**Table S1.** Primers used in the study for validation of microarray data

Table S1 continued overleaf

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene Symbol | Ref Seq ID | Primer (5’ – 3’) | Product size (bp) | Annealing Temperature |
| Β-ACTIN | NM\_001101.3 | Forward: CATGTACGTTGCTATCCAGGC | 184 | 60 |
| Reverse: CTCCTTAATGTCACGCACGAT |
| GAPDH | NM\_001289745.1 | Forward: TGTTGCCATCAATGACCCCTT | 202 | 60 |
| Reverse: CTCCACGACGTACTCAGCG |
| SESN1 | NM\_001199934.1 | Forward: TGCTTTGGGCCGTTTGGATAA | 131 | 60 |
| Reverse: TGTAGTGACGATAATGTAGGGGT |
| CNOT8 | NM\_004779.4 | Forward: GGCGGCTCTATGGCTTAGG | 140 | 60 |
| Reverse: CATGTTGTTGATAATCGCCAGGA |
| DDIT4 | NM\_019058.2 | Forward: AGTGCCCTCCAAGACAGAGA | 110 | 60 |
| Reverse: GTATGTGTCCCCAATGCACA |
| MAP2K1 | NM\_002755 | Forward: TTCAAGGTCTCCCACAAGCCATCT | 213 | 60 |
| Reverse: TTGATCCAAGGACCCACCATCCAT |
| UCP3 | NM\_022803.2 | Forward: AAGGTCCAAGGTCAGGCCAG | 89 | 60 |
| Reverse: GCGATGGTTCTGTAGGCGTC |
| ABCA9 | NM\_080283.3 | Forward: TTGCACCTGAATCCAAAACTACC | 147 | 59 |
| Reverse: TGACTCTCACTGCGTCTATTGAA |

**Table S2.** Medication history

Table S2 continued overleaf

|  |  |  |  |
| --- | --- | --- | --- |
| Drugs | AD (*n*=90) | NDC (*n*=90) | *p*-value† |
| AChEI  NMDA antagonist  Antidepressant  Biguanides  Antiplatlet  β- blocker  Benzodiazepine  Calcium channel blocker  Diuretics  Calcium supplement  Digitalis  Atypical antipsychotic  Antipsychotic  A2RA  Steroids  Statins  Sulphonylurea  Insulin  Nitrates  α blocker  Proton pump inhibitor  Antihistamine  ACE-inhibitor  Vitamin and supplements | 49 (54.4%)  25 (27.8%)  14 (15.6%)  7 (7.8%)  5 (5.6%)  2 (2.2%)  1 (1.1%)  9 (10.0%)  1 (1.1%)  9 (10.0%)  1 (1.1%)  2 (2.2%)  3 (3.3%)  2 (2.2%)  1 (1.1%)  9 (10.0%)  2 (2.2%)  0 (0%)  0 (0%)  0 (0%)  0 (0%)  0 (0%)  5 (5.6%)  7 (7.8%) | 0 (0%)  0 (0%)  7 (7.8%)  16 (17.8%)  12 (13.3%)  9 (10.0%)  1 (1.1%)  17 (18.9%)  1 (1.1%)  17 (18.9%)  1 (1.1%)  0 (0%)  0 (0%)  7 (7.8%)  7 (7.8%)  26 (28.9%)  6 (6.7%)  3 (3.3%)  2 (2.2%)  2 (2.2%)  5 (5.6%)  5 (5.6%)  13 (14.4%)  9 (10.0%) | <0.0001\*\*\*  <0.0001\*\*\*  0.16  0.07  0.12  0.06  1.0  0.14  1.0  0.14  1.0  0.5  0.25  0.17  0.06  0.02\*  0.28  0.25  0.5  0.5  0.62  0.62  0.08  0.59 |

A2RA, angiotensin-II receptor antagonist; ACE, Angiotensin-converting-enzyme; AChEI, acetylcholinesterase inhibitor; AD, Alzheimer’s disease; NMDA, N-methyl-D-aspartate

\**p* < 0.05; \*\*\**p* < 0.001. †*p*-value of signiﬁcant difference when compared with NDCs.

F = Fisher’s exact test. By default, all χ² tests are two-sided

**Table S3.** Fifty genes that best differentiated probable AD patients from NDC subjects in the training cohort

Table S3 continued overleaf

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene Probe | *p* | FC | Gene Name | Z-score |
| A\_23\_P45871|10964 | 4.42E-05 | 2.449567 | IFI44L | 2.018718363 |
| A\_23\_P110846|9337 | 1.54E-07 | 2.400566 | CNOT8 | 1.855511 |
| A\_23\_P64828|4938 | 1.96E-07 | 2.078037 | OAS1 | 1.852635 |
| A\_24\_P296070|83548 | 3.30E-07 | 2.412684 | COG3 | 1.84605 |
| A\_23\_P206107|25989 | 6.08E-10 | 2.163369 | ULK3 | 1.832737 |
| A\_23\_P33216|506 | 1.75E-08 | 2.268937 | ATP5B | 1.822349 |
| A\_23\_P104318|54541 | 1.96E-07 | 2.287799 | DDIT4 | 1.819804 |
| A\_23\_P152272|10921 | 4.75E-08 | 2.315216 | RNPS1 | 1.818584 |
| A\_23\_P20248|5604 | 1.63E-07 | 2.345263 | MAP2K1 | 1.801754 |
| A\_23\_P13359|10482 | 1.66E-07 | 2.250579 | NXF1 | 1.794138 |
| A\_24\_P299911|23178 | 1.89E-08 | 2.081516 | PASK | 1.793923 |
| A\_23\_P431388|90853 | 1.94E-08 | 2.001294 | SPOCD1 | 1.785833 |
| A\_24\_P361006|4704 | 2.61E-07 | 2.17775 | NDUFA9 | 1.782192 |
| A\_23\_P93562|27244 | 6.08E-10 | 2.086743 | SESN1 | 1.775372 |
| A\_24\_P156388|55020 | 2.60E-06 | 2.231063 | TTC38 | 1.764472 |
| A\_32\_P156963|71 | 7.23E-07 | 2.237426 | ACTG1 | 1.76122 |
| A\_32\_P101689|10447 | 1.63E-07 | 2.013296 | FAM3C | 1.755287 |
| A\_23\_P77228|64784 | 1.94E-08 | 2.148847 | CRTC3 | 1.740672 |
| A\_23\_P433990|6687 | 1.94E-08 | 2.097137 | SPG7 | 1.739235 |
| A\_23\_P252653|10494 | 6.08E-10 | 2.077479 | STK25 | 1.727794 |
| A\_23\_P212159|23225 | 6.51E-09 | 2.019442 | NUP210 | 1.709894 |
| A\_23\_P149470|4720 | 3.65E-08 | 2.123214 | NDUFS2 | 1.708397 |
| A\_24\_P21044|84262 | 8.90E-09 | 2.101381 | PSMG3 | 1.703799 |
| A\_24\_P278747|894 | 1.16E-07 | 2.058847 | CCND2 | 1.701463 |
| A\_24\_P319715|10130 | 3.65E-08 | 2.004103 | PDIA6 | 1.694528 |
| A\_33\_P3237225|55030 | 5.41E-08 | 2.095568 | FBXO34 | 1.685884 |
| A\_23\_P193|64789 | 1.94E-08 | 2.016798 | EXO5 | 1.68577 |
| A\_24\_P253215|10436 | 1.75E-08 | 2.022757 | EMG1 | 1.681429 |
| A\_23\_P251303|9919 | 2.88E-08 | 2.015887 | SEC16A | 1.679321 |
| A\_32\_P191084|54442 | 7.33E-08 | 2.072382 | KCTD5 | 1.678761 |
| A\_24\_P313186|801 | 9.26E-07 | 2.080052 | CALM1 | 1.647336 |
| A\_23\_P88249|55147 | 2.36E-06 | 2.025029 | RBM23 | 1.644011 |
| A\_23\_P144311|5431 | 1.94E-08 | 2.004818 | POLR2B | 1.636125 |
| A\_23\_P68892|51493 | 1.89E-08 | 2.000509 | RTCB | 1.629909 |
| A\_24\_P322741|3588 | 2.66E-05 | 2.036489 | IL10RB | 1.628496 |
| A\_23\_P252700|25972 | 2.10E-06 | 2.011735 | UNC50 | 1.623706 |
| A\_23\_P31816|1668 | 1.89E-08 | -3.03403 | DEFA3 | -0.95574 |
| A\_24\_P292470|7352 | 6.08E-10 | -3.06828 | UCP3 | -0.96049 |
| A\_23\_P153562|728 | 5.56E-11 | -3.20331 | C5AR1 | -0.97664 |
| A\_33\_P3234457 | 1.35E-10 | -2.24142 | FBRSL1 | -0.97785 |
| A\_33\_P3226542 | 4.84E-05 | -3.49026 | SNORD3B-1 | -1.03336 |
| A\_33\_P3316505|6080 | 3.93E-06 | -4.77601 | SNORA73A | -1.17746 |
| A\_33\_P3382595|125050 | 7.23E-07 | -4.20134 | RN7SK | -1.22478 |
| A\_33\_P3399064|100008587 | 3.00E-07 | -5.3465 | RNA5-8S5 | -1.59326 |
| A\_23\_P253791|820 | 4.92E-07 | -2.43783 | CAMP | -1.61097 |
| A\_33\_P3414799|6452 | 1.61E-06 | -2.25861 | SH3BP2 | -1.69143 |
| A\_33\_P3220425 | 2.50E-07 | -3.20409 | XLOC\_014512 | -1.743862 |
| A\_33\_P3292179|10350 | 1.25E-05 | -2.12523 | ABCA9 | -1.80913 |
| A\_33\_P3318946|60484 | 1.66E-07 | -5.49406 | HAPLN2 | -1.82571 |
| A\_33\_P3209581|440073 | 3.41E-08 | -2.47946 | IQSEC3 | -2.49206 |

**Table S4.** Canonical pathways that were significant in AD based on the Ingenuity Pathway Analysis

|  |  |
| --- | --- |
| Canonical pathways | *p*-value |
| Granzyme A signaling  Chemokine signaling  Oxidative phosphorylation  Protein Kinase A signaling  STAT3 pathway  Mitochondrial dysfunction  Integrin signalling  VEGF signalling  Natural killer cell signaling  IL-6 signaling  Complement system  Interferon signaling  Axonal guidance signaling  iNOS signalling  Gap junction signaling | 1.20E-04  6.86E-03  2.36E-02  5.31E-02  6.01E-02  7.39E-02  8.68E-02  9.26E-02  1.25E-01  1.29E-01  1.63E-01  1.68E-01  2.01E-01  2.20E-01  2.20E-01 |

**Table S5.** Standard curves of the RT-qPCR analysis

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Quantitation Information | β-actin | GAPDH | SESN1 | CNOT8 | DDIT4 | MAP2K1 | | ABCA9 | | UCP3 |
| Threshold | 0.174 | 0.19189 | 0.1396 | 0.03651 | 0.25004 | 0.08171 | 0.09288 | | 0.2366 | |
| Left Threshold | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | |
| Standard Curve Imported | No | No | No | No | No | No | No | | No | |
| Standard Curve (1) | conc= 10^(-0.319\*CT + 9.887) | conc= 10^(-0.313\*CT + 10.721) | conc= 10^(-0.318\*CT + 11.196) | conc= 10^(-0.313\*CT + 10.165) | conc= 10^(-0.282\*CT + 9.933) | conc= 10^(-0.277\*CT + 9.762) | conc= 10^(-0.297\*CT + 10.619) | | conc= 10^(-0.333\*CT + 11.915) | |
| Standard Curve (2) | CT = -3.135\*log(conc) + 30.993 | CT = -3.196\*log(conc) + 34.268 | CT = -3.147\*log(conc) + 35.237 | CT = -3.200\*log(conc) + 32.523 | CT = -3.543\*log(conc) + 35.191 | CT = -3.611\*log(conc) + 35.246 | CT = -3.372\*log(conc) + 35.807 | | CT = -3.004\*log(conc) + 35.792 | |
| Reaction efficiency (\*) | 1.08456 (\* = 10^(-1/m) - 1) | 1.05526 (\* = 10^(-1/m) - 1) | 1.07838 (\* = 10^(-1/m) - 1) | 1.05371 (\* = 10^(-1/m) - 1) | 0.91534 (\* = 10^(-1/m) - 1) | 0.89215 (\* = 10^(-1/m) - 1) | 0.97961 (\* = 10^(-1/m) - 1) | | 1.1523 (\* = 10^(-1/m) - 1) | |
| M | -3.13466 | -3.19624 | -3.14737 | -3.19961 | -3.54301 | -3.61069 | -3.37177 | | -3.00389 | |
| B | 30.99274 | 34.26825 | 35.23706 | 32.52251 | 35.191 | 35.24626 | 35.80652 | | 37.40839 | |
| R Value | 0.99737 | 0.99852 | 0.99648 | 0.99364 | 0.995 | 0.98919 | 0.99719 | | 0.99709 | |
| R^2 Value | 0.99475 | 0.99704 | 0.99298 | 0.98732 | 0.99003 | 0.97851 | 0.9944 | | 0.99419 | |
| Start normalising from cycle | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | |
| Noise Slope Correction | Yes | Yes | No | No | No | No | No | | Yes | |