          | ZKH3      | 0.74594                    |
| 85          | TSPO      | 0.74816                    |
| 86          | STAT5A    | 0.74994                    |
| 87          | AFF1      | 0.75039                    |
| 88          | RASL12    | 0.75217                    |
| 89          | AL359052  | 0.75528                    |
| 90          | ALDH3A2   | 0.75706                    |
| 91          | C15       | 0.76062                    |
| 92          | AV700298  | 0.76062                    |
| 93          | HBEGF     | 0.76819                    |
| 94          | B2G251521 | 0.77086                    |
| 95          | ZBTB20    | 0.77353                    |
| 96          | ALD49443  | 0.78109                    |
The divergence of the average control profile together with a convergence to the severe AD profile; these correlations computed over all sample types. We need to check both groups according to their correlations to the average profile. The first group of probes we are interested in are those that have a positive correlation with \( \sqrt{JSD(P,C)} \) and a negative correlation with \( \sqrt{JSD(P,S)} \). The probes in this group are those probes with under-expression in the non-disease sample but are over-expressed in the severe AD cases. The second group has the opposite behaviour, the probes’ expression values have a negative correlation with \( \sqrt{JSD(P,C)} \) and a positive correlation with \( \sqrt{JSD(P,S)} \). This pattern can be visualised in Figure 4, where the elliptical shape of the dispersion of the probes in this scatter plot indicates that our methodology has preserved all the significant probes for both classes and that there are no probes (after the filter) presenting a high correlation simultaneously with the control and severe AD profiles.

On these values a new selection criterion is applied, as we wanted to identify the group of probes that have strong correlations to both groups in absolute value. This symmetry of our argument stems from the interest in understanding the biology of the progression of the disease. For identifying disease biomarkers we may just concentrate in finding the probes that present an upregulation trend when progressing from “Control” to “Disease”. However, here we would also like to identify those probes that become increasingly downregulated, which, in turn, would help us to identify significantly dysregulated biological pathways (as members of the pathway will be either up or downregulated). Towards this end, we rank the probes in the order given by their Euclidean distance from the origin of coordinates in Figure 4. We selected an arbitrary cut-off value of fifty probes (the selected probes are marked in red). These fifty probes are also identified by their Gene Symbols in Figures 5 and 6.

Calculating the distance of each probe to the origin, on the \( \sqrt{JSD} \) system of coordinates, we further selected the 50 most distant probes and analysed their behaviour. Table 3 presents the 50 probes (corresponding to 48 genes), their correlation to each group and their distance to the origin of coordinates. File S2 sheet ‘correlation Analysis’ column ‘E - Distance’ of the supplementary

**Table 2.**

| Gene symbol | Probe    | Spearman rank correlation |
|-------------|----------|----------------------------|
| 97          | PTTG1P   | -0.78154                   |
| 98          | FYCO1    | -0.78598                   |
| 99          | ATP6VOE1 | -0.802                     |
| 100         | SERTAD2  | -0.84338                   |

We listed the top fifty probes with positive and negative correlation. Rows in boldface indicate the cases for which a putative relationship exist in the published literature between the gene and AD. A probe that has a positive correlation with the square root of the Jensen-Shannon divergence with the average Control gene expression profile roughly indicates a probe that, over all samples in the set, tends to increase its expression from their values in the “Control” group to the “Severe AD”.

**Figure 5.** Zoom of Figure 4, identifying the most distant probes from the origin with negative correlation with the control profile, \( \delta(\sqrt{JSD}(P,C)) \) and positive correlation with the severe profile, \( \delta(\sqrt{JSD}(P,S)) \).

doi:10.1371/journal.pone.0010153.g005
material presents the distance to the origin of the 1,372 probes analysed. In Table 3, it can be seen which genes have some putative annotation that links them to AD (17 genes out of 48).

Figure 7 shows the heat map of the 50-probe signature, where the probes and patient samples are ordered by considering the similarity of their gene-expression values only. It can be observed that the Memetic Algorithm (MA), a high performance combinatorial optimization ordering method [12] for microarray datasets introduced in 2007, ordered most of the patients with or without an incipient level of AD on the left and the more severe cases on the right. When ordering the probes' gene expression, the MA perfectly sort the groups previously described. We refer to [12,13] for details of the MA. The supplementary material (File S2 ‘1372 norm. +heat map+GO’) presents the heat map of the 1,372 gene-probes, with samples and probes sorted by the MA.

Transcription factors analysis of most correlated probes

The signature of 50 probes we present in Figure 7 has 48 different genes (some probes are related to the same gene). The two repeated genes in this 50-probe list are ATP5C1 (ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1) and PPIA (peptidylprolyl isomerase A (cyclophilin A)) [14,15,16,17], a calcineurin regulatory protein. A recent study that used RT-PCR to examine tissue from 90 AD and 81 control human brains reports that cyclophilin is reduced in AD (both for females and males as compared with their gender-matched groups) [18]. We note here that the cutoff of 50 probes circumscribes the initial description a little, but most of the later discussion uses information from the whole signature to identify dysregulated pathways. Figure 8 presents the heat map of the 1,372-probe signature. The probes were sorted with the MA but the samples remain in the same position as obtained previously with the 50-probe signature.

We analysed this list of genes using GATHER [19], an online tool for annotating signatures. Forty-one genes out of fifty have a motif for EVI1 (ecotropic viral integration site 1) and thirty-nine of them have a binding motif with $\text{VSTC1F1PQ6}$ (TCF1: transcription factor 1, hepatic; LF-B1, hepatic nuclear factor (HNF1), albumin proximal factor). The same analysis can be done if we divide the set of genes in two groups. The first group has positive correlation with the control profile and are overexpressed in AD; the second group has a positive correlation with the severe profile, and tend towards being underexpressed in AD (see Table 3). Table 4 presents the overrepresented motifs. We note, however, that we believe that the best results to identify putative overrepresented regulatory motifs can be obtained using the whole signature of 1,372 probes, and we will present the results of this investigation after presenting the case of the most correlated probes.

Another interesting pattern emerged when analysing the KEGG Pathways of the 50-probe signature using GATHER and PATHWAY Studio [20]. Using GATHER, three KEGG Pathways appear significantly represented, Amyotrophic lateral sclerosis (ALS), Oxidative phosphorylation and ATP synthesis. Using PATHWAY Studio, we automatically built the “common-regulators” diagram by selecting a filter that only considers protein interactions and binding. The resulting diagram is presented in Figure 9. As can be seen from the figure, we have chosen a circular
Table 3. The 50 genes most distant to the origin of the coordinates space $\delta (\sqrt{s}JSD (P, P_C)) \times \delta (\sqrt{s}JSD (P, P_S))$.

| Probe Set ID | Gene Symbol | Gene Title | $\delta (\sqrt{s}JSD (P, P_C))$ | $\delta (\sqrt{s}JSD (P, P_S))$ | Dist O | Ref (ADG) |
|--------------|-------------|------------|-------------------------------|-------------------------------|--------|-----------|
| 206481_s_at  | LDB2        | LIM domain binding 2 | -0.7988                       | 0.7427                        | 1.0907 |
| 219736_at    | TRIM36      | tripartite motif-containing 36 | -0.8077                       | 0.7242                        | 1.0848 |
| 200650_s_at  | LDHA        | lactate dehydrogenase A | -0.8210                       | 0.6984                        | 1.0778 |
| 205113_at    | NEFM        | neurofilament, medium polypeptide 150kDa | -0.7448                       | 0.7742                        | 1.0743 |

| Probe Set ID | Gene Symbol | Gene Title | $\delta (\sqrt{s}JSD (P, P_C))$ | $\delta (\sqrt{s}JSD (P, P_S))$ | Dist O | Ref (ADG) |
|--------------|-------------|------------|-------------------------------|-------------------------------|--------|-----------|
| 202656_s_at  | SERTAD2     | SERTA domain containing 2 | 0.7343                        | -0.7827                       | 1.0732 |
| 203798_s_at  | VSNL1       | visinin-like 1 | -0.7093                       | 0.7923                        | 1.0634 |
| 205352_at    | SERPIN1I    | serpin peptidase inhibitor, clade I (neuroserpin), member 1 | -0.7432                       | 0.7496                        | 1.0555 |
| 213667_s_at  | ZHX3        | zinc fingers and homeoboxes 3 | 0.7677                        | -0.7129                       | 1.0477 |
| 209975_s_at  | NEFL        | neurofilament, light polypeptide 68kDa | 0.7153                        | 0.7552                        | 1.0402 |
| 213366_x_at  | ATP5C1      | ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1 | -0.7302                       | 0.7327                        | 1.0344 |
| 203340_s_at  | SLC25A12    | solute carrier family 25 (mitochondrial carrier, Aralar), member 12 | -0.7141                       | 0.7444                        | 1.0315 |
| 213967_at    | CAMK1G      | calcium/calmodulin-dependent protein kinase IG | -0.7819                       | 0.6682                        | 1.0285 |
| 218204_s_at  | FYCO1       | FYVE and coiled-coil domain containing 1 | 0.7250                        | -0.7222                       | 1.0233 |
| 213222_at    | PLCB1       | phospholipase C, beta 1 (phosphoinositide-specific) | -0.7694                       | 0.6738                        | 1.0227 |
| 200925_at    | COX6A1      | cytochrome c oxidase subunit Vla polypeptide 1 | -0.7532                       | 0.6883                        | 1.0204 |
| 38037_at     | HBEFG       | heparin-binding EGF-like growth factor | 0.7222                        | -0.7194                       | 1.0193 |
| 209481_at    | SNRK        | SNF related kinase | -0.7048                       | 0.7331                        | 1.0169 |
| 201142_at    | LRPI0       | low density lipoprotein receptor-related protein 10 | 0.6964                        | -0.7399                       | 1.0161 |
| 202941_at    | NDUFV2      | NADH dehydrogenase (ubiquinone) flavoprotein 2, 244Da | -0.6984                       | 0.7379                        | 1.0160 |
| 205383_s_at  | ZBTB20      | zinc finger and BTB domain containing 20 | 0.6774                        | -0.7569                       | 1.0157 |
| 206384_at    | CACNG3      | calcium channel, voltage-dependent, gamma subunit 3 | -0.7778                       | 0.6516                        | 1.0147 |
| 218888_s_at  | NETO2       | neurophin (NRP) and tolloid (TLL)-like 2 | -0.7899                       | 0.6246                        | 1.0070 |
| 212214_at    | OPA1        | optic atrophy 1 (autosomal dominant) | -0.7194                       | 0.7024                        | 1.0054 |
| 218456_at    | CAPRI1N2    | caprin family member 2 | -0.7915                       | 0.6186                        | 1.0046 |
| 211307_s_at  | FCAR        | Fc fragment of IgA, receptor for | 0.7297                        | -0.6886                       | 1.0033 |
| 202724_s_at  | FOXO1       | forkhead box O1 | 0.6875                        | -0.7270                       | 1.0006 |
| 219145_at    | LPHN1       | latrophilin 1 | -0.7293                       | 0.6826                        | 0.9989 |
| 205711_x_at  | ATP5C1      | ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1 | -0.7153                       | 0.6968                        | 0.9986 |
| 55662_at     | C10orf76    | chromosome 10 open reading frame 76 | 0.7632                        | -0.6420                       | 0.9973 |
| 211978_x_at  | PPIA        | peptidylprolyl isomerase A (cyclophilin A) | -0.7363                       | 0.6726                        | 0.9972 |
| 210016_at    | MYT1L       | myelin transcription factor 1-like // hypothetical protein LOC100134306 | -0.7577                       | 0.6395                        | 0.9915 |
| 204072_s_at  | FRY         | furry homolog (Drosophila) | -0.7456                       | 0.6512                        | 0.9899 |
| 219497_s_at  | BCL11A      | B-cell CLL/lymphoma 11A (zinc finger protein) | -0.6843                       | 0.7117                        | 0.9873 |
| 201125_s_at  | ITGB5       | integrin, beta 5 | 0.7323                        | -0.6613                       | 0.9867 |
Table 3. Cont.

| Probe Set ID | Gene Symbol | Gene Title | \(\delta(\text{sqrt}JSD(P,Pc))\) | \(\delta(\text{sqrt}JSD(P,Pc))\) | Dist O | Ref (ADG) |
|--------------|-------------|------------|-----------------|-----------------|--------|-----------|
| 211765_at     | PPIA        | peptidylprolyl isomerase A (cyclophilin A) | 0.7230          | 0.7014          | 0.9866 | [747,748,749,750] |
| 214057_at     | MCL1        | Myeloid cell leukemia sequence 1 (BCL2-related) | 0.7722          | 0.6137          | 0.9864 | [751] |
| 211839_at     | CSF1        | colony stimulating factor 1 (macrophage) | 0.8120          | 0.5590          | 0.9858 | [752,753,754,755,756, 757,758,759,760,761, 762,763,764,765,766, 767,768,769,770,771, 772,773,774,775,776, 777,778,779,780,781, 782] |
| 205551_at     | SV2B        | synaptic vesicle glycoprotein 2B | 0.6915          | 0.7008          | 0.9846 | [66] |
| 219167_at     | RASL12      | RAS-like, family 12 | 0.6226          | 0.7605          | 0.9828 | |
| 214393_at     | RND2        | Rho family GTPase 2 | 0.7051          | 0.6799          | 0.9796 | |
| 212899_at     | CDC2L6      | cell division cycle 2-like 6 (CDK8-like) | 0.7319          | 0.6504          | 0.9791 | |
| 220615_at     | MLSTD1      | male sterility domain containing 1 | 0.6665          | 0.7149          | 0.9774 | |
| 201681_at     | DLG5        | discs, large homolog 5 (Drosophila) | 0.7993          | 0.6706          | 0.9761 | |
| 208195_at     | TTN         | titin       | 0.7173          | 0.6617          | 0.9759 | [783] |
| 202457_at     | PPP3CA      | protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform | 0.7661          | 0.6016          | 0.9741 | |
| 214150_at     | ATP6VOE1    | ATPase, H+ transporting, lysosomal 9Na+, V0 subunit e1 | 0.6230          | 0.7488          | 0.9741 | |
| 204743_at     | TAGLN3      | transgelin 3 | 0.6952          | 0.6802          | 0.9726 | |
| 213197_at     | ASTN1       | astrotactin 1 | 0.7069          | 0.6673          | 0.9721 | |

The column “Dist O” shows the Euclidean distance from the origin for each gene. If the gene has a known relation with AD (ADG), the reference’s codes are display in column “Ref ADG”.
doi:10.1371/journal.pone.0010153.t003

In addition to these five, we observed the reduced expression of the glycolytic enzyme LDHA, which may also indicate another challenge for energy metabolism in these neurons. Although glucose is generally considered to be the only substrate for brain energy metabolism, monocarboxylates have also been hypothesised as alternative substrates [37]. Laughton et al. report segregation in the hippocampus, with LDHA present in astrocytes and not in neurons. Instead, it is pyruvate dehydrogenase that is present in neurons but not in astrocytes and as a consequence of this study they support the argument that a metabolic compartmentalization exists in the human cortex and hippocampus where lactate produced by astrocytes could be oxidized by neurons [37]. We have also observed a reduction in expression of a probe that corresponds to PDHAI1 (Pyruvate dehydrogenase (lipomide) alpha 1, 200980_s_at) with increasing AD severity. The reduction of PDH expression, and the concurrent increase in pyruvate carboxylase gene expression, was discussed by Landfield et al. [38], who argue that: “These changes suggest that reduced pyruvate flux through PDH and increased oxidative metabolism of glucose may develop early in AD. Interestingly, the inactivation of PDH is also a major pathway through which glucocorticoid activity acts to conserve glucose, and apparently, to induce insulin resistance [65,66]. Thus, our data are consistent with the possibility that GC effects on this and other important target pathways in brain are enhanced in both aging and AD. If so, such alterations in glucocorticoid efficacy may have implications for AD pathogenesis as well as for the increased risk of AD associated with normal aging.” Our results seem to indicate that LDHA might also be discussed within the extended metabolic pathways that serve as the basic framework of this novel, more complex hypothesis [38,39,40,41,42,43,44,45,46,47,48,49,50,51, 52,53,54,55].

Four of the 50 most correlated gene probes are linked to synaptic function and neurofilament bundle assembly and also have reduced expressions with AD severity: NEFM, NRXN1, SV2B, and NEFL all have a similar pattern of reduced gene expression with AD severity. Experiments with mice depleted of the NEFL have been previously reported in the...
literature. Dubois et al. state that this procedure: “mimics the reduced NFL mRNA levels seen in amyotrophic lateral sclerosis and causes perikaryal accumulation of neurofilament proteins and axonal hypotrophy in motoneurons. NFL−/− mice was evaluated for regional brain metabolism by means of quantitative histochemical estimation of cytochrome oxidase activity.”

Mutations in the NEFL gene [56,57,58,59,60,61,62] and in the NEFM [63] have been linked to Charcot-Marie-Tooth disease. We will discuss the loss of expression of NRXN1 (Neurexin 1) later, when we comment on its presence in a panel of putative genes linked to prion-induced neurodegeneration [64]. However, we note here that both NRXN1 and NEFL appeared to be downregulated on a transcriptional profiling study of prion infection in mice [65].

The loss of expression of SV2B is also interesting. In 2001, Heese et al. [66] reported “a new transcript of SV2B (SV2Bb) mRNA that is up-regulated at mRNA level in neurons by amyloid beta peptide (Abeta) fragment (1–42). In comparison to SV2B this new mRNA encodes for the same protein but it has an elongated 3′-untranslated region (3′UTR) that contains several AU-rich (AUR) cis-acting elements which are probably involved in posttranscriptional regulating of SV2Bb translation. In conclusion, alteration of SV2B(b) expression appears to be involved in processes of neuronal degeneration” (see also [67]). We note that SV2B is only expressed in vesicles that undergo calcium-regulated exocytosis [68] and is a regulator of synaptotagmin 1 [69], which is a synaptic calcium sensor with a role in neurotransmitter release previously studied in AD [70,71,72,73,74,75]. We present a number of genes related to synaptic function and neuronal plasticity which are increasingly down/up regulated later on the manuscript and on the supplementary material (File S3 Sheet ‘Synapse’).

Analysis of the 1,372-probe signature reveals alterations in calcium and insulin signalling

Using GATHER, we have identified 32 genes in the Calcium signalling pathway http://www.genome.jp/dbget-bin/show_pathway?hsa04020 ($p$-value<0.009). They are ADCY2, ADORA2B, AGTR1, ATP2A3, ATP2B1, ATP2B2, ATP2B4, AVPR1A, CALM1, CALM3, CREBBP, GNA14, GNAS, GRM5, HTR2A, ITPR1, ITPR2, LHCGR, NEATCI, PHKA2, PLCB1, PLCE1, PPP3CA, PPP3R1, PRKCB1, PTAFR, SLC25A6, SLC3A2, SYK, TBX2A2, TNNC2, and TTN. We cannot do enough justice in this manuscript to the several different hypotheses that point at imbalances/deregulation in calcium signalling and AD pathology. Instead, we contribute to these interesting discussions with our findings of genes related to this pathway within this group of 32 genes. The gene symbols in boldface can be mapped to the KEGG Pathway hsa04080, Neuroactive ligand-receptor interaction; those in italics can be mapped to KEGG Pathway hsa04310, Wnt Signalling. Being aware of the existing interest on Wnt signalling and AD, we went back to the list of genes present in our (alpha,beta)-k-feature set signature and we identified others that can also be linked to Wnt signalling, like CSNK1G3, CSNK2A2, FRAT1[76,77,78,79,
In addition, most of the remaining 32 genes in the Calcium signaling pathway can be mapped to KEGG Pathway hsa04070, Phosphatidylinositol signalling system (CALM1, CALM3, ITPT1, ITPT2, PLCB1, PLCG1, PRKCB1), and Gap junction (ADCY2, GNAI4, GNAS, GRM5, HTR2A, ITPT1, ITPT2, PLCB1, PRKCB1).

This fact suggested that we should check how many genes were mapped to these pathways. We found that Phosphatidylinositol signalling system was indeed the third pathway with most “hits” in our signature, and also with other 12 genes (CDIPT, CSNK1G3, PIK3C3, PIK3R1, PIK3R4, PHKB, PIP5K1A, PIP5K1C, PIP4K2C, PTEN, SKIP and TTK) which brings the total number to 19. We have also found (CCND3, CSNK1A1, CSNK2A2, CTBP1, CTBP2, FRAT1, FZD5, PPARD, PPP2CA, PPP2R2B, RBX1, SMAD3, TBL1X, TCF7L1, TCF7L2, VANGL1) bringing the total to 22 genes. We refer the reader to the supplementary material (File S3 Sheet ‘Phosphatidylinositol signalling’) for inspection of the individual pattern of expression of all these genes.

Together with the 20 genes mapped to the Insulin signalling pathway KEGG hsa04910 (ACACA, CALM1, CALM3, EIF4E2, FOXO1A, INSR, MAPK1, PDE3A, PHKA2, PIK3R1, PIK3R4, PPP1CC, PIP5K1A, PIP5K1C, PIP4K2C, PTEN, SKIP and TSC2), our results seem to give some support to the hypothesis of altered calcium dynamics [35,118,119,120,121,122,123,124,125,126,127], deregulation of insulin signalling [36,41,113,114,115,116,128,129,130,131,132,133,134,135,136,137,138,139,140,141,142,143,144,145,146,147,148,149,150,151,152,153,154,155,156,157,158,159,160,161,162,163,164,165] and the implication of the Wnt pathway [166,167,168,169,170,171,172,173,174,175,176,177,178,179,180,181,182,183,184,185,186,187,188,189,190,191,192,193,194,195,196,197,198,199] in AD pathogenesis.

Figures 10, 11, 12, 13, and 14 illustrate down(up)-regulation of genes in these signalling pathways (Calcium signalling, Neuroactive ligand receptor pathway, WNT, Phosphatidylinositol and Insulin signalling, respectively). Figure 15 shows the expression of probes corresponding to genes for which there are known associations to synaptic function and neuronal plasticity. We refer the reader to the supplementary material (File S3) for more searchable information.

Transcription factors analysis of 1,372-probe signature reveals significant associations with the EGR/KROX family of proteins, MAZ, and E2F1

The analysis of the 1,372-probe signature indicates that they can be linked to putative transcription factors that have been previously implicated in AD and other neurodegenerative diseases. Using GATHER, we have observed that there is a strong association with motif V\$KROX_Q6 (p-value, 0.0004) with 719 out of 1294 genes in our signature; V\$MAZ_Q6 (p-value, 0.001, with 1003 genes); and V\$E2F1_Q6_01 and V\$E2F1_Q3_01 (with p-values which are smaller than 0.002 and 0.009 respectively). Of the 1294 genes associated with the 1,372 probes (by GATHER), more than half of them (656) have a motif for V\$E2F1_Q6_01 and 603 have a motif for V\$E2F1_Q3_01.

MAZ (MYC-associated zinc finger protein (purine-binding transcription factor)), also known as ZFB7 and Cys2His2-type zinc finger transcription factor serum amyloid A activating factor 1 [200], has been previously implicated in Alzheimer’s disease [201] and as a blood biomarker in schizophrenia [202]. MAZ interacts with DCC, the receptor for netrin-1, a neuronal survival factor [203]. Deregulation of cyclin-dependent kinases and abnormal patterns of E2F1 regulation have also been linked with Alzheimer’s

Table 4. Binding factors related to two groups of genes.

| Transcription Factors | Description | P value |
|----------------------|-------------|---------|
| **First group**      |             |         |
| V$EV1_04            | Ectopic viral integration site 1 encoded factor | 0.00069 |
| V$SMAD4_Q6          | SMAD family member 4 | 0.0033  |
| **Second Group**    |             |         |
| V$HF1_C             | Hepatic nuclear factor 1 | 0.0022  |
| V$CAAT_B            | Avian C-type CCAAT box | 0.0015  |

The second group has the opposite behaviour, that is, positive correlation with the severe profile. The first group has positive correlation with the control profile.

doi:10.1371/journal.pone.0010153.t004
disease [204,205,206,207,208], neurodegeneration [205,207,209,210,211,212,213,214,215], and neuronal apoptosis [216,217,218,219,220].

The involvement of the EGR/KROX (immediate early genes) family of proteins in the pathogenesis of Alzheimer’s disease was first suggested in [221]. Studies of the behavioural consequences of stress have shown a link between the activation of the glucocorticoid receptor mediated response and EGR1, one of the members of this family [222]. It has been recently proposed that different members of the EGR/KROX family have different roles in learning and memory and cognitive functions [223,224,225,226,227,228]. Mutant mice experiments showed that EGR1/KROX24 is required for the consolidation of long-term memory, while it is EGR3 the one linked to short-term memory [229], with EGR2 having perhaps other type of phenotypic characteristics not yet mapped [230]. In rat hippocampus, EGR1 decreases with aging [231]. In a recent study, it has been shown that initial playbacks of novel songs transiently increase EGR1 but that the observed response selectively

Figure 9. ‘Common-regulators’ 50-probes’ signature. The figure was obtained using Pathway Studio [569]. The program received as input the 50-probes displayed in Fig. 7 and automatically searched all the known putative common regulators relationships. The highlighted proteins are the 5-protein signature (IL1-α, TNF-α, IL-3, EGF and GCSF) of [1]. We have also highlighted IL-6 (discussed in [1] in the context of results of classifiers that also use it) and CSF1, Colony-stimulating factor 1, (macrophage).

doi:10.1371/journal.pone.0010153.g009
Figure 10. Calcium signaling pathway. The upper graph presents the stacked normalized expression values of all the probes involved in the Calcium signaling with an upregulation trend. The lower graph analyses the genes involved in the pathway with a downregulation tendency. In the supplementary material (File S3 sheet ‘Calcium signalling pathway’), the reader will find all the individual gene expression values, normalised and not normalised.

doi:10.1371/journal.pone.0010153.g010
Figure 11. Neuroactive ligand-receptor interaction pathway. The upper graph presents the stacked normalized expression values of all the probes involved in the pathway with an upregulation trend. The lower graph analyzes the genes involved in the pathway with a downregulation tendency. In the supplementary material (File S3 sheet 'Neuroactive ligand-receptor'), the reader will find all the individual gene expression values, normalized and not normalized.

doi:10.1371/journal.pone.0010153.g011
Figure 12. WNT signaling pathway. The upper graph presents the stacked normalized expression values of all the probes involved in the pathway with an upregulation trend. The lower graph analyses the genes involved in the pathway with a downregulation tendency. In the supplementary material (File S3 sheet ‘Wnt Signalling’), the reader will find all the individual gene expression values, normalised and not normalised. doi:10.1371/journal.pone.0010153.g012
Figure 13. Phosphatidylinositol signaling pathway. The upper graph presents the stacked normalized expression values of all the probes involved in the pathway with an upregulation trend. The lower graph analyses the genes involved in the pathway with a downregulation tendency. In the supplementary material (File S3 sheet ‘Phosphatidylinositol signalling’), the reader will find all the individual gene expression values, normalized and not normalized.

doi:10.1371/journal.pone.0010153.g013
habituated after repetition of the stimulus, with a different expression profile after one day [232] (see [233] and also [234] in which the homolog of NEFM, one of our biomarkers of reduced expression with increasing ‘AD severity‘ called NF-M, is showed to be involved in the development and/or maturation of the oscine song control system).

We found the following connection between EGR/KROX, E2F1 and MAZ transcription factors that makes their concurrent finding notable. A recent study of microRNA signature of prion-induced neurodegeneration [64] has shown that EGR1, E2F1 and MAZ might be also implicated in the putative deregulation of immune response related genes by miRNAs via modulation of transcriptional regulators in scrapie-infected mice. We leave these findings for the next section of the manuscript where we will discuss them and present a list of common differentially expressed genes in these two neurodegenerative processes.

The 1,372-probe signature contains a significant number of genes differentially expressed that are linked to synaptic function and neuronal plasticity.

The existence of several genes among the most correlated ones (NRXN1, SV2B, NEFM, etc.) motivated us to try to identify which genes were present in the 1,372-probe signature that are also related to synaptic function and neuronal plasticity. We have identified 42 probes that can be divided into two groups, those that seem to be increasingly downregulated with AD severity (CABP1 [235, 236, 237, 238, 239, 240, 241, 242, 243], CADPS2 [244, 245, 246, 247, 248, 249], COLQ [250], DMD [251, 252, 253, 254, 255, 256], ELOVL2 [257], FAIM2/LFG [258, 259, 260, 261], GABBR2 [262, 263, 264, 265], GRIA2/GLUR2 [266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277], ITFPR1 [278, 279, 280, 281, 282, 283], KIAA0528, LZT51/FEZ1 [284, 285], NEMF, NRG1, NRXN1, NUFIP1 [286, 287, 288], PPT1 [289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301], PSD3, RAB3B [302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321], SHANK2 [322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340], SV2B [68, 69, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359] and those that present an upregulation pattern (CASK [360, 361, 362], CDK5R1 [363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379], CHRNA1, CHRNA9, CHRNBA3, CTBP2, DLG1/SAP97 [380, 381, 382, 383, 384, 385, 386, 387, 388], DLAG2, GARAP5 [389, 390, 391, 392, 393, 394], GABRQ [395], GLRA3 [396, 397, 398], GIRK3/GLUR7 [399], HOMER3 [400], ICA1 [401], ITGB1 [402, 403], MCTP1 [404, 405], PPP1CC [406], SNPH [407, 408, 409, 410, 411, 412, 413, 414], SSPN [415], SYNC1, and USH1C [416, 417, 418]). The reader can consult the supplementary material (File S2) for the individual expression patterns of these genes. If, in agreement with Klemmer et al. [362], consider synapses as the most complex cellular organelle, with approximately 1500 proteins interacting in an activity dependent manner, we can argue that we must be inclusive with our list of references to help other researchers map the literature of their functions. Our aim is that experts can use this information to find ways of building novel testable hypotheses of AD neuronal plasticity impairment in the hippocampus. Our approach here has been to map what is currently known, and link it with the current biomedical literature, to facilitate experts that understand processes in detail.

We have already discussed some of the increasingly downregulated genes, another important candidate for further study is NRG1 (Neuregulin 1), a gene that has already been linked to several neuronal diseases. It is a candidate for susceptibility to schizophrenia and bipolar disorder [see [419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434] and references therein]. There have been reported links of NRG1 with AD. BACE1 (beta-Site APP-cleaving enzyme) is necessary for the cleavage of the amyloid-beta precursor protein, and BACE1 participates in the proteolytic processing of NRG1 [435, 436], and there exists some concerns about BACE1 inhibition as a potential therapeutic intervention due to its interaction with NRG1 and potential effects on remyelination [437]. In particular, NRG1 has been reported as a possible biomarker in cerebral spinal fluid, since its levels have been reported to be significantly increased in AD. Pankonin et al. suggest that: “While (NRG1) is not detected in human serum, a novel neuregulin antagonist activity was identified in human serum that could have presented its detection. These results suggest that human neuregulin is selectively targeted from cortical neurons to white matter extracellular matrix where it exists in steady-state equilibrium with cerebral spinal fluid where it has the potential to serve as a biological marker in human neuronal disorders“ [438]. NRG1 seems to collaborate with the EBBR4 receptor, and Li et al. propose that together they control glutamatergic synapse maturation and plasticity [439]. A single nucleotide polymorphism in NRG1 has also been associated as a risk factor to positive symptoms of psychosis in a proportion of late-onset AD [440]. With this evidence it is clear that NGR1 [439, 441, 442, 443, 444, 445, 446, 447] as well as the whole panel presented here are excellent candidates for further studies due to their well supported role in synaptic function in health and disease states.

Other biomarkers of interest

We should also mention some other biomarkers that could be interesting for further studies, including imaging purposes, like TSPO/PBR (translocator protein (18kDa)) also known as Mitochondrial Benzodiazepine Receptor (peripheral), thus supporting its current role as a putative imaging biomarker for AD [448, 449, 450, 451, 452, 453, 454, 455], CIS (complement component 1, s subcomponent) [456, 457, 458, 459, 460, 461], DFD1 (the squalene synthase gene), which is critical for cholesterol synthesis [462, 463], BMP4 [92, 96, 464, 465], CD68 (as marker of enhanced lysosomal activity) [450, 466, 467, 468, 469, 470, 471, 472], SERTAD2/TRIP-Bt2 [473, 474, 475], LTF (Lactotransferrin) [474, 477, 478], FTL (ferritin, light polypeptide; Ferritin light chain) [479, 480, 481, 482], MT1F (Metal-regulatory transcription factor 1) [483, 484, 485], GSTA3 (Glutathione S-transferase A3), GSTM4 (Glutathione S-transferase M4), MT1L (Metallothionein 1L (gene/pseudogene) [481, 482], MTF1 (Metallothionein 1L s subcomponent) [486] (a human-specific truncated protein which may have changed its function or suppressed it [487]), MT1H (Metallothionein 1H) [488], MT1F (Metallothionein 1F) [488, 489] (Figure 16). These last three upregulated genes need to be put in concert with other reports on metallothioneins in AD brains [490, 491, 492]. Figure 16 shows the upregulation of Lactotransferrin, FTL (ferritin, light polypeptide; Ferritin light chain), and the Metallothionein family with increasing AD severity.

Other probes which present an upregulation trend that we would like to highlight are BCL2 [493, 494], FYCO1 [495, 496], PAX6 [111, 497, 498, 499] (Figure 17), and QKI [500] (Figure 18).
The increase of expression of these probes, together with SOX2, is intriguing as they are related to differentiation from stem cells and are considered critical in neurogenesis [501,502,503,504,505,506, 507,508,509,510]. Our results support the combined use of them in tracking AD progression in this tissue. In addition, we have previously mentioned the relevance of EGR1 in coordinating a large number of genes that seem to be differentially expressed in this study. EGR1 also appears with a marked upregulation in severe AD patients (we refer to the supplementary material File S2 Sheet ‘1372 norm. +heat map+GO’ for its gene expression profile).

We found that this link is very important, as the homologues of EGR1, zif268, Egr-1 or ZENK, together with other members of the EGR family, are consolidating a key role in the neuronal plasticity in the brain [226,230,511,512,513,514,515,516,517, 518,519,520,521,522,523,524,525,526,527,528,529,530,531,532, 533,534,535,536,537,538,539,540,541,542,543,544,545,546,547, 548,549] and links with AD and cognitive decline progression are starting to be reported [514,515,550,551,552,553,554].

At the same time, prospective studies should encompass some other genes which appear downregulated with increasing AD severity. Top of the list is perhaps LDB2/CLIM1 (LIM domain binding 2), recently pointed as a marker (with LMO4 [555,556]) of the control program of the development of neuronal subtype diversity of the cerebral cortex [557]. TRIM36 is another interesting candidate for further studies [558]. A gene that shares the same trend of downregulation is CAMK1G (calcium/calmodulin-dependent protein kinase 1G) [559,560,561,562, 563,564]. When analysing prefrontal cortical tissue from mice with inducible deletions of BDNF (Brain-derived neurotrophic factor), Glorioso et al. employed microarray gene expression profiling to show that there were alterations to early-immediate genes (including EGR1) and CAMK1G [563]. They conclude their manuscript stating that: “while altered BDNF expression may not represent the primary disturbance in AD, changed expression of, or altered responsiveness to BDNF (and subsequently decreased SST levels) may represent a critical feature of Alzheimer’s disease progression.”

VSNL1 (Visinin-like protein 1) [565], a Ca++ sensor protein is also down-regulated (see Figure 19), a finding which is paralleled in the work of Youn et al. [566], who found similar changes in hippocampus.

Figure 15. Genes related to synapse and neuronal plasticity. The upper graph presents the stacked normalized expression values of all the related probes with an upregulation trend. The lower graph analyses the genes involved with a downregulation inclination. In the supplementary material (File S3, Sheet ‘Synapse’), the reader will find all the individual gene expression values, normalised and not normalised.
doi:10.1371/journal.pone.0010153.g015

Figure 16. Metallothionein family. Stacked line graph of the probes related to the Metallothionein family in the 1372-probe signature.
doi:10.1371/journal.pone.0010153.g016
Discussion

Putative common genes involved in Alzheimer’s disease and prion-induced neurodegenerative processes

In late 2008, a paper was published in PLoS ONE, shortly after the publication of our signature for prediction of clinical symptoms of AD [1] appeared online [64]. In this contribution, Saba et al. present a microRNA signature of prion-induced neurodegeneration [64]. By examination of the promoter regions of putative microRNA targets, they found that some transcription factor motifs were significantly enriched, E2F-1 (p-value = 6.01 \times 10^{-14}), KROX (p-value = 9.34 \times 10^{-14}), MAZ (p-value = 2.23 \times 10^{-15}) and PAX6 (p-value = 1.76 \times 10^{-18}). Our identification of EGR1/KROX-24 and PAX-6 as upregulated with AD progression, and the identification of motif V$KROX_Q6, V$MAZ_Q6, V$E2F1_Q6_01, V$E2F1_Q3_01 as enriched in our signature were two contributing factors that motivated us to explore any further similarities that we could find.

In [64], an analysis of the predicted target genes of their microRNA signature, linked with differentially expressed genes in scrapie-infected mice [65] as well as two other publications [567,568], led Saba et al. [64] to identify a network of de-regulated immune response-related genes. Additionally, they identified the putative transcription regulator genes that are targets of miRNAs similarly de-regulated. In essence, a possible hierarchy of deregulations of microRNAs, which, deregulated transcription factors that then, modify 1282 target genes. A Gene Ontology analysis also indicated that the “data sets were found to be in the significant enrichment for genes involved in cell death, regulation of the cell cycle, nervous system development and function and cell signalling pathways.”

As a consequence, we have investigated if some of the 1,282 putative target genes of the microRNA signature of prion induced neurodegeneration also appear in our lists. Of those 1,282 genes we immediately noticed that there were 9 genes listed in our list of the 50 most correlated genes (Table 3). These genes are BCL11A, CSF1, DLG5, FOXO1, HBEGF, NRXN1, SERTAD2, SNRK and ZBTB20. Two of these genes, CSF1 (colony stimulating factor 1 [macrophage]) and HBEGF (heparin-binding EGF-like growth factor) appear to be conspicuous mediators of cytokine and growth factor signalling as Figure 9 illustrates (we obtained this network using Pathway Studio [569] as described in the previous section), and CSF1 and HBEGF seems to be increasing with AD severity. In opposition, the probe corresponding to NRXN1 (Neurexin 1, 209915_s_at) has decreasing expression (Figure 20). Although no connection has been found between NRXN1 and AD yet, this gene has been implicated in autism [570,571,572,573,574,575,576], schizophrenia [577,578,579,580,581], nicotine and
alcoholism dependence [582, 583, 584], and mental retardation [585]. SERTAD2 (SERTA domain containing 2), mentioned in the previous section, is also known as Transcriptional regulator interacting with the PHD-bromodomain 2, TRIP-Br2, a member of the TRIP-Br family of transcriptional regulators, required for the transduction of mitogenic signals and the execution of serum-inducible E2F-mediated cell cycle progression [473]. In our data, the probe for SERTAD2 is increasing with AD severity. It has also been reported that overexpression of SERTAD2 is sufficient to transform murine fibroblasts and promotes tumorigenesis in athymic nude mice due to the deregulation of the E2F/DP-transcriptional pathway thanks to the upregulation of the key E2F-responsive genes [474]. FOXO1 (Forkhead box O1) also appears upregulated with increasing AD severity, and has been reported as a negative regulator of EGR1 expression via the activation of the PI3K/Akt/Forkhead pathway [586]. The expression of FOXO1 is also induced by E2F1 [587]. The product of this gene has also been reported as a survival factor in deprivation-induced neuronal cell death [588, 589] (see also the review in [590]). Although FOXO1 has not been previously implicated in AD, an exception may exist. van Der Heide et al. describe in [591] how the Forkhead transcription factors are involved in insulin signalling. The “PI3K route” is a name given to common signal transduction cascade that links neuronal survival, synaptic plasticity (and, as a consequence, learning and memory) [592]. This “PI3K-Akt-FOXO1 mechanism” and its role in neurons warrant the current intensive investigation [593, 594, 595, 596, 597, 598, 599, 600]. From this group of 9 genes, seven of them (NRX1, SERTAD2, SNRK, HBGEF, FOXO1, CSF1, BCL11A) and QKI have been predicted to be targeted by mmu-mir128 by two or more microRNA prediction tools. We found this to be a connection that is worth exploring. Lukiw and Pogue have reported that following metal-induced reactive oxygen species production (by iron and aluminium-sulfate at nanomolar concentrations) upregulates miR-128 in human neural cells in primary culture [601]. They also report that, together with miR-9, mir-125a, mir-128 is upregulated in AD brain. In the previously cited reference Lukiw reported that: “miR-9, miR-124a, miR-125b, miR-128, miR-132 and miR-219 are abundantly represented in fetal hippocampus, are differentially regulated in aged brain, and an alteration in specific micro-RNA complexity occurs in Alzheimer hippocampus.”

The expression of probes corresponding to PP2A and PP2B catalytic subunits (i.e. PPP2CA, Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform, and PPP3CA, Protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform, Calcineurin A1) shows increasing downregualtion with the progression of AD., see Figure 21. This finding supports a role for downregulation of PPP2CA, PPP3CA in AD pathology [619–647].

Finally, in addition to the presence of hyperphosphorylated tau, the accumulation of Amyloid-beta (Abeta) peptide in brain tissue is a hallmark of AD [602]. The identification of the genes involved in
the proteolytic processing of APP (beta-amyloid precursor protein), which in turn produces Abeta, is a subject of intense research. Researchers are currently looking at the alterations of APP cellular localization and endocytic trafficking as one mechanism that can modify the processing of APP to Abeta. LRPs are known to regulate APP’s endocytic trafficking [603,604,605,606], and seem to be a hub of a number of mounting evidences on processes that link to cholesterol metabolism and atherosclerosis [607]. In our selected panel of 50 proteins we have one member of this family, LRP10 (low density lipoprotein receptor-related protein 10), as one of the most correlated gene expression profiles. In our list of 1372 gene probe signature we also have another member of this family, LRP1B (low density lipoprotein-related protein 1B (deleted in tumors))[608], While LRP10 appears to be positively upregulated with cognitive decline an inverse relationship is observed for LRP1B.

LRPs are also known to linked to APP via a mechanism that involves the alternative splicing of APBB3/Fe65L2 [609,610,611]. Tanahashi and Tabira have proposed that the splicing of APBB3/Fe65L2 alters the ability to bind with APP and low-density-lipoprotein-receptor-related protein. They propose that the secretion of beta-amyloid peptide Abeta40 and Abeta42 is increased following the overexpression of APBB3, but there are no visible changes of half-life and maturation of APP, or the secretion of secreted APP [612]. In our dataset, we observe APBB3 expression being upregulated with the increasing cognitive decline, following the same pattern of LRP10.

Polymorphisms on these genes have previously been linked to AD. Tanahashi, Asada and Tabira have reported an association between a polymorphism in APBB3/Fe65L2 and early-onset AD [612] (the link between APBB3 and AD is being increasingly explored, we refer to [613,614,615,616] for further references). Using 500K SNP microarray technology, Poduslo, Huang and Spiro have identified haplotypes in LRP1B as significant for successful aging without cognitive decline in a study involving individuals that were 85 years old or older, had MMSE scores greater than 26, no history of dementia in their families, and no major illnesses (i.e. no cardiovascular problems, diabetes, obesity, or major cancer diseases) and most of them had normal cholesterol levels. Their genome-wide association screening compared these individuals with those that have late-onset AD [617]. Poduslo et al. have suggested that if the decreased production of Abeta42 in successful aging is due to the haplotypes they describe, then LRP1B may be a new target for treatment of AD [608,617]. Taken together these results indicate that integrative bioinformatics analytic

Figure 19. The expression of a probe for VSNL1 (Visinin-like protein-1) shows increasing downregualtion with AD severity. VSNL1, a neuronal calcium sensor that has received recent attention in AD [636,637,638,639] has also been linked to model systems of schizophrenia, where it has been found upregulated in hippocampus [640]. A previous result by Schnurra et al. raised the possibility that the reduction of VSNL1 expressing neurons indicate a selective vulnerability of these cells, since they observed that VSNL1 expression enhanced hyperphosphorylation of tau protein (in contrast with nontransfected or calbindin-D28K-transfected cells) [641]. In 2001, Braunewell et al. had already reported the reduction of VSNL1-immunoactive neurons in the temporal cortex of AD patients as compared with controls [642].

doi:10.1371/journal.pone.0010153.g019
Conclusions

This re-analysis of the microarray dataset hippocampal gene expression contributed by Blalock et al. has shown that there exist a relatively large number of probes (1,372) that present a clear pattern of either up or down regulation with increasing AD severity. The signature reveals alterations in calcium, insulin, phosphatidylinositol and Wnt-signalling. Among the group of most correlated gene probes with AD severity we found some linked to synaptic function, neurofilament bundle assembly, neuronal plasticity and inflammation.

A transcription factors analysis of 1,372-probe gene expression signature reveals significant associations with the EGR/KROX family of proteins, MAZ, and E2F1. The gene homologous of EGR1, zif268, Egr-1 or ZENK, together with other members of the EGR family, are consolidating as key players in short and long-term memory and neuronal plasticity in the brain. We have also uncovered a large consensus of this gene expression signature with current genes putatively involved in AD progression. Our results also indicate a degree of commonality between putative genes involved in AD and prion-induced neurodegenerative processes that warrants further investigation.

Materials and Methods

Dataset

In this contribution, we have used a MIAME compliant, Affymetrix gene expression dataset that is public available and was contributed by Blalock et al [3] in 2004. We thank the authors of that publication for making this useful dataset available to the research community at large allowing further exploration and reanalysis.

The dataset is available from GEO Dataset Browser, accession number GDS1297 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1297). The Affymetrix human GeneChip, HG-U133A, containing 22,283 targets was used. The dataset is de-identified and the methods for disease classification, based on MMSE and NFT scores, are described in full detail by Blalock et al in Ref. [3].

The hippocampal samples used by Blalock et al. were obtained from the autopsy of 31 subjects through the Brain Bank of the University of Kentucky Alzheimer’s Disease Research Center (ADRC), Sanders-Brown Center on Aging, University of Kentucky. The ADRC was established in 1985 and in operation since 1989 a pool of research volunteers that have agreed in principle to be research participants. Participants were asked questions based on NINCDS/ADRDA criteria [618] to establish their physical...
and mental condition to determine if they were eligible for the study. When a mutual agreement existed, the individuals were visited in their homes to review and sign the informed-consent document (which was approved by the University of Kentucky Institutional Review Board). Participants also signed a donor card, and the visit also aimed to establish their baseline mental-status testing. Eligibility for the purpose of the study included having a Mini-Mental State Exam score above 24 [619], passing a series of cognitive tests, and a previous history of absence of neurological disease [620], as well as neither substance abuse nor major psychiatric illnesses. All eligible volunteers were 60 years of age or older and satisfactorily performed normal activities of daily living. The Wechsler Adult Intelligence Scale (Vocabulary) was also applied to exclude significant other medical diseases that could affect cognition and eligible participants must had no previous history of head injury with loss of consciousness.

The research participants that were deemed eligible also signed a form (in addition to the consent document) indicating their agreement to donate their brain to the Sanders-Brown Center on Aging. A full description of the methods used can be found in Brain Donation in Normal Aging Procedures, Motivations, and Donor Characteristics from the Biologically Resilient Adults in Neuronal Studies (BRAiNS) Project [621].

Blalock et al. [3] categorized the samples in four groups, with a labelling that indicates different “levels of severity”. These labels were decided based on the Mini-Mental State Examination (MMSE) and the Neurofibrillary Tangle count (NFT) of each sample [622]. Samples are then separated in the types ‘Control’, ‘Incipient AD’, ‘Moderate AD’ and ‘Severe AD’. Table 1 of Blalock et al. shows the mean values of MMSE and NFT for each one of these groups. In addition, they give the mean Braak stage [623,624,625] for each one of the groups (2.1 for ‘Control’, 5 for ‘Incipient’, 5.6 for ‘Moderate’ and 5.9 for ‘Severe’). We are grateful to Dr. Blalock who has kindly given us these values of the Braak stage for each sample in the dataset. Together with the individual values of MMSE, NFT, the Braak stage of each sample is included in the Supplementary Material (File S2 sheet ‘Braak’) section of this publication.

Methodology

Our analysis method consisted of four steps: abundance quantization and filtering of probes; a feature selection algorithm to refine the probe selection; a Jensen-Shannon divergence computation; and finally, a correlation analysis. Each of these steps is described below.

As mentioned in the Results section, we only used the samples labelled as “Control” or “Severe AD” for feature selection, thus we have a two-class probe/gene selection task. We did not use the samples labelled as “Incipient AD” or “Moderate AD” for the probe selection steps. Those samples were only used in the final step, at the time of computing the correlation of the gene profile, across all samples, with the Jensen-Shannon divergences computed for the “Control” and “Severe” classes as explained later in this section.

For the first step, the quantization of the expression values, as well as for the initial data pruning, we used Fayyad and Irani’s algorithm [626]. The heuristic algorithm minimises the feature-class entropy and discards genes according to the Minimum Description Length principle. The application of Fayyad and Irani’s algorithm not only filters several thousand genes, it also provides thresholds for each probe remaining in the dataset. These quantized values of gene expression leave us with an instance of a combinatorial optimization problem, the (x, β)-k-Feature Set problem [13,627,628].

The (x, β)-k-Feature Set problem is a combinatorial optimization problem introduced by Cotta, Sloper and Moscato [629] in 2004 to address the problem of feature selection in high-dimensional datasets. We solve an instance of this problem numerically using an integer programming formulation. This approach has been previously employed to obtain molecular biomarker signatures in Alzheimer’s Disease [1,629], models of Parkinson disease [630], prostate cancer [631], electrode selection in EEGs [632], and elsewhere. To obtain mathematically proven optimal solutions of the integer programming formulation, the CPLEX commercial optimization solver was used. As in previous contributions of our group, we found gene expression signatures corresponding to values of x maximum and β maximal [1,13,627,628,633]. We refer the reader to these previous contributions for a detailed explanation of the methodology.

At this point, we have a selection of 1,372 probes, a set which we denote as Ω. For each sample m and probe i ∈ Ω, let \( f_{im} \) be its expression value. We now define a probability distribution function (PDF) for each sample. For sample, m its PDF \( p_{i}^{(m)} = \{ p_{i}, \forall i \in \Omega \} \), is given by

\[
p_{i}^{(m)} = \frac{f_{im}}{\sum_{i \in \Omega} f_{im}}
\]

We can now compute an average PDF profile for samples in the “Control” and “Severe AD” groups, denoted by \( P_{C} \) and \( P_{S} \) respectively. Let \( C \) and \( S \) be the set of samples with the labels “Control” and “Severe AD” respectively. The average profile \( P_{C} = \{ p_{i}^{(C)}, \forall i \in \Omega \} \), is then:

\[
p_{i}^{(C)} = \frac{\sum_{j \in C} f_{ij}^{(C)}}{N_{C}}, \forall i \in \Omega,
\]

where

\[
f_{ij}^{(C)} = \frac{1}{N_{C}} \sum_{m \in C} f_{im}, \forall i \in \Omega,
\]

where \( N_{C} \) represents the number of samples in class \( C \). \( P_{S} \) is analogously defined.

The Jensen-Shannon divergence between two sample PDFs, i.e. samples \( l \) and \( k \) (\( P^{(l)} \) and \( P^{(k)} \)) is defined as

\[
JSD[P^{(l)}, P^{(k)}] = S \left[ \frac{P^{(l)} + P^{(k)}}{2} \right] - \frac{S[P^{(l)}]}{2} - \frac{S[P^{(k)}]}{2}
\]

where

\[
P^{(l)} + P^{(k)}
\]

\[
S
\]

\[
\frac{1}{2}
\]

\[
2
\]

\[
S
\]

\[
2
\]
**Supporting Information**

**File S1** IHop Glossary of Genes. Found at: doi:10.1371/journal.pone.0010153.s001 (0.15 MB DOC)

**File S2** Supplementary Data 1. Found at: doi:10.1371/journal.pone.0010153.s002 (4.10 MB XLS)

**File S3** Supplementary Data 2. Found at: doi:10.1371/journal.pone.0010153.s003 (1.26 MB XLS)

**Acknowledgments**

The authors wish to thank the late Dr. William Markesbery, Dr. Erik Blalock and through them the whole team of the University of Kentucky’s Sanders-Brown Center on Aging who have contributed a very valuable dataset.

**Author Contributions**

Conceived and designed the experiments: MGR OAR RB PM. Performed the experiments: MGR OAR RB PM. Analyzed the data: MGR OAR RB. Wrote the paper: MGR OAR RB PM.

**References**

1. Gomez Ravetti M, Moscati P (2008) Identification of a 5-Protein Biomarker Molecular Signature for Predicting Alzheimer’s Disease. PLoS One 3: e3111.

2. Ray S, Britschgi M, Herbert C, Takenaka-Uchimura Y, Boxer A, et al. (2007) Classification and prediction of clinical Alzheimer’s disease based on plasma protein signatures. Nat Med 13: 1359-1362.

3. Blalock EM, Geddes JW, Chen KC, Porter NM, Markesbery WR, et al. (2004) Incipient Alzheimer’s disease: Microarray correlation analyses reveal major transcriptional and tumor suppressor responses. Proceedings of the National Academy of Sciences of the United States of America 101: 2173-2178.

4. Schmitt FA, Davis DG, Wekstein DR, Smith CD, Ashford JW, et al. (2000) “Preclinical” AD revisited: Neuropathology of cognitively normal older adults. Neurology 55: 370-376.

5. Haroutunian V, Purdon DT, Perl DP, Khan K, et al. (1999) Neurofibrillary Tangles in Nondemented Elderly Subjects and Mild Alzheimer Disease. Arch Neurol 56: 713-718.

6. Price DL, Sisodia SS (1998) Mutant genes in familial Alzheimer’s disease and transgenic models. Annual Review of Neuroscience 21: 479-505.

7. Price JL (1993) The relationship between tangle and plaque formation during aging and mild dementia. Neurobiology of Aging 14: 661-663.

8. Price JL, Davis PB, Morris JC, White DL (1991) The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer’s disease. Neurobiology of Aging 12: 295-312.

9. Robbins K, Sandeep J, Zhang W, Rekaya R (2006) Classification of incipient Alzheimer patients using gene expression data: Dealing with potential maldistribution. Online Journal of Bioinformatics 7: 9.

10. Sandeep J, Robbins K, Zhang W, Rekaya R (2008) Effects of Maldistribution in Input Data on the Identification of Differential Expression Genes in Incipient Alzheimer Patients. In Silico Biology 8: 9.

11. Gross E, Bernaola-Galvan P, Cepeda P, Roman-Roldan R, Oliver J, et al. (2002) Analysis of symbolic sequences using the Jensen-Shannon divergence. Phys Rev E Stat Nonlin Soft Matter Phys 65: 041905.

12. Moscati P, Mendes A, Berretta R (2007) Benchmarking a memetic algorithm for ordering microarray data. Biosystems 88: 56-75.

13. Berretta R, Costa W, Moscati P (2008) Combinatorial Optimization Models for Finding Genetic Signatures from Gene Expression Datasets. In: Keith JM, ed. Bioinformatics. Humana Press. pp 363-377.

14. Blalock EM, Geddes JW, Chen KC, Porter NM, Markesbery WR, et al. (2004) Incipient Alzheimer’s disease: Microarray correlation analyses reveal major transcriptional and tumor suppressor responses. Proceedings of the National Academy of Sciences of the United States of America 101: 2173-2178.

15. Schmitt FA, Davis DG, Wekstein DR, Smith CD, Ashford JW, et al. (2000) “Preclinical” AD revisited: Neuropathology of cognitively normal older adults. Neurology 55: 370-376.

16. Price DL, Sisodia SS (1998) Mutant genes in familial Alzheimer’s disease and transgenic models. Annual Review of Neuroscience 21: 479-505.

17. Hughes V, Smith S, Garcia-Sanchez A, Sales J, Stevenson K (2007) Proteomic comparison of Mycobacterium avium subspecies paratuberculosis grown in vitro and isolated from clinical cases of ovine paratuberculosis. Microbiology 153: 196-205.

18. Preece P, Cairns NJ (2003) Quantifying mRNA in postmortem human brain: influence of gender, age at death, postmortem interval, brain pH, agonal state and inter-lode mRNA variance. Brain Res Mol Brain Res 118: 60-71.

19. Chang JT, Nevin JK (2006) GATHER: a systems approach to interpreting genomic signatures. Bioinformatics 22: 2926-2933.

20. Ariasde Genoveses I (2007) Pathway Studio™. 5.0 ed.

21. Ongwiwitth S, Wong-Riley MT (2004) Functional analysis of the rat cytochrome c oxidase subunit 6A1 promoter in primary neurons. Gene 337: 163-171.

22. Wong-Riley M, Gao A, Bachman NJ, Limax MI (2000) Human COX6A1 gene: promoter analysis, cDNA isolation and expression in the monkey brain. Gene 247: 63-75.

23. Ben-Shachar D, Karry R (2008) Neuroanatomical pattern of mitochondrial complex I pathology varies between schizophrenia, bipolar disorder and major depression. PLoS One 3: e3676.

24. Ji B, La Y, Gao L, Zhu H, Tian N, et al. (2009) A Comparative Proteomics Analysis of Rat Mitochondria from the Cerebral Cortex and Hippocampus in Response to Antipsychotic Medications. J Proteome Res 8: 3633-3641.

25. Zhang J, Li X, Wang Y, J, J, Yang F, et al. (2009) Association study on the mitochondrial gene NDUFV2 and bipolar disorder in the Chinese Han population. J Neural Tranms 116: 357-361.

26. Washuzuka S, Iwamoto K, Kizakurai B, Bandro M, Kato T (2009) Expression of mitochondrial complex I subunit gene NDUFV2 in the lymphoblastoid cells derived from patients with bipolar disorder and schizophrenia. Neurosci Res 63: 199-204.

27. Xu C, Li PP, Kennedy JL, Green M, Hughes B, et al. (2008) Further support for association of the mitochondrial complex I subunit gene NDUFV2 with bipolar disorder. Bipolar Disord 10: 105-110.

28. Ben-Shachar D, Karry R (2007) Spl1 expression is disrupted in schizophrenia; a possible mechanism for the abnormal expression of mitochondrial complex I genes, NDUFV1 and NDUFV2. PLoS One 2: e2017.

29. Lazarou M, McKenzie M, Oktame A, Thorburn DR, Ryan MT (2007) Analysis of the assembly profiles for mitochondrial- and nuclear-DNA-encoded subunits into complex I. Mol Cell Biol 27: 4220-4257.

30. Nakatanai N, Hattori E, Ohnishi T, Dean B, Iwayama Y, et al. (2006) Genome-wide expression analysis detects eight genes with robust alterations specific to bipolar I disorder: relevance to neuronal network perturbation. Hum Mol Genet 15: 1949-1962.

31. Syrjäla TL, Mohar Z, Kind PC, Cordery PM, Upton AL, et al. (2005) Activity-dependent regulation of synapse and dendritic spine morphology in developing barrel cortex requires phospholipase C-bta1 signalling. Cereb Cortex 15: 385-393.
32. Litoch I (2002) Novel mechanisms for feedback regulation of phospholipase C-

beta activity. IUBMB Life 54: 253–260.

33. Bohm D, Schweger H, Kotthaus I, Neyarina K, Rickmann M, et al. (2002) Disruption of PLC-beta 1-mediated signal transduction in mutant mice causes age-dependent hippocampal mossy fiber sprouting and neurodegeneration. Mol Cell Neurosci 21: 584–601.

34. Hannan AJ, Blakemore C, Katsnelson A, Vitalis T, Huber KM, et al. (2001) PLC-beta1, activated via mGluRs, mediates activity-dependent differentiation in cortical neurons. Nat Neurosci 4: 282–288.

35. Rasmuson S, Andrew R, Nasman B, Seckl JR, Walker BR, et al. (2001) The role of the HPA axis in Alzheimer’s disease. J Neurol Neurosurg Psychiatry 70: 639–645.

36. Polleri A, Gianelli MV, Murialdo G (2002) Dementia: a neuroendocrine perspective. J Endocrinol Invest 25: 73–83.

37. de Quervain DJ, Poirier R, Wollmer MA, Grimaldi LM, Tsolaki M, et al. (2007) Multisignal regulation of the rat NMDA1 receptor subunit gene–a pivotal role in neurotoxicology. Neurosci Biobehav Rev 32: 1161–1173.

38. Landfield PW, Blalock EM, Chen KC, Porter NM (2007) A new glucocorticoid hypothesis of brain aging: implications for Alzheimer’s disease. Curr Alzheimer Res 4: 205–212.

39. Elgärd E, Lindqvist Astot A, Fagerlund M, Eriksson S, Olsson T, et al. (2006) Cognitive dysfunction, hippocampal atrophy and glucocorticoid feedback in Alzheimer’s disease. Biochem Pharmacol 72: 153–161.

40. Bao AM, Meynen G, Swaab DF (2008) The stress system in depression and neurodegeneration: focus on the human hypothalamus. Brain Res Rev 57: 151–162.

41. Lee JK, Kumar P, Fu Q, Rosen KM, Querfurth HW (2009) The insulin/Akt signaling pathway is targeted by intracellular beta-amyloid. Mol Biol Cell 20: 1533–1544.

42. Escrich L, Simon AM, Perre-Medravilla A, Salazar-Colocho P, Del Rio J, et al. (2008) Decreased somatic and synaptic expression of the synaptotagmin 1 receptor in an Alzheimer’s mouse model. Biochem Biophys Res Commun 379: 406–410.

43. Bonomo SM, Ragnamonti AE, Giunta M, Golimberti D, Guaina A, et al. (2009) Menopausal transition: a possible risk factor for brain pathologic events. Neurobiol Aging 30: 71–80.

44. Aisa B, Gil-Bea FJ, Marcos B, Tordera R, Lasheras B, et al. (2009) Neonatal lead exposure affects hippocampal and cerebellar neurons in a cell- and region-specific localization of lactate dehydrogenase 5 and pyruvate dehydrogenase. BMC Neurosci 8: 33.

45. Landfield PW, Blalock EM, Chen KC, Porter NM (2007) A new glucocorticoid hypothesis of brain aging: implications for Alzheimer’s disease. Curr Alzheimer Res 4: 205–212.

46. Elgärd E, Lindqvist Astot A, Fagerlund M, Eriksson S, Olsson T, et al. (2006) Cognitive dysfunction, hippocampal atrophy and glucocorticoid feedback in Alzheimer’s disease. Biochem Pharmacol 72: 153–161.

47. Bao AM, Meynen G, Swaab DF (2008) The stress system in depression and neurodegeneration: focus on the human hypothalamus. Brain Res Rev 57: 151–162.

48. Bao AM, Meynen G, Swaab DF (2008) The stress system in depression and neurodegeneration: focus on the human hypothalamus. Brain Res Rev 57: 151–162.

49. Lee JK, Kumar P, Fu Q, Rosen KM, Querfurth HW (2009) The insulin/Akt signaling pathway is targeted by intracellular beta-amyloid. Mol Biol Cell 20: 1533–1544.

50. Escrich L, Simon AM, Perre-Medravilla A, Salazar-Colocho P, Del Rio J, et al. (2008) Decreased somatic and synaptic expression of the synaptotagmin 1 receptor in an Alzheimer’s mouse model. Biochem Biophys Res Commun 379: 406–410.

51. Bonomo SM, Ragnamonti AE, Giunta M, Golimberti D, Guaina A, et al. (2009) Menopausal transition: a possible risk factor for brain pathologic events. Neurobiol Aging 30: 71–80.

52. Aisa B, Gil-Bea FJ, Marcos B, Tordera R, Lasheras B, et al. (2009) Neonatal lead exposure affects hippocampal and cerebellar neurons in a cell- and region-specific localization of lactate dehydrogenase 5 and pyruvate dehydrogenase. BMC Neurosci 8: 33.

53. Landfield PW, Blalock EM, Chen KC, Porter NM (2007) A new glucocorticoid hypothesis of brain aging: implications for Alzheimer’s disease. Curr Alzheimer Res 4: 205–212.

54. Elgärd E, Lindqvist Astot A, Fagerlund M, Eriksson S, Olsson T, et al. (2006) Cognitive dysfunction, hippocampal atrophy and glucocorticoid feedback in Alzheimer’s disease. Biochem Pharmacol 72: 153–161.

55. Bao AM, Meynen G, Swaab DF (2008) The stress system in depression and neurodegeneration: focus on the human hypothalamus. Brain Res Rev 57: 151–162.

56. Bao AM, Meynen G, Swaab DF (2008) The stress system in depression and neurodegeneration: focus on the human hypothalamus. Brain Res Rev 57: 151–162.
88. Crowder RJ, Freeman RS (2000) Glycogen synthase kinase-3 beta activity is critical for neuronal death caused by inhibiting phosphatidylinositol-3-kinase or Akt but not for death caused by nerve growth factor withdrawal. J Biol Chem 275: 34266–34271.

89. Kubas J, Varnum-Finck H, Bao X, Gao H, Long J, et al. (2009) BMP signaling pathways in PC12 cells. Stem Cell 27: 30–38.

90. Liu C, Wang Y, Smallwood PM, Nathans J (2008) A novel role for frizzled5 in neuronal survival in the paraffasicular nucleus of the mouse. J Neurosci 28: 16415–16433.

91. Carmon KS, Loose DS (2008) Wnt7a interaction with Fzd5 and 55 - A function of Wnt signaling utilizing a split eGFP. Biochem Biophys Res Commun 368: 285–291.

92. Steventon B, Araya C, Linker C, Kariyama S, Movey R (2009) Differential requirements of Wnt5a and Wnt7a signaling during gastrulation and neurulation. Development 136: 771–780.

93. Lee MY, Lim HW, Lee SH, Han HJ (2009) Smad, FZK/Akt, and Wnt-Dependent Signaling Pathways are Involved in BMP-4-induced ES Cell Self-Reisal in Stem Cells.

94. Kelberman D, de Castro SC, Huang S, Crolla JA, Palmer R, et al. (2008) SOX2 plays a critical role in the pituitary, forebrain, and eye during human embryonic development. J Clin Endocrinol Metab 93: 1865–1873.

95. Shi Y, Sun G, Zhao C, Stewart R (2008) Neural induction requires BMP inhibition only as a late step, and involves signals other than FGF and Wnt antagonists. Development 135: 5671–5681.

96. Tam WL, Lim CV, Han J, Zhang J, Ang YS, et al. (2008) T-cell factor 3 regulates embryonic stem cell pluripotency and self-renewal by the transcriptional control of multiple lineage pathways. Stem Cells 26: 2019–2031.

97. Kuwabara S, Hattori S, Tanaka K, Nakatsukasa T, Nakano S, et al. (2008) Tcell factor 3 binds to the Wnt signaling component Dazap2 modulates transcription driven by the Wnt effector TCF-4. Nucleic Acids Res 37: 3007–3020.

98. Li L, Holscher C (2007) Common pathological processes in Alzheimer disease and type 2 diabetes. Diabetes Metab 12: 364–371.

99. Yamaoka S, Hara N, Aoyama S, Shioda S, Itoh Y, et al. (2009) The interaction of xKaiso with xTcf3: a revised model for integration of epigenetic and Wnt signaling pathways. Development 136: 723–727.

100. Fancy SP, Baranzini SE, Zhao C, Yuk DI, Irvine KA, et al. (2009) Genetic variants of Wnt transcription factor TCF-4 (TCF7L2) putative enhancer region in Alzheimer Biomarker Discovery entry is associated with the Alzheimer-like disturbances in oxidative/energy brain metabolism and in behavior in adult rats. Ann N Y Acad Sci 1153: 301–303.

101. Noble S, Lee SK, Loffler T, Schliebs R (2006) Inhibition of the neuronal insulin receptor. An in vivo model for sporadic Alzheimer disease? Ann N Y Acad Sci 920: 256–258.

102. Hoyer S (1996) Oxidative metabolism deficiencies in brains of patients with Alzheimer's disease. Acta Neurol Scand Transm Suppl: 217–233.

103. Verkhratsky A, Toescu EC (2005) Endoplasmic reticulum Ca(2+) homeostasis and neuronal death. J Cell Mol Med 7: 351–361.

104. Amin S, Kusakabe K, Blalock EM, Chen KC, Thibault V, et al. (2005) Calcium influx triggers reactive/inflammatory processes in astrocytes and is upregulated in aging and Alzheimer's model. J Neurosci 25: 4649–4658.

105. Bojarski L, Hermja J, Kurnicki J (2008) Calcium dysregulation in Alzheimer's disease. Neurosci Letr 362: 621–633.

106. Cowburn RF, Popescu BA, Ankarcrona M, Delvar N, Cedazo-Minguez A (2007) Presenilin-mediated signal transduction. Physiol Behav 92: 95–97.

107. Giacomello M, Barbiero L, Zatti G, Squitti R, Binetti G, et al. (2005) Reduction of Ca2+ stores and capacitance Ca2+ entry is associated with the familial Alzheimer's disease presenilin-1 mutation and anticipates the onset of dementia. Neurobiol Dis 18: 638–648.

108. Peers G, Smith IF, Boyle JP, Pearson HA (2004) Remodelling of Ca2 + channels in sporadic Alzheimer-like pathology: an experimental approach. J Neurotransm 110: 211–217.

109. Qin W, Zhao W, Ho L, Wang J, Wahl K, et al. (2008) Regulation of forkhead transcription factor FoxO1 expression in astrocytes to caloric restriction-induced prevention of Alzheimer's disease-type amyloid neuropathology and spatial memory deterioration. Ann N Y Acad Sci 1147: 335–347.

110. Carro E, Trejo JL, Spuch C, Bold D, Heard JM, et al. (2006) Blockade of the insulin-like growth factor I receptor in the choroid plexus augments Alzheimer’s-like neuropathology in rodents: new cues into the human disease? Neurobiol Aging 27: 1618–1631.

111. Rincon V, Eckert A (2007) Effects of Alzheimer's amyloid-beta and tau protein on mitochondrial function. J Biol Chem 282: 285–291.

112. Abbas T, Faivre E, Holocher C (2009) Impairment of synaptic plasticity and memory formation in GLP-1 receptor KO mice: Interaction between type 2 diabetes and Alzheimer's disease. Behav Brain Res.

113. Castri P, Iacovelli L, De Blasi A, Ghibelli F, Moretti A, et al. (2007) Reduced insulin-induced phosphatidylinositol-3-kinase activation in peripheral blood mononuclear leucocytes from patients with Alzheimer’s disease. Eur J Neurosci 26: 2469–2472.

114. Li L, Holocher C (2007) Common pathological processes in Alzheimer disease and type 2 diabetes: a review. Brain Res Rev 56: 384–402.

115. Salkovic-Petrisic M, Hoyer S (2007) Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: a molecular foundation for sporadic Alzheimer-like pathology: a molecular approach. Ann N Y Acad Sci 1147: 335–347.

116. Biesalski HJ, Kappelle LJ (2003) Increased risk of Alzheimer's disease in Type II diabetes: insulin resistance of the brain or insulin-induced amyloidopathy? Biochem Soc Trans 31: 1041–1044.

117. Nelson TJ, Alkon DL (2005) Insulin and cholesterol pathways in neuronal function, memory and neurodegeneration. Biochem Soc Trans 33: 1033–1036.

118. Biessels GJ, Braverbroecker BO, Giesen WH (2004) Glucose, insulin and the brain: modulation of cognition and synaptic plasticity in health and disease: a preface. Eur J Pharmacol 490: 1–4.

119. Cortes GM, Colomares M, Nunez MT, Inestrosa NC (2004) Structure and function of amyloid in Alzheimer's disease. Prog Neurobiol 74: 323–349.

120. Zhao WQ, Townsend M (2009) Insulin resistance and amyloidogenesis as a common molecular foundation for type 2 diabetes and Alzheimer's disease. Biochem Biophys Acta 1792: 492–496.

121. De Felice FG, Vieira MN, Bomfim TR, Decker H, Velasco PT, et al. (2009) Genetic variants of Wnt transcription factor TCF-4 (TCF7L2) define two steps in neural crest induction. Development 136: 771–779.

122. De Felice GS, Peers G, Smith IF, Boyle JP, Pearson HA (2004) Remodelling of Ca2+ channels in sporadic Alzheimer-like pathology: an experimental approach. J Neurotransm 110: 211–217.
activity in the brain of diabetic mice: parallels with Alzheimer's disease and correction by insulin. J Neurosci Res 86: 3265–3274.

145. de Tullio MB, Morelli L, Castano EM (2008) The irreversible binding of amyloid peptide substrates to insulin-degrading enzyme: a biological perspective. Proc Natl Acad Sci U S A 105: 21–26.

146. Dore S, Kar S, Quirion R (1997) Insulin-like growth factor I protects and rescues hippocampal neurons against beta-amyloid- and human amylin-induced toxicity. Proc Natl Acad Sci U S A 94: 4772–4777.

147. Grandy S, Crestani V, Ayto F, Robitaille Y, Quirion R, et al. (2000) Insulin-like growth factor I and its receptor in the frontal cortex, hippocampus, and cerebellum of normal human and Alzheimer disease brains. Synapse 38: 150–160.

148. Deutsch Si, Rosse RB, Lakshman RM (2006) Dysregulation of tau phosphorylation is a hypothesized point of convergence in the pathogenesis of Alzheimer's disease: from neurotransmitter receptors and schizophrenia with therapeutic implications. Prog Neuropsychopharmacol Biol Psychiatry 30: 1369–1380.

149. Wickelgren I (1998) Tracking insulin to the mind. Science 280: 517–519.

150. Jafferali S, Dumont Y, Sotty F, Robitaille Y, Quirion R, et al. (2000) Insulin-like growth factor I regulates presenilin expression and cell survival in the hippocampus of normal adult rats. Exp Cell Res 257: 151–161.

151. Hoyer S, Nitsch R, Oesterreich K (1991) Predominant abnormality in cerebral glucose metabolism in transgenic mice expressing resistant insulin receptors. J Neurosci 11: 2367–2386.

152. Frolich L, Blum-Degen D, Bernstein HG, Engelsberger S, Humrich J, et al. (1994) Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. J Neurochem 63: 423–438.

153. Grunblatt E, Hoyer S, Riederer P (2004) Gene expression profile in sporadic (type II) Alzheimer disease: an update. J Neural Transm 109: 341–360.

154. Hoyer S (1998) Is sporadic Alzheimer disease the brain type of non-insulin dependent diabetes mellitus? A challenging hypothesis. J Neurochem 70: 415–422.

155. Hoyer S (2002) The aging brain. Changes in the neuronal insulin/insulin receptor signal transduction cascade trigger late-onset sporadic Alzheimer disease (SAD). A mini-review. J Neurochem 81: 991–1002.

156. Hoyer S (2002) The brain insulin signal transduction system and sporadic Alzheimer disease: an update. J Neurochem 79: 341–360.

157. Hoyer S, Nitsch R (1993) Cerebral excess release of neurotransmitter amino acids subsequent to reduced cerebral glucose metabolism in early-onset dementia of the Alzheimer type. J Neurochem 60: 227–232.

158. Xu WH, Huber R, Kirpe MW (2007) Gender- and region-specific expression of insulin receptors in mouse brain: effect of mild inhibition of oxidative phosphorylation. J Neurochem 114: 373–377.

159. Hoyer S, Nitsch R, Oesterreich K (1991) Predominant abnormality in cerebral glucose utilization in late-onset dementia of the Alzheimer type: a cross-sectional comparison against advanced late-onset and incipient early-onset cases. J Neural Transm Park Dis Dement Sect 3: 1–14.

160. Blum-Degen D, Frolich L, Hoyer S, Riederer P (1995) Altered regulation of brain glucose metabolism as a cause of neurodegenerative disorders? J Neural Transm Suppl 46: 139–147.

161. Hoyer S (1997) Models of Alzheimer's disease: cellular and molecular aspects. J Neural Transm Suppl 49: 11–21.

162. Hoyer S, Muller D, Phaschke K (1994) Desensitization of brain insulin receptor. Effect on glucose/energy and related metabolism. J Neural Transm Suppl 44: 259–268.

163. Aguado-Llera D, Arrila-Ferreiro E, Campos-Barros A, Puebla-Jimenez L, Barrios V (2005) Protective effects of insulin-like growth factor-I on the somatosensory system in the temporal cortex of beta-amyloid-treated rats. J Neurochem 92: 607–615.

164. Creses FT, McElhaney R, Freund G, Ballinger WE, Jr., Rayaizda MK (1992) Insulin-like growth factor I receptor binding in brains of Alzheimer's and alcoholic subjects. J Neurochem 58: 1205–1210.

165. Hoyer S (1987) Somatostatin and Alzheimer's disease. J Neurosci 254: 266–267.

166. De Ferrari GV, Moon RT (2006) The ups and downs of Wnt signaling in prevalent neurodegenerative disorders. Oncogene 25: 7545–7553.

167. Balaraman V, Lamaye AR, Leeve AL, Sturman S (2006) Glycogen synthase kinase-3beta and Alzheimer's disease: neurophysiological and therapeutic significance. Cell Mol Life Sci 63: 1226–1235.

168. Widelitz R (2005) Wnt signaling through canonical and non-canonical pathways: recent progress. Growth Factors 23: 111–116.

169. Copolla W, Ding J, Insinuosa NC (2006) Structure-function implications in Alzheimer's disease: effect of Abeta oligomers at central synapses. Curr Alzheimer Res 3: 233–243.

170. Mercado-Gomez O, Hernandez-Fonseca K, Villavicencio-Quejiero A, Masucci L, Chiron MC, Inestrosa NC (2008) Pathogenesis of Alzheimer's disease: effect of Abeta oligomers at central synapses. Curr Alzheimer Res 5: 233–243.

171. Ferrero A, Gessa GT, Cordero V, Pradhan B, Sorensen JS, et al. (2003) Wnt signaling as a therapeutic target for Alzheimer's disease. FEBS J 269: 9729–9738.

172. Ray A, Dhar S, Shaktot A, Roy P, Okada Y, et al. (2009) SAF-3, a novel splice variant of the SAF-1/MAZ/Pur-1 family, is expressed during inflammation. FEBS J.

173. De Ferrari GV, Papastasiopoulos A, Biochele T, Warrant De-Vrieze F, Avila MI, et al. (2007) Common genetic variation within the low-density lipoprotein receptor-related protein 6 and late-onset Alzheimer's disease. Proc Natl Acad Sci U S A 104: 9834–9839.

174. Rosales NG, Varela-Sanjuán L, Grzubowski CP, Colombos M (2007) Synaptotoxicity in Alzheimer's disease: the Wnt signaling pathway as a molecular target. JUBMB Life 59: 316–321.

175. Maiese K, Chong ZZ, Shing VC (2007) Mechanistic insights into diabetes mellitus and Alzheimer's disease. Cur Med Chem 14: 233–246.

176. Rebecchi E, Yuen BS, Zheng H, Kang DE. (2007) Presenilin 1 regulates growth of mapped control cell-fiber system to the Wnt pathway in human neuroepithelial stem cells. J Cell Mol Med 12: 914–927.

177. Ferrari A, Cseresato M, Sifionios L (2006) [The relationship between the Wnt signaling and the psychiatric diseases]. Vertex 17: 165–171.

178. De Ferrari GV, Papastasiopoulos A, Biochele T, Warrant De-Vrieze F, Avila MI, et al. (2007) Common genetic variation within the low-density lipoprotein receptor-related protein 6 and late-onset Alzheimer's disease. Proc Natl Acad Sci U S A 104: 9834–9839.
201. Jordan-Sciutto KL, Dragich JM, Caltagarone J, Hall DJ, Bowser R (2000) Fetal actions of VGF in hippocampal synaptic plasticity. J Neurosci 23: 1750–1760.

202. Bowden NA, Weidenhofer J, Scott RJ, Schall U, Todd J, et al. (2006) Altered synaptic plasticity and expressions of immediate early genes in schizophrenia. Schizophr Res 82: 175–183.

203. Bataller L, Wade DF, Graus F, Rosendal MR, Dalmau J (2003) The MAZ protein is a mediator of Hodgkin’s disease and paraneoplastic cerebral dysautonomia. Ann Neurol 53: 123–127.

204. Sajjan FD, Mariniuk F, Marcos DL, Frey WH, 2nd, Hite R, et al. (2007) Apoptotic gene expression in Alzheimer’s disease hippocampal tissue. J Alzheimers Dis Other Dement 21: 319–328.

205. Lim AC, Qi RZ (2003) Cyclin-dependent kinases in neural development and degeneration. J Alzheimers Dis 5: 329–335.

206. Bozon B, Davis S, Laroche S (2003) A requirement for the immediate early gene CREB in the consolidation of recognition memory. Philos Trans R Soc Lond B Biol Sci 358: 805–814.

207. Revest JM, Di Blasi F, Kitchener P, Rouge-Pont F, Desmedt A, et al. (2005) The E2F-cdc2 cell-cycle pathway specifically mediates activity deprivation-induced apoptosis of postmitotic neurons. J Neurosci 25: 10059–10069.

208. Motonaga K, Itoh M, Hirayama A, Hirano S, Becker LE, et al. (2001) Up-regulation of ZF87/MAP2K7, a neuronal-specific MAPK, and CREB in the hippocampus. Neurosci Lett 308: 365–369.

209. Putzer BM (2006) Targeting E2F1 Death Signaling: Opposing Role in Cancer Control and Neurodegeneration. Discov Med 6: 193–199.

210. Fortin A, MacLaurin JG, Arbour N, Cregan SP, Kushwaha N, et al. (2004) Expression of Fos, Jun, and Krox family proteins in Alzheimer’s disease. Exp Neurol 175: 226–244.

211. Lim AC, Qi RZ (2003) Cyclin-dependent kinases in neural development and degeneration. J Neurosci 23: 10800–10808.

212. Ranganathan S, Bowser R (2003) Alterations in G1 to S phase cell-cycle regulatory pathways in simian immunodeficiency virus encephalitis. Am J Pathol 163: 1383–1392.

213. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

214. Ranganathan S, Bowser R (2003) Altered subcellular distribution of transcriptional regulators in response to Beta amyloid and during Alzheimer’s disease. Mech Ageing Dev 125: 11–20.

215. Motonaga K, Itoh M, Hirayama A, Hirano S, Becker LE, et al. (2003) Structural analysis of Mg2+ and Ca2+ binding to CaBP1, a neuron-specific regulator of calcium channels. J Biol Chem 278: 37461–37470.

216. Li C, Chan J, Haeseeler F, Mikolahiia K, Palczewskia K, et al. (2009) Structural insights into Ca2+-dependent regulation of inositol 1,4,5-trisphosphate receptors by CaBP1. J Biol Chem 284: 2471–2481.

217. Chen ML, Chen YC, Peng IW, Kang RL, Wu JP, et al. (2008) Ca2+ binding protein 1 inhibits Ca2+ currents and exocytosis in bovine chromaffin cells. J Mol Cell Biol 15: 169–181.

218. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

219. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

220. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

221. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

222. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

223. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

224. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

225. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

226. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

227. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

228. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

229. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

230. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

231. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

232. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

233. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

234. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

235. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

236. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

237. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

238. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

239. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

240. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

241. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.

242. Jordan-Sciutto KL, Murray Fenner BA, Wilies CA, Achim CL (2001) Response of cell cycle proteins to neurotoxic factor and chemokine stimulation in human neuroglia. Exp Neurol 167: 205–214.
329. Kim JY, Han W, Namkung W, Lee JH, Kim KH, et al. (2004) Inhibitory
328. Hwang JI, Kim HS, Lee JR, Kim E, Ryu SH, et al. (2005) The interaction of
327. Qualmann B, Boeckers TM, Jeromin M, Gundelfinger ED, Kessels MM (2004)
326. Uemura T, Mori H, Mishina M (2004) Direct interaction of GluRdelta2 with
325. Brandstatter JH, Dick O, Boeckers TM (2004) The postsynaptic scaffold
324. Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, et al. (2000) cAMP-
323. Fujimoto K, Shibasaki T, Yokoi N, Kashima Y, Matsumoto M, et al. (2002)
322. Weidenhofer J, Scott RJ, Tooney PA (2009) Investigation of the expression of
321. Karniguian A, Zahraoui A, Tavitian A (1993) Identification of small GTP-
320. Lledo PM, Johannes L, Vernier P, Henry JP, Vincent JD, et al. (1993)
319. Lledo PM, Vernier P, Vincent JD, Mason WT, Zorec R (1993) Inhibition of
318. Masumoto N, Ikebuchi Y, Tahara M, Yokoi T, Tasaka K, et al. (1998)
317. Toribara K, Hanada K, Takai Y, Kato K, et al. (1993) The non-catalytic domain
316. Lledo PM, Vernier P, Vincent JD, Mason WT, Zorec R (1993) Inhibition of
315. Grabs D, Bergmann M, Urban M, Post A, Gratzl M (1996) Rab3 proteins and
314. Madison DL, Kruger WH, Kim T, Pfeiffer SE (1996) Differential expression of
313. Redecker P, Cetin Y, Grube D (1995) Differential distribution of synapto-
312. Toma H, Takahashi K, Bockmann J, Hofmann K, Sudhof TC (1998) Tyrosine kinase
311. Grabs D, Bergmann M, Urban M, Post A, Gratzl M (1996) Rab3 proteins and
310. Madison DL, Kruger WH, Kim T, Pfeiffer SE (1996) Differential expression of
309. Masumoto N, Ikebuchi Y, Tahara M, Yokoi T, Tasaka K, et al. (1998)
308. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
307. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
306. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
305. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
304. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
303. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
302. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
301. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
300. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
302. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
303. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
304. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
305. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
306. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
307. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
308. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
309. Kim CH, Bockmann J, Hofmann K, Sudhof TC (1998) The presence of small GTP-
310. Madison DL, Kruger WH, Kim T, Pfeiffer SE (1996) Differential expression of
311. Grabs D, Bergmann M, Urban M, Post A, Gratzl M (1996) Rab3 proteins and
312. Toma H, Takahashi K, Bockmann J, Hofmann K, Sudhof TC (1998) Tyrosine kinase
313. Redecker P, Cetin Y, Grube D (1995) Differential distribution of synapto-
314. Madison DL, Kruger WH, Kim T, Pfeiffer SE (1996) Differential expression of
315. Grabs D, Bergmann M, Urban M, Post A, Gratzl M (1996) Rab3 proteins and
316. Lledo PM, Vernier P, Vincent JD, Mason WT, Zorec R (1993) Inhibition of
317. Toribara K, Hanada K, Takai Y, Kato K, et al. (1993) The non-catalytic domain
318. Masumoto N, Ikebuchi Y, Tahara M, Yokoi T, Tasaka K, et al. (1998)
319. Lledo PM, Johannes L, Vernier P, Henry JP, Vincent JD, et al. (1993)
320. Weidenhofer J, Scott RJ, Tooney PA (2009) Investigation of the expression of
321. Karniguian A, Zahraoui A, Tavitian A (1993) Identification of small GTP-
322. Brandstatter JH, Dick O, Boeckers TM (2004) The postsynaptic scaffold
323. Uemura T, Mori H, Mishina M (2004) Direct interaction of GluRdelta2 with
324. Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, et al. (2000) cAMP-
325. Brandstatter JH, Dick O, Boeckers TM (2004) The postsynaptic scaffold
326. Uemura T, Mori H, Mishina M (2004) Direct interaction of GluRdelta2 with
327. Qualmann B, Boeckers TM, Jeromin M, Gundelfinger ED, Kessels MM (2004)
328. de Bartolomeis A, Fiore G (2004) Postsynaptic density scaffolding proteins at
329. Kim JY, Han W, Namkung W, Lee JH, Kim KH, et al. (2004) Inhibitory
330. Masumoto N, Ikebuchi Y, Tahara M, Yokoi T, Tasaka K, et al. (1998)
331. Grabs D, Bergmann M, Urban M, Post A, Gratzl M (1996) Rab3 proteins and
332. Park E, Na M, Choi J, Kim S, Lee JR, et al. (2003) The Shank family of
d-postsynaptic density proteins interacts with and promotes synaptic accumula-
tion of the beta PIX guanine nucleotide exchange factor for Rac1 and Cdc42.
J Biol Chem 278: 19220–19229.
333. Kriencikamp HJ, Zitzer H, Richter D (2000) Identification of proteins
interacting with the rat somatostatin receptor subtype 2. J Physiol Paris 94:
193–198.
334. Shibasaki T, Kashima Y, Matsumoto M, et al. (2002) The interaction of
synaptic proteins - a family of highly order organizing molecules of the
postsynaptic density with an emerging role in human neurological disease.
J Neurochem 81: 903–910.
335. Elders MD (2002) Molecular morphogens for dendritic spines. Trends Neurosci
25: 64–67.
336. Hernandez-Ortega K, Ferrera P, Arias C (2007) Sequential expression of cell-
cycle regulators and Alzheimer’s disease-related proteins in entorhinal cortex
after hippocampal excitotoxic damage. J Neurosci Res 85: 1744–1751.
364. Moncini S, Maccioni R, Bevilacqua A, Venturin M, Fallini C, Ratti A, et al. (2007) The cyclin-dependent kinase 5 activator p35 over-expression and amyloid beta synregia increase apoptosis in cultured neuronal cells. Neuroscience 161: 978–987.

367. Utreras E, Maccioni R, Gonzalez-Billault C (2009) Cyclin-dependent kinase 5 activator p35 is involved in Alzheimer's disease. J Alzheimer's Dis 11: 191–201.

367. Utreras E, Maccioni R, Gonzalez-Billault C (2009) Cyclin-dependent kinase 5 activator p35 involved in Alzheimer's disease regulates insulin gene transcription in pancreatic beta-cells. Endocrinology 145: 3023–3031.

368. Matro I, Vazquez-Higuera JL, Sanchez-Juan P, Rodriguez-Rodriguez E, Infante J, et al. (2006) Epistasis between tau phosphorylation-regulating genes (CDK5R1 and GSK3beta) and Alzheimer's disease risk. Acta Neurol Scand. 114: 196–203.

369. Moncini S, Bevilacqua A, Venturin M, Fallini C, Ratti A, et al. (2007) The 3 untranslated region of human Cyclin-Dependent Kinase 5 Regulatory subunit 1 contains regulatory elements affecting transcript stability. BMC Mol Biol 8: 111.

370. Rademakers R, Sleevers K, Thunn J, Van den Broeck M, Bel Kacem S, et al. (2005) Association of cyclin-dependent kinase 5 and neuronal activators p35 and p39 complex in early-onset Alzheimer's disease. Neurobiol Aging 26: 1149–1151.

371. Lopes JP, Oliveira CR, Agostinho P (2007) Role of cyclin-dependent kinase 5 in the neuroregenerative process triggered by amyloid-beta and prion peptides: implications for Alzheimer's disease and prion-related encephalopathies. Cell Mol Neurobiol 27: 945–957.

372. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

373. Patrick GN, Zukerberg L, Nikolic M, de la Monte S, Dikkes P, et al. (1999) Conversion of p35 to p25 deregulates CDK5 activity and promotes neurodegeneration. Nature 402: 615–622.

374. Orellana DI, Quintanilla RA, Maccioni RB (2007) Neuroprotective effect of interleukin-6 induces Alzheimer-type phosphorylation of tau protein by inhibiting the beta-amyloid precursor protein and inhibit Abeta production. Neurobiol Dis 30: 335–346.

375. Ramanathan S, Woodroffe A, Flodman PL, Mays LZ, Hansoni S, et al. (2004) A case of autism with an interstitial deletion on 4q leading to hemizygosity for genes encoding for glutamatergic and glutamate neurotransmitter receptor subunits (AMPa 2, GLRA3, GLRB and neuropeptide receptors NPY1R, NPY3R. BMC Med Genet 5: 10.

376. Camins A, Verdaguer E, Folch J, Canudas AM, Pallas M (2006) The role of PSD-95, two Maguk proteins involved in synaptic trafficking of AMPA receptors. J Neurochem 97: 1 contains regulatory elements affecting transcript stability. BMC Mol Biol 8: 111.

377. Quintanilla RA, Orellana DI, Gonzalez-Billault C, Maccioni RB (2004) Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdK5/p35 pathway. Exp Cell Res 295: 245–257.

378. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

379. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

380. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

381. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

382. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

383. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

384. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

385. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

386. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

387. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

388. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

389. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

390. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

391. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

392. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

393. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

394. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

395. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

396. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

397. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

398. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

399. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

400. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

401. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

402. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

403. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

404. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

405. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

406. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

407. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

408. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

409. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

410. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.

411. Monaco EA, 3rd (2004) Recent evidence regarding a role for CDk5 dysregulation in Alzheimer's disease. Curr Alzheimer Res 1: 53–58.
443. MacDonald AW, 3rd, Chafee MV (2006) Translational and developmental perspective on N-methyl-D-aspartate synaptic deficits in schizophrenia. Dev Psychopathol 18: 833–876.

444. Jaworski A, Burden SJ (2006) Neurovascular syncytium formation in mice lacking motor neuron- and skeletal muscle-derived Neuroglian-1. J Neurosci 26: 653–661.

445. Jacobsen C, Duggan D, Fischbach G (2004) Neurogliin induces the expression of transcription factors and myosin heavy chains in mouse spindle cells cultured in human cultured muscle. Proc Natl Acad Sci U S A 101: 12218–12223.

446. Okada M, Corfas G (2004) Neurogliin downregulates postsynaptic GABAA receptors at the hippocampal inhibitory synapse. Hippocampus 14: 337–344.

447. Omer AO (2000) Dual controls on synaptic formation and regression in schizophrenia: neurogliin, neuregulin, dystrobrevin, DSC1, M6K and agrin. Neuropharmacology 41: 662–677.

448. Papadopoulos V, Lecanu L, Brown RC, Han Z, Yao ZX (2006) Peripheral-type benzodiazepine receptors in the hippocampus, neuropathology and neurological disorders. Neuroscience 138: 749–756.

449. Hazell AS (2002) Astrocytes and manganese neurotoxicity. Neurochem Int 41: 271–277.

450. Roberts JC, Friel SL, Roman S, Ferren M, Harper A, et al. (2009) Anatomical and functional imaging paradigm in a potential model system for Alzheimer disease. J Neurosci 30: 271–277.

451. Reiners J, Nagel-Wolfrum K, Jurgens K, Marker T, Wolfrum U (2006) Neuregulin 1-stimulated phosphorylation of Akt, Psychosis Proneness, and Habituation of Arousal in Nonclinical Individuals. Schizophren Bull 32: 939–948.

452. Schijndel JE, Loo KM, Zweeden MV, Djurovic S, Andreasen NA, et al. (2009) Three-cohort targeted gene screening reveals a non-synonymous TRKA polymorphism associated with schizophrenia. J Psychiatr Res 43: 177–186.

453. So HC, Fong PY, Chen HY, Hui TC, Ng MY, et al. (2009) Identification of neuroregulin 1C and interacting partners as potential susceptibility genes for schizophrenia in a Southern Chinese population. Am J Med Genet B Neuropsychiatr Genet 149B: 82–89.

454. Wood JD, Bonath F, Kumar S, Ross CA, Cunliffe VT (2009) Disrupted-in- development of peripheral benzodiazepine receptor expression as biomarkers of detrimental versus beneficial glial responses in mouse models of Alzheimer's and other CNS neurological disorders. J Neurosci 29: 12253–12267.

455. Gulyas B, Makai B, Kasa P, Gulya B, Bakota I, et al. (2009) A Combined pharmacological and autoradiography study in post mortem whole hemisphere human brain slices taken from Alzheimer's and age-matched controls using two radio labelled DAA1106 analogues with high affinity to the peripheral benzodiazepine receptor (PBR) system. Neurochem Int 54: 28–36.

456. Wang M, Gao M, Hutchins GD, Zheng QH (2009) Synthesis of [11C]PE20D1106 as a new imaging probe for peripheral benzodiazepine receptor and microglial cell visualization. Bioconjug Chem 18: 1397–1407.

457. Walker DG, Dahling-Hernandez JE, Lue LF (2008) Human postmortem brain-derived cerebrovascular smooth muscle cells express all genes of the classical complement pathway: a potential mechanism for vascular damage in cerebral amyloid angiopathy and Alzheimer's disease. Microvasc Res 75: 411–419.

458. Vreven R, Jansen I, De Groot CJ, Van Muiswinkel FL, Hack CE, et al. (1999) Cytochrome P450 enzymes at amyloid plaques in Alzheimer's disease brain stimulate human glut and neuronal cell cultures to secrete early complement proteins, but not C1-inhibitor. Exp Neurol 160: 289–299.

459. Yasojima K, McGeer EG, McGeer PL (1999) Complement regulators C1 inhibitor and CD59 do not significantly inhibit complement activation in Alzheimer brain cortex. Brain Res 832: 297–301.

460. Bergamaschi L, Cianziani S, Bottasso B, Cugno M, Bradotti P, et al. (1999) Alzheimer's beta-amyloid peptides can activate the early components of complement classical pathway in a C1q-independent manner. J Biol Chem 274: 526–533.

461. Yasojima K, Schwab C, McGeer EG, McGeer PL (1999) Up-regulated production and activation of the complement system in Alzheimer's disease brain. J Alzheimers Dis 1: 93–107.

462. Terai K, Walker DG, McGeer EG, McGeer PL (1997) Neurons express proteins of the classical complement pathway in Alzheimer disease brain. Brain Res 769: 385–390.

463. Mori M, Sawahita J, Higuchi K (2007) Functional polymorphisms of the Las and Fdb1 genes in laboratory rats. Exp Anim 56: 93–101.

464. Funechill U, Saher G, Xiao L, Mobius W, Nave KA (2007) Survival of mature photoreceptors at the retinal boundary is dependent on the transcription factors and myosin heavy chains typical of muscle spindles in cultured human muscle. Proc Natl Acad Sci U S A 104: 3369–3374.

465. Le Strat Y, Ramoz G, Gloor P (2009) The role of genes involved in neuroplasticity and neurogenesis in the observation of a gene-environment interaction (GxE) in schizophrenia. Curr Mol Med 9: 506–518.

466. Schijndel JE, Loo KM, Zweeden MV, Djurovic S, Andreasen NA, et al. (2009) Three-cohort targeted gene screening reveals a non-synonymous TRKA polymorphism associated with schizophrenia. J Psychiatr Res 43: 177–186.

467. Kobayashi K, Hayashi M, Nakano H, Fukutani Y, Sasaki K, et al. (2002) A neuregulin 1 variant is associated with increased lateral ventricle volume in patients with first-episode schizophrenia. Biol Psychiatr Res 59: 205–212.

468. Mata I, Perez-Iglesias R, Roiz-Santianez R, Tordesillas-Gutierrez D, Gonzalez-Mandly A, et al. (2009) A neuregulin 1 variant is associated with increased lateral ventricle volume in patients with first-episode schizophrenia. Biol Psychiatry 65: 535–540.

469. Reiners J, Nagel-Wolfrum K, Jurgens K, Marker T, Wolfrum U (2006) Neuregulin 1 ICE-single-nucleotide polymorphism in first episode schizophrenia correlates with cerebral activation in frontal-temporal areas. Eur Arch Psychiatry Clin Neurosci 259: 72–77.

470. Prata DP, Breen G, Osborne S, Munro J, St Clair D, et al. (2009) An association study of the neuregulin 1 gene, bipolar affective disorder and neurocognitive functions. Neurosci Lett 499: 113–116.

471. Pedrosa E, Nolan KA, Stefanescu R, Hershcovitz P, Novak T, et al. (2009) Three-cohort targeted gene screening reveals a non-synonymous TRKA polymorphism associated with schizophrenia. J Psychiatr Res 43: 177–186.

472. Papadopoulos V, Lecanu L, Brown RC, Han Z, Yao ZX (2006) Peripheral-type benzodiazepine receptors in the hippocampus, neuropathology and neurological disorders. Neuroscience 138: 749–756.

473. Yasojima K, Schwab C, McGeer EG, McGeer PL (1999) Up-regulated production and activation of the complement system in Alzheimer's disease brain. J Alzheimers Dis 1: 93–107.

474. Bergamaschi L, Cianziani S, Bottasso B, Cugno M, Bradotti P, et al. (1999) Alzheimer's beta-amyloid peptides can activate the early components of complement classical pathway in a C1q-independent manner. J Biol Chem 274: 526–533.

475. Yasojima K, Schwab C, McGeer EG, McGeer PL (1999) Up-regulated production and activation of the complement system in Alzheimer's disease brain. J Alzheimers Dis 1: 93–107.
537. Nishimura H, Sakagami H, Ueno A, Fukunaga K, Watanabe M, et al. (2003) Cloning, characterization and expression of two alternatively splicing isoforms of Ca2+/calmodulin-dependent protein kinase I gamma in the rat brain. J Neurochem 85: 1216–1227.

538. Zhou C, Braeunewell KH (2008) Expression of the neuronal calcium sensor visinin-like protein-1 in the rat hippocampus. Neuroscience 153: 1202–1212.

539. Youn H, Jenseung M, Koo Y, Ji H, Markesbery WR, et al. (2007) Kalirin is under-expressed in Alzheimer’s disease hippocampus. J Alzheimers Dis 11: 453–457.

540. Huang JC, Bahak T, Corson TW, Chua G, Khan S, et al. (2007) Using expression profiling data to identify human microRNA targets. Nat Methods 4: 1045–1049.

541. Liu T, Papagiannakopoulos T, Puskar K, Qi S, Santiago F, et al. (2007) Detection of a microRNA signal in an in vivo expression set of rat brains. PLoS One 2: e804.

542. Nikitin A, Egorov S, Daraselia N, Mazo I (2003) Pathway studies—the analysis and navigation of molecular networks. Bioinformatics 19: 2153–2157.

543. Bucan M, Abrahms BS, Wang K, Glesner JT, Herman EL, et al. (2009) Genome-wide analyses of exonic copy number variants in a family-based study point to novel autism susceptibility genes. PLoS Genet 5: e1000536.

544. Bourgeron T (2009) A synaptic trek to autism. Curr Opin Neurobiol. 545. Youn H, Chun D, Park J, Koo Y, Ji H, Markesbery WR, et al. (2007) Kalirin is under-expressed in Alzheimer’s disease hippocampus. J Alzheimers Dis 11: 453–457.

546. Nissen R, Nyberg E, Reitan R, Brevig B (2009) Genetic risk of schizophrenia. Schizophr Res. 547. Youn H, Chun D, Park J, Koo Y, Ji H, Markesbery WR, et al. (2007) Kalirin is under-expressed in Alzheimer’s disease hippocampus. J Alzheimers Dis 11: 453–457.

548. Kim HG, Kishikawa S, Higgins AW, Seong IS, Donovan DJ, et al. (2008) Recurrent CNVs disrupt three candidate genes in schizophrenia. Hum Mol Genet 18: 988–996.

549. Northrup H, Buxar-Vokampje, van der Stelt I, Strengman E, Sabatti C, et al. (2008) Recurrent CNVs disrupt three candidate genes in schizophrenia patients. Am J Hum Genet 82: 199–207.

550. Owen MJ, Williams HJ, O’Donovan MC (2009) Schizophrenia genetics: advancing on two fronts. Curr Opin Genet Dev 19: 266–270.

551. Need AC, Ge D, Weale ME, Maia J, Feng S, et al. (2009) A genome-wide investigation of SNPs and CNVs in schizophrenia. PLoS Genet 5: e1000373.

552. Roepstorff D, Ingason A, Cichon S, Petersen OP, Barnes MR, et al. (2009) Disruption of the neurexin 1 gene is associated with schizophrenia. Hum Mol Genet 18: 988–996.

553. Vrijenhout T, Buizer-Voskampje, van der Stelt I, Strengman E, Sabatti C, et al. (2008) Recurrent CNVs disrupt three candidate genes in schizophrenia patients. Am J Hum Genet 82: 199–207.

554. Kirov G, Gusum D, Chen W, Norton N, Georgieva L, et al. (2008) Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia. Hum Mol Genet 17: 430–436.

555. Niuhaus J, Xie Y, Payal, Ma J, Hu X, Harel M, et al. (2008) Significant association of the neurexin-1 gene (NRXN1) with nicotine dependence in European- and African-American smokers. Hum Mol Genet 17: 1569–1577.

556. Tyburski A, Schuck, Shanks L, Johnson EO, Hatsuaki D, et al. (2007) Novelenty-related genome wide association study for nicotine dependence. Hum Mol Genet 16: 24–35.

557. Yang HC, Chang CC, Lin CY, Chen CL, Fann CS (2005) A genome-wide scanning and fine mapping study of COG4A data. BMC Genet 6 Suppl 1: S30.
domain of the NF-M protein and its relevance to Alzheimer’s disease. Biochemistry 33: 9627–9636.

Troxanojki QJ, Schmidt ML, Orvos L, Jr., Gur RC, Gur RE, et al. (1989) Selective expression of epitopes in multipolymerization repeats of the high and middle molecular weight neurofilament proteins in Alzheimer neurofibrillary tangles. Ann Med 21: 113–116.

Troy CM, Greene LA, Shelanski ML (1992) Neurite outgrowth in peripherin-depleted PC12 cells. J Cell Biol 117: 1085–1092.

Wang J, Tung YC, Wang Y, Li XT, Ijbaal K, et al. (2001) Hyperphosphorylation and accumulation of neurofilament proteins in Alzheimer disease brain and in okadic acid-treated SY5Y cells. FEBS Lett 507: 81–87.

Wang Y, Wang Q, Wang J (2002) [Detection of level and mutation of neurofilament rRNA in Alzheimer’s disease]. Zhongguo Yi Xue Za Zhi 82: 519–522.

Wang YP, Wei ZL, Wang XC, Wang Q, Wang JZ (2001) [Comparative study of the expression and phosphorylation of neurofilament proteins of brain gray matter area in Alzheimer’s disease]. Zhonggou Yi Xue Ke Xue Yuan Xue Bao 23: 445–449.

Wurman RJ, Ulis HJ, Cansev M, Watkins CJ, Wang L, et al. (2006) Synaptic proteins and phosphoproteins are increased in gerbil brain by administering tritiated plus docosahexaenoic acid orally. Brain Res 1080: 83–92.

Yang X, Yang Y, Luo Y, Li G, Wang J, et al. (2009) Hyperphosphorylation and Accumulation of Neurofilament Proteins in Transgenic Mice with Alzheimer Presenilin 1 Mutation. Cell Mol Neurobiol.

Yeap PY, Codner MD (1990) Reduced glycylated clathrin assembly protein API80: implication for synaptic vestige recycling dysfunction in Alzheimer’s disease. Neurosci Lett 252: 33–36.

Laterza OF, Modur VR, Crimmins DL, Olander JV, Landt Y, et al. (2006) Identification of novel brain biomarkers. Clin Chem 52: 1713–1721.

Zhao C, Anand R, Brauneck WH (2008) Nitric-oxide-induced Ca(2+)/mynitroso Switch of Neurofilament Ca(2+) Sensor VILIP1 in Hippocampal Neurons: A Possible Crotstask Mechanism for Nicotinic Receptors. Cell Mol Neurobiol.

Zhan G, Nguyen X, Brandmark M, Gloe T, Macleish A, et al. (2000) Neonatal Ca(2+) Sensor caV1.1A activates the upregulation of functional alpha2beta2 nicotinic acetylcholine receptors in hippocampal neurons. Mol Cell Neurosci.

Brian C, Kodroz SV, Sonderegger P, Grutter MG (2001) Crystal structure of neuroserpin: a neuronal serpin involved in a conformational disease. FEBS Lett 505: 18–22.

Bruno MA, Cuello AC (2006) Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor, and its proteolysis by a protease cascade. Proc Natl Acad Sci U S A 103: 6735–6740.

Carrell RW (2005) Cell toxicity and conformational disease. Trends Cell Biol 15: 574–580.

Crowther DC (2002) Familial conformational diseases and dementia. Hum Mol Genet 20: 1–14.

Dufour A, Osio LA, Gelati M, Massa G, Taricci N, et al. (2000) Mutations in the neuroserpin gene are rare in familial dementia. French Alzheimer’s Disease and Fronto-Temporal Dementia Genetics Study Groups. Ann Neurol 47: 680–686.

Kinghorn RJ, Crowther DC, Sharp LK, Nerelius C, Davis RL, et al. (2006) Neuroserpin binds Abeta and is a neuroprotective component of amyloid plaques in Alzheimer disease. J Biol Chem 281: 29268–29277.

Nielson HM, Limon L, Londo E, Blennow K, Miranda A, et al. (2007) Plasma and CSF serpins in Alzheimer disease and dementia with Lewy bodies. Neurology 69: 1569–1579.

Tabira T (2003) [Familial non-Alzheimer dementia]. Rinsho Shinkeigaku 43: 757–769.

Yamashiki M, Li W, Johnson DJ, Huntington JA (2008) Crystal structure of a stable dimer reveals the molecular basis of serpin polymerization. Nature 455: 1253–1258.

Opi WO, Joshl G, Head E, Milgram NW, Muggenburg BA, et al. (2008) Proteomic identification of brain proteins in the canine model of human aging following a long-term treatment with antioxidants and a program of behavioral enrichment: relevance to Alzheimer’s disease. Neurobiol Aging 29: 51–70.

Poon HF, Farr SA, Thongboondee Y, Lynn BC, Banks WA, et al. (2008) Proteomic analysis of specific brain proteins in aged SAMP8 mice treated with alpha-lipoic acid: implications for aging and age-related neurodegenerative disorders. Neurochem Res 33: 902–911.

Zhuo JM, Prakasam A, Murray ME, Zhang HY, Baxter MG, et al. (2008) An Alzheimer Biomarker Discovery

Wishart TM, Paterson JM, Short DM, Meredith S, Robertson KA, et al. (2007) Differential proteomics analysis of synaptic proteins identifies potential cellular targets and protein mediators of synaptic neuroprotection conferred by the slow Wallerian degeneration (Wlds) gene. Mol Cell Proteomics 6: 418–428.

Zu Campo S, Satake M, Matsushima H (1995) Signal transduction mechanisms in presenilin 1 Mutation. Cell Mol Neurobiol.

Wishart TM, Paterson JM, Short DM, Meredith S, Robertson KA, et al. (2007) Differential proteomics analysis of synaptic proteins identifies potential cellular targets and protein mediators of synaptic neuroprotection conferred by the slow Wallerian degeneration (Wlds) gene. Mol Cell Proteomics 6: 418–428.

Zhuo JM, Prakasam A, Murray ME, Zhang HY, Baxter MG, et al. (2008) An Alzheimer Biomarker Discovery

Zu Campo S, Satake M, Matsushima H (1995) Signal transduction mechanisms in presenilin 1 Mutation. Cell Mol Neurobiol.

Wishart TM, Paterson JM, Short DM, Meredith S, Robertson KA, et al. (2007) Differential proteomics analysis of synaptic proteins identifies potential cellular targets and protein mediators of synaptic neuroprotection conferred by the slow Wallerian degeneration (Wlds) gene. Mol Cell Proteomics 6: 418–428.

Zhuo JM, Prakasam A, Murray ME, Zhang HY, Baxter MG, et al. (2008) An Alzheimer Biomarker Discovery

Wishart TM, Paterson JM, Short DM, Meredith S, Robertson KA, et al. (2007) Differential proteomics analysis of synaptic proteins identifies potential cellular targets and protein mediators of synaptic neuroprotection conferred by the slow Wallerian degeneration (Wlds) gene. Mol Cell Proteomics 6: 418–428.

Zhuo JM, Prakasam A, Murray ME, Zhang HY, Baxter MG, et al. (2008) An Alzheimer Biomarker Discovery

Wishart TM, Paterson JM, Short DM, Meredith S, Robertson KA, et al. (2007) Differential proteomics analysis of synaptic proteins identifies potential cellular targets and protein mediators of synaptic neuroprotection conferred by the slow Wallerian degeneration (Wlds) gene. Mol Cell Proteomics 6: 418–428.
765. Kumar AP, Piedrafita FJ, Reynolds WF (2004) Peroxisome proliferator-
764. Kong QL, Zhang JM, Zhang ZX, Ge PJ, Xu YJ, et al. (2002) [Serum levels of
763. Kondo Y, Lemere CA, Seabrook TJ (2007) Osteopetrotic (op/op) mice have
762. Kawata T, Tsutsui K, Kohno S, Kaku M, Fujita T, et al. (2005) Amyloid beta
761. Kaku M, Tsutsui K, Motokawa M, Kawata T, Fujita T, et al. (2003) Amyloid
760. Ito S, Sawada M, Haneda M, Fujii S, Oh-Hashi K, et al. (2005) Amyloid-beta
759. Hasegawa Y, Sawada M, Ozaki N, Inagaki T, Suzumura A (2000) Increased
758. Hamilton JA, Whitty G, White AR, Jobling MF, Thompson A, et al. (2002)
756. Flanagan AM, Lader CS (1998) Update on the biologic effects of macrophage
755. Desjardins P, Ledoux S (1998) Expression of ced-3 and ced-9 homologs in
754. Du Yan S, Zhi H, Fu J, Yan SF, Roher A, et al. (1997) Amyloid-beta peptide-
753. Boissonnault V, Filali M, Lessard M, Relton J, Wong G, et al. (2009) Powerful
752. Akiyama H, Nishimura T, Kondo H, Ikeda K, Hayashi Y, et al. (1994)
751. Desjardins P, Ledoux S (1998) Expression of ced-3 and ced-9 homologs in
750. Yang G, Wang L, Zhu M, Xu D (2008) Identification of non-Alzheimer's
749. Wang H, Dong K, Li G, Peng X, Zhu H (2009) [Effect of yizhi jiannao granules on the expression of Pa1 and HMGB1 mRNA in the hippocampus of SAMP8 mice]. Zhong Nan Da Xue Xue Bao Yi Xue Ban 34: 63–66.
748. Yang G, Wang L, Zhu M, Xu D (2008) Identification of non-Alzheimer's disease tauopathy-related proteins by proteomic analysis. Neurol Sci 30: 613–622.
747. Desjardins P, Ledoux S (1998) Expression of ced-3 and ced-9 homologs in Alzheimer's disease cerebral cortex. Neurosci Lett 244: 69–72.
746. Akiyama H, Nishimura T, Kondo H, Ikeda K, Hayashi Y, et al. (1994) Expression of the receptor for macrophage colony stimulating factor by brain microglia and its upregulation in brains of patients with Alzheimer's disease and amyotrophic lateral sclerosis. Brain Res 639: 171–174.
745. Boissonnault V, Filali M, Lessard M, Relton J, Wong G, et al. (2009) Powerful beneficial effects of macrophage colony-stimulating factor on [beta]-amyloid deposition and cognitive impairment in Alzheimer's disease. Brain.
744. Du Yan S, Zhi H, Fu J, Yan SF, Roher A, et al. (1997) Amyloid-beta peptide-receptor for advanced glycation endproduct interaction elicits neuronal expression of macrophage-colony stimulating factor: a proinflammatory pathway in Alzheimer disease. Proc Natl Acad Sci U S A 94: 5296–5301.
743. Ebadi M, Bashir RM, Heidrick ML, Hamada FM, Refaey HE, et al. (1997) Neurotrophins and their receptors in nerve injury and repair. Neurochem Int 30: 347–374.
742. Flanagan AM, Lader CS (1998) Update on the biologic effects of macrophage colony-stimulating factor. Curr Opin Hematol 5: 181–183.
741. Georganopoulou DG, Chang L, Nam JM, Thaxton CS, Murison EJ, et al. (2005) Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer’s disease. Proc Natl Acad Sci U S A 102: 2274–2276.
740. Hamilton JA, Whitty G, White AR, Jobling MF, Thompson A, et al. (2002) Alzheimer’s disease amyloid beta and prion protein amyloidogenic peptides promote macrophage survival, DNA synthesis and enhanced proliferative response to CSF-1 (M-CSF). Brain Res 940: 49–54.
739. Haegawa Y, Sawada M, Ozaki N, Inagaki T, Suzumura A (2000) Increased soluble tumor necrosis factor receptor levels in the serum of elderly people. Gerontology 46: 105–108.
738. Ito S, Sawada M, Haneda M, Fujiyama O, Oishi A, Kishida K, et al. (2005) Amyloid-beta peptides induce cell proliferation and macrophage colony-stimulating factor expression via the PI3-kinase/Akt pathway in cultured Ra2 macroglial cells. FEBS Lett 579: 1995–2000.
737. Koku M, Tsutsui K, Motokawa M, Kawata T, Fujita T, et al. (2003) Amyloid beta protein deposition and neuron loss in osteopetrotic (op/op) mice. Brain Res Brain Res Protoc 12: 104–108.
736. Koku T, Leber EM, Sealbrook TJ (2007) Osteopetrotic (op/op) mice have reduced microglia, no Alzheimer deposition, and no changes in dopaminergic neurons. J Neuroinflammation 4: 51.
735. Kong QL, Zhang JM, Zhang ZX, Ge PJ, Xu YJ, et al. (2002) [Serum levels of macrophage colony stimulating factor in the patients with Alzheimer’s disease]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 34: 298–301.
734. Kumar AP, Piedrafita FJ, Reynolds WF (2004) Peroxisome proliferator-
733. Kong QL, Zhang JM, Zhang ZX, Ge PJ, Xu YJ, et al. (2002) [Serum levels of macrophage colony stimulating factor in the patients with Alzheimer’s disease]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 34: 298–301.
732. Kumar AP, Piedrafita FJ, Reynolds WF (2004) Peroxisome proliferator-
731. Kong QL, Zhang JM, Zhang ZX, Ge PJ, Xu YJ, et al. (2002) [Serum levels of macrophage colony stimulating factor in the patients with Alzheimer’s disease]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 34: 298–301.