Supplementary figures and tables for

Alzheimer's brains show inter-related changes in RNA and lipid metabolism

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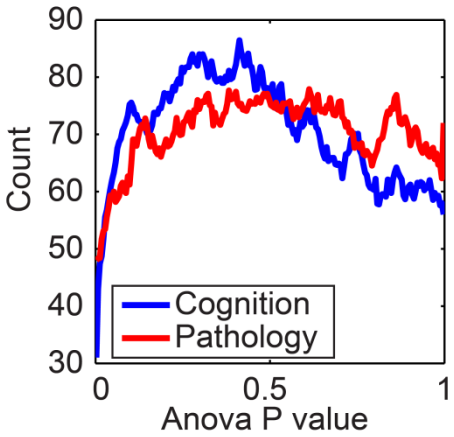
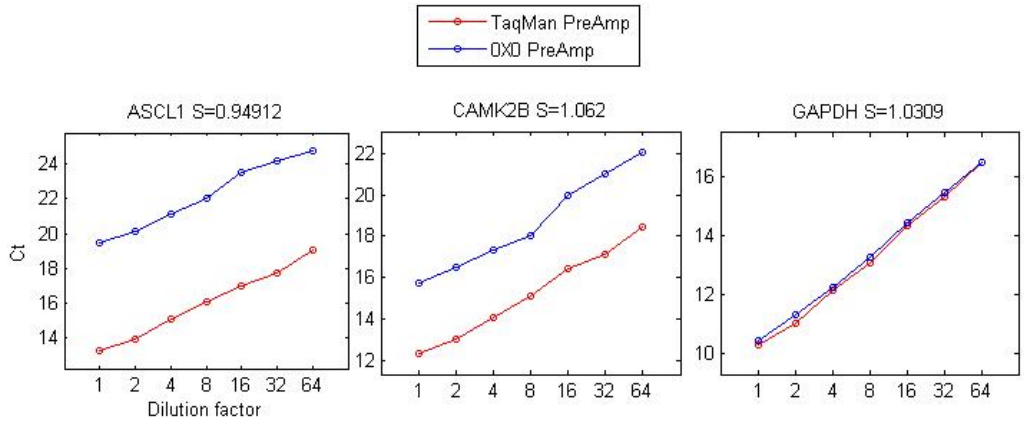
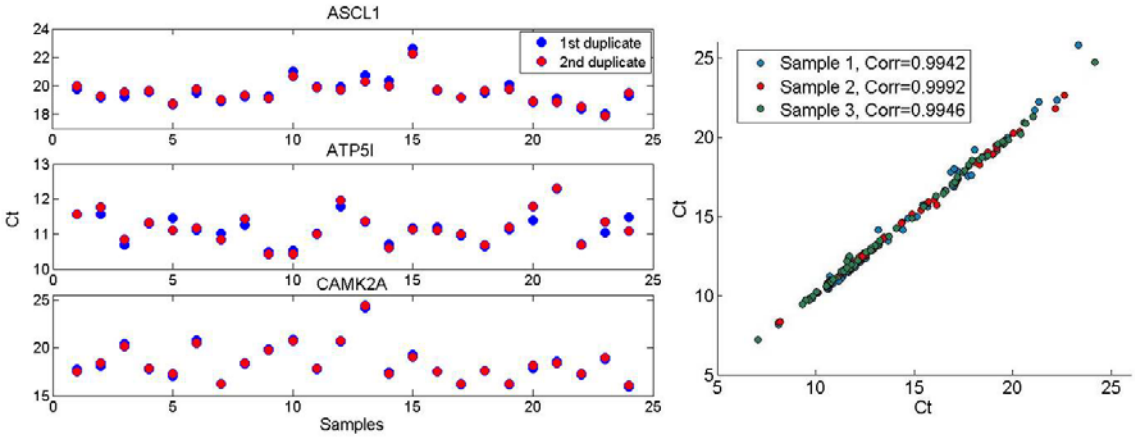
Supplementary Figures



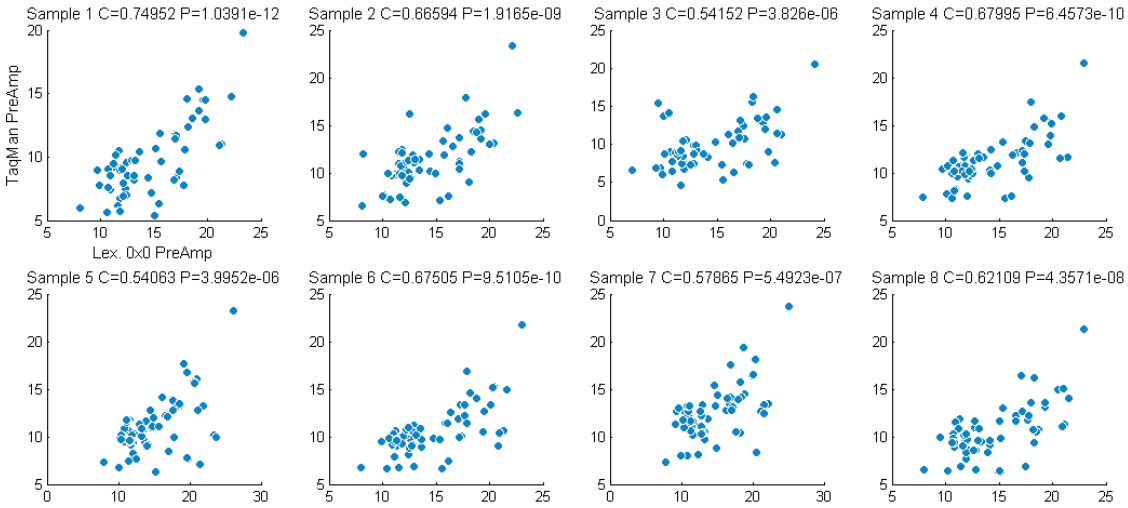
Supplementary Figure 1: strong association with cognitive deterioration. Sequencing data of the RUSH cohort was 2-Way-ANOVA analyzed with cognitive deterioration levels and pathology level being the two parameters. Shown are distributions of the 2-Way-ANOVA P values for each of the parameters.



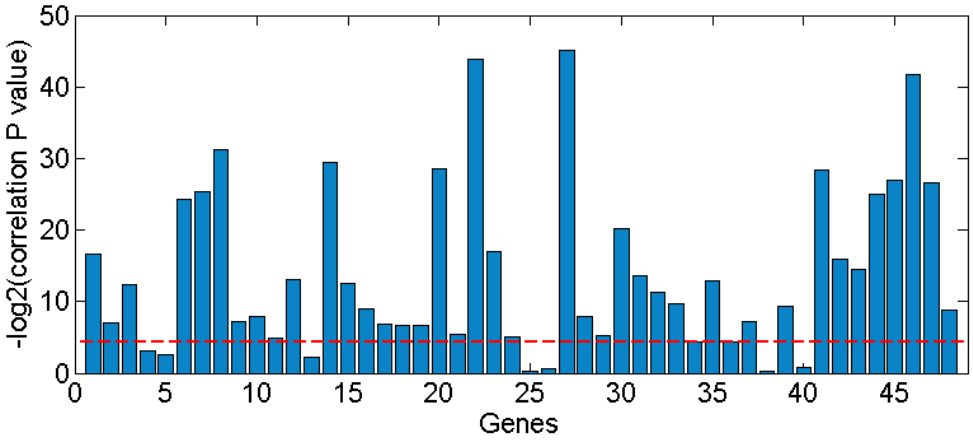
Supplementary Figure 2: Calibration curve for the ASCL1, CAMK2B and GAPDH genes which have been investigated in the Fluidigm RT experiment. The calculated slope, S, was 0.94912. The calculated slope for the calibration curves of the entire gene list is shown in Supplementary Table 3. Two cDNA synthesis reactions were examined; using a single universal primer from both 5' and 3' ends (0×0 PreAmp; blue) and pooling the primers for the Fluidigm examined genes (TaqMan PreAmp; red).



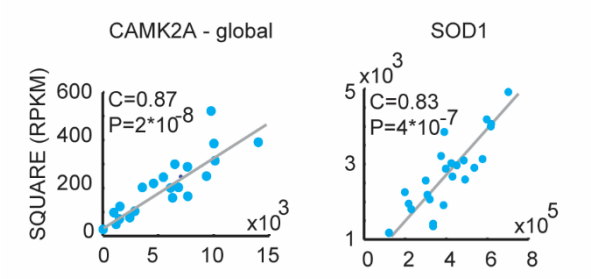
Supplementary Figure 3: Signal to noise in the Fluidigm system. Shown are technical duplicates from each of the 24 patients for three exemplary genes (left). The technical variance constitutes less than 1% of the biological variance between samples. For the first three samples the technical duplicates of all 65 validated genes are plotted in one scatter plot (right).



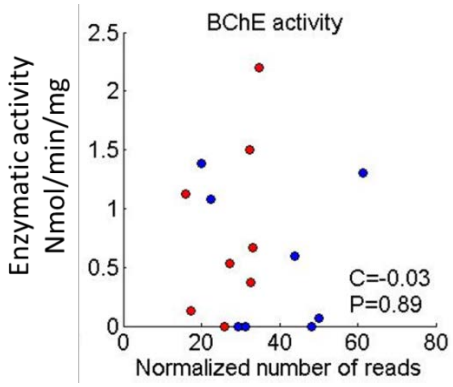
Supplementary Figure 4: High correlation between two methods of DNA pre-amplification. The DNA was amplified either with one universal primer pair or by pooling the primers of the 80 chosen genes and running 10 RT cycles. Shown are scatter plots for the threshold cycle, ct, of the RT reaction for 65 genes for each Control sample separately and the correlation coefficient and P value for each sample.



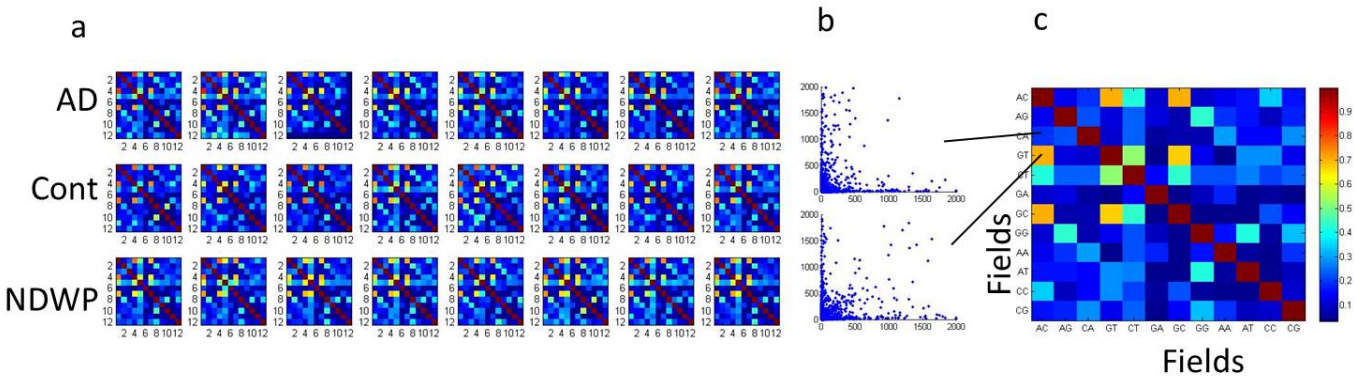
Supplementary Figure 5: Correlation P values (-log2 scale) for gene expression measured by the SQUARE sequencing and the Fluidigm system. Red line marks significant level of 0.05.



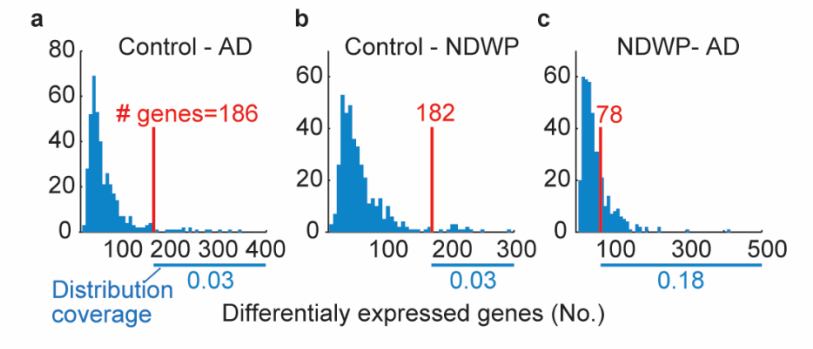
Supplementary Figure 6: SQUARE sequencing results (Y axis, RPKM) show high correlation to Fluidigm RT-PCR measurements (X axis, arbitrary units; A.U., delta-delta-CT normalized) for global gene expression of the CAMK2A gene and for the known distal and proximal 3'UTR variants of SOD1.



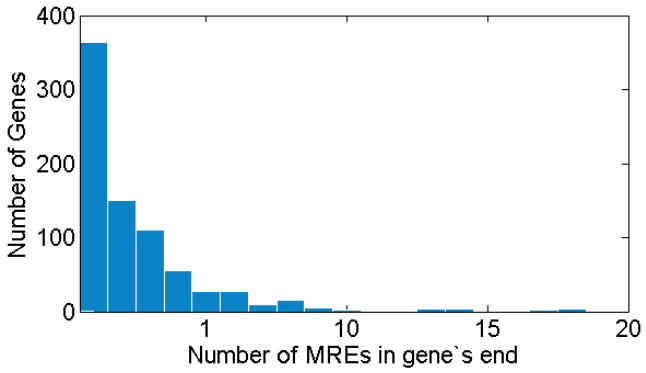
Supplementary Figure 7: Scatter plot of butyrylcholinesterase (BChE) enzymatic activity (Y axis) and normalized number of reads (X axis) shows no correlation between the two measures.



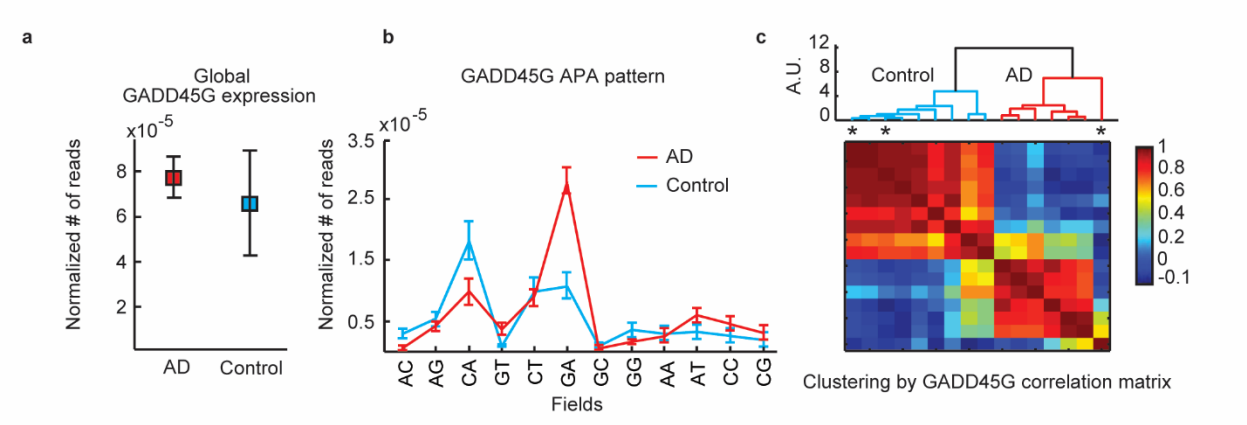
Supplementary Figure 8: No correlation between SQUARE fields. Shown are correlation matrices between the different SQUARE fields, across all genes, for each patient separately (a) and averaged across the patient cohort (c). Shown in (b) are two examples of a scatter plot of expression for all genes in two fields, AC compared to CA (upper graph) and AC compared to GT (lower graph). Color bar on the right represents correlation coefficient from 0 to 1.



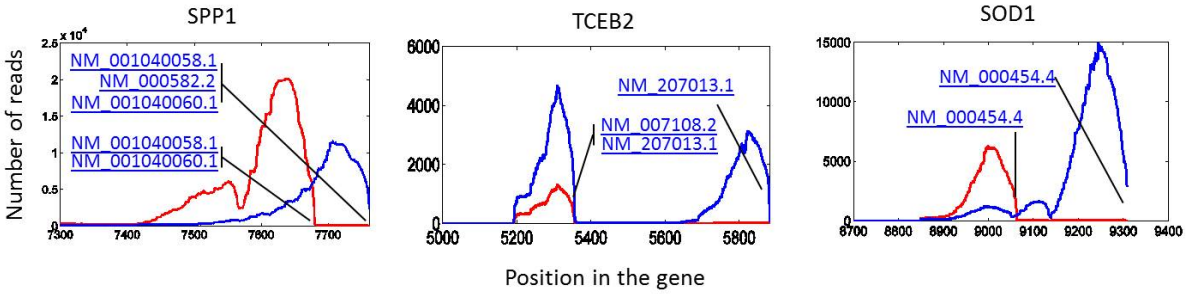
Supplementary Figure 9: Number of iterations in which specified numbers of differentially expressed genes were identified in SQUARE field 'AC' (see Supplemental Table 4 for all fields' analyses). Note the distinct comparisons of distributions coverage for the controls vs. AD (a), controls vs. NDWP (b) and NDWP vs. AD cohorts (c).



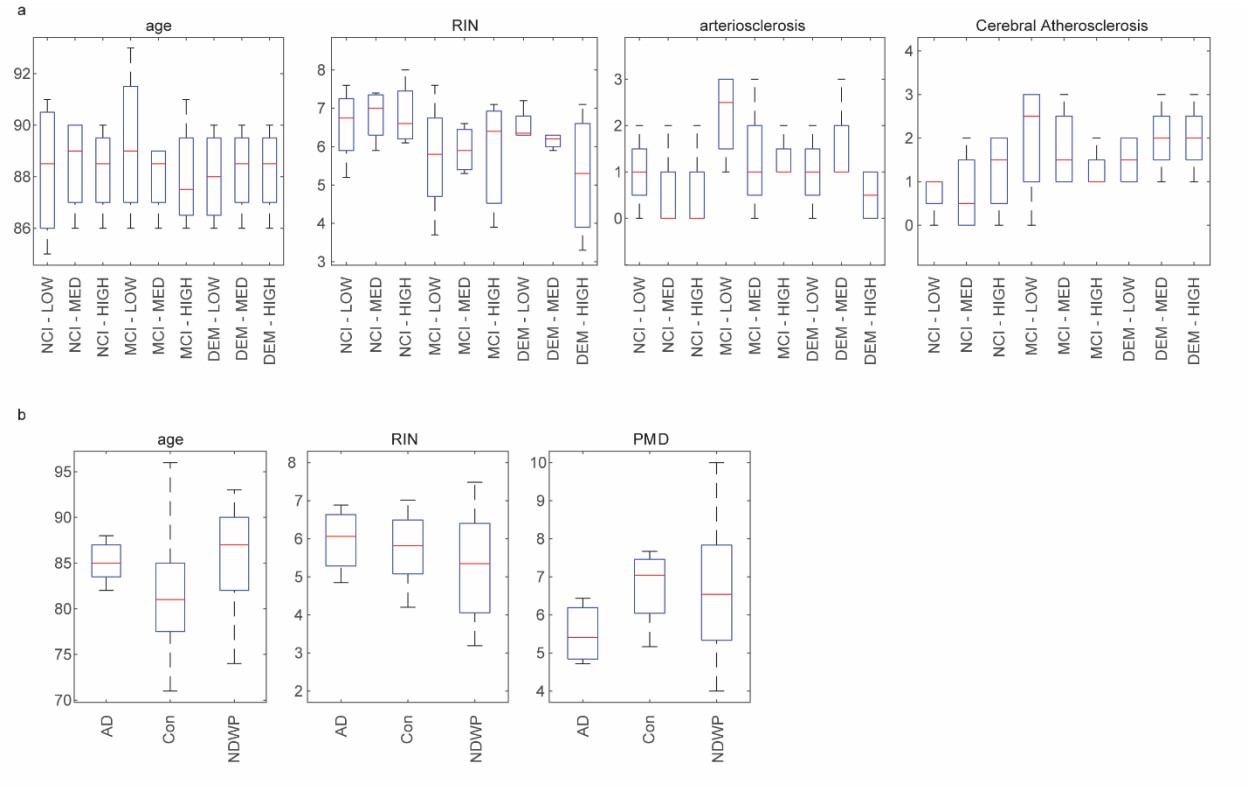
Supplementary Figure 10: Histogram showing numbers of genes with different numbers of detected MREs overlapping the region of 5 nucleotides upstream of the putative distal poly(A) site. MREs were identified with the microT prediction algorithm.



Supplementary Figure 11: (a) Expression levels of GADD45G in AD patients and controls. (b) APA pattern of GADD45G in AD (1) and controls (blue). Shown are averages ± SEM of expression in each SQUARE field in the patient groups, note switch between the proximal and distal products from controls to AD. Fields 'CA' and 'GA', but none of the others neither the global transcript counts show significant change. (c) APA ratio correlation matrix for the GADD45G gene in AD and controls.



Supplementary Figure 12: Read distribution in the 3'UTR of the two major SQUARE fields for the SPP1, TCEB2 and SOD1 genes. Also shown are the RefSeq IDs of the APA variants.



Supplementary Figure 13: Boxplot showing median (red line), 25th and 75th percentiles (box edges) and 2 standard deviations (whiskers) of measured confounders across the patient groups for available sample parameters for the 36 human sample cohort (a) and the 24 human sample cohort (b). Confounders include age, RNA integrity number (RIN), post mortem delay (PMD) and the risk factors Arteriosclerosis and Cerebral Atherosclerosis as measured by Buchman et. al. (2).

Supplementary Tables

Supplementary Table 1: Full data on first cohort (n=36, RUSH) of human brain samples from which total RNA was extracted and sequenced. Cognitive State shown in 'group', NCI=no cognitive impairments, MCI=mild cognitive impairments, DEM=demented. 'Low', 'MED' and 'HIGH' refers to low, medium and high Braak staging. Arteriosclerosis: describes the histological changes commonly found in the small vessels of the brain in aging.

index	group	Braak	Age of death	Arteriosclerosis	Cerebral Atherosclerosis
1	MCI - LOW	1	90	3.0	3.0
2	NCI - MED	4	90	0.0	0.0

3	DEM - HIGH	5	89	1.0	3.0
4	NCI - HIGH	5	90	0.0	2.0
5	NCI - HIGH	5	89	0.0	1.0
6	NCI - MED	3	88	2.0	2.0
7	MCI - MED	4	89	0.0	1.0
8	DEM - MED	3	89	1.0	2.0
9	NCI - LOW	2	91	1.0	1.0
10	NCI - MED	4	86	0.0	0.0
11	DEM - HIGH	5	90	0.0	2.0
12	DEM - HIGH	5	86	1.0	2.0
13	MCI - HIGH	5	87	2.0	1.0
14	DEM - LOW	2	87	1.0	1.0
15	NCI - HIGH	5	86	2.0	2.0
16	MCI - HIGH	5	86	1.0	1.0
17	DEM - LOW	1	89	0.0	1.0
18	NCI - LOW	2	87	2.0	1.0
19	DEM - MED	4	90	1.0	3.0
20	DEM - MED	3	86	1.0	2.0
21	MCI - MED	4	88	1.0	1.0
22	DEM - MED	3	88	3.0	1.0
23	NCI - LOW	2	90	0.0	1.0
24	MCI - LOW	1	86	3.0	3.0
25	DEM - LOW	1	86	1.0	2.0
26	MCI - LOW	2	88	2.0	0.0
27	MCI - LOW	2	93	1.0	2.0
28	DEM - LOW	2	90	2.0	2.0
29	MCI - MED	4	89	3.0	3.0
30	MCI - HIGH	5	91	1.0	1.0
31	NCI - LOW	1	85	1.0	0.0
32	DEM - HIGH	5	88	0.0	1.0
33	NCI - MED	3	90	0.0	1.0
34	NCI - HIGH	5	88	0.0	0.0
35	MCI - MED	3	86	1.0	2.0
36	MCI - HIGH	5	88	1.0	2.0

Supplementary Table 2: Full data on the second cohort (n=24, NBB) of human brain samples from which total RNA was extracted and sequenced.

nbb id	autopsy	sex	age	pmd	ph	weight	region	storage
2008-047	S08/132	M	77	06:35	6.10	1250	superior temporalis gyrus	plastic bag -80°C
2000-066	S00/141	M	80	04:20	7.08	1160	inferior temporalis gyrus	plastic bag -80°C

2007-025	S07/107	M	82	05:15	6.34	1182	superior temporalis gyrus	plastic bag -80°C
2007-052	S07/197	M	82	04:15	6.41	1205	medial temporalis gyrus	plastic bag -80°C
2009-040	S09/129	M	83	06:10	5.91	1102	superior temporalis gyrus	plastic bag -80°C
2008-029	S08/085	M	84	08:05	5.95	1195	superior temporalis gyrus	plastic bag -80°C
2001-044	S01/110	M	85	04:25	6.20	1383	superior temporalis gyrus	plastic bag -80°C
2001-063	S01/145	M	85	04:45	6.38	1215	superior temporalis gyrus	plastic bag -80°C
2010-016	S10/036	M	86	06:15	?	1211	superior temporalis gyrus	plastic bag -80°C
2009-107	S09/324	M	88	04:40	6.22	1054	superior temporalis gyrus	plastic bag -80°C
2005-010	S05/039	M	93	04:30	6.46	1040	superior temporalis gyrus	plastic bag -80°C
2002-087	S02/258	M	71	07:40	6.20	1150	superior temporalis gyrus	plastic bag -80°C
2001-016	S01/054	M	77	08:25	7.19	1118	superior temporalis gyrus	plastic bag -80°C
2000-015	S00/034	M	78	05:35	6.63	1417	inferior temporalis gyrus	cryovial -80°C
2005-044	S05/159	M	80	07:15	5.80	1331	superior temporalis gyrus	plastic bag -80°C
2001-021	S01/064	M	82	07:40	6.07	1318	inferior temporalis gyrus	plastic bag -80°C
2009-005	S09/009	M	82	05:10	6.75	1087	superior temporalis gyrus	plastic bag -80°C
2001-086	S01/194	M	88	07:00	6.84	1368	superior temporalis gyrus	plastic bag -80°C
2003-035	S03/084	M	96	06:30	6.65	1300	superior temporalis gyrus	plastic bag -80°C
2005-019	S05/072	M	74	05:00	6.70	1115	superior temporalis gyrus	plastic bag -80°C
2003-084	S03/254	M	82	10:00	6.53	1488	superior temporalis gyrus	plastic bag -80°C
2009-039	S09/128	M	82	12:55	6.21	1406	superior temporalis gyrus	plastic bag -80°C
2005-073	S05/269	M	87	06:05	6.96	1468	superior temporalis gyrus	plastic bag -80°C
2009-075	S09/244	M	88	07:00	6.76	1230	superior temporalis gyrus	plastic bag -80°C

Supplementary Table 3: Full enrichment analysis results. Linked to Figure 2a.

		P value	% gene
Cognition	Organelle luman	3.39E-05	8.1
	Proteinlocalization and transoprt	2.95E-04	11
	Translation	4.37E-04	17
	Mitochondrion	1.00E-03	10
	GTP binding and activity	0.0046	5.5
	Golgi	0.0089	4.3
	Apoptosis	0.01	8.8
	Cytoskeleton	0.012	8.1
	RNA processing	0.0123	16
	Lipid synthesis	0.0182	5.2
Patholgy	Ion binding	1.00E-03	3
	Plecksterin homology	0.01	2.2
	Cell adhesion	0.0148	4.2
	Positive regulation of immune response	0.017	2.1
	Secretion by cell	0.0309	2.1
	Cytoskeleton organization	0.05	1.4

Supplementary Table 4: P values of statistical significant genes in the 2-Way-ANOVA model, separately for the parameters 'cognition' and 'pathology'. Attached as an excel file.

Supplementary Table 5: primer design for assessing gene expression in the Fluidigm system**.**

	gene	sequence 5' to 3'
1	ALDOC-L	GAAGGCAGTGGAGAAGATGG
2	ALDOC-R	CAGCAATGAGAGAGGGGAAG
3	ATP5I-L	TACCTAAAACCTCGGGCAGA
4	ATP5I-R	TTTATTCATCCGCTGCTGGT
5	MT1F-L	TCCTGCAAGTGCAAAGAGTG
6	MT1F-R	GCAAATGGGTCAAGGTGGTA
7	PINK1-L	CTGGAGTGTGAAACGCTCTG
8	PINK1-R	TCTCTGCGCTGTCTTGTCTC
9	APOE-L	ACGAGGTGAAGGAGCAGGT
10	APOE-R	GCTGCATGTCTTCCACCAG
11	MAPT-L	CATGCCTCCCTAAGACCTTG
12	MAPT-R	CCTCGTGGCTTTACTTGGAG
13	Proximal SOD1-L	AGAAAACACGGTGGGCCAAA
14	Proximal SOD1-R	TTTTTTCCACCAGTGTGCGGCCAAT
15	Distal SOD1-L	TTGTGTGACTTTTTCAGAGTTGC
16	Distal SOD1-R	TTCACAGGCTTGAATGACAAA
17	SPP1-L	GCTTCATGGAAACTCCCTGT
18	SPP1-R	TCACACCACAAAAAGATAATCACA
19	CAMK2A-L	TTCATTGGCTGACACACCTC

20	CAMK2A-R	TTTGTCTGGTGAGGGTAGGC
21	TMSB10-L	CCACAAGCTGCACTGTGAAC
22	TMSB10-R	TGCCACGAGGTGTGTTTTAT
23	CST3-L	ATTCCCACCCCTGGACTG
24	CST3-R	ACACCGGGGCTATGAGAAG
25	COX5B-L	CAGCTGGCACACTGAGCAC
26	COX5B-R	TATGGGAGAAGGAGCCAATG
27	FTL-L	CGAAAGGCTCACTCTCAAGC
28	FTL-R	CATTTGGTCCAAGGCTTGTT
29	HBB-L	GGCTGCCTATCAGAAAGTGG
30	HBB-R	GATGCTCAAGGCCCTTCATA
31	SEPW1-L	TGTCCCGTGGTTTCATCATA
32	SEPW1-R	CAAACGTTGCCACAATCAGA
33	MT3-L	TGTGGAGCACGTGGAGATAG
34	MT3-R	GTCATTCCTCCAAGGTCAGC
35	MYL6-L	CTGAATGGCTGAGGACCTTC
36	MYL6-R	GGCCCAGAGAGCAAGTTTATT
37	ATP5I-L	GGAGAGGAGGATAGCAGCAG
38	ATP5I-R	TCACACAACACAGGAAGCTTTA
39	CRYAB-L	AGCCCCCAAGAAATAGATGC
40	CRYAB-R	TTGCTGAATGATATTTTATTAGCTTGA
41	MT2A-L	GTGGGCTGTGCCAAGTGT
42	MT2A-R	ATAGCAAACGGTCACGGTCA
43	RPL37A-L	TGCCTGGACGTACAATACCA
44	RPL37A-R	GGCCAGTGATGTCTCAAAGAG
45	S100B-L	TTAAATGCGTTCCTCATCCA
46	S100B-R	TGCGAGTTCTGATGGAGTTG
47	COX6B1-L	GTCACAGACTGGGATGAGCA
48	COX6B1-R	TTCCAATGAGTTTTTATTAGCAGGT
49	CKB-L	TTCTCAGAGGTGGAGCTGGT
50	CKB-R	TACCAAGGGTGACGGAAGTC
51	HIST1H4C-L	CGAGGTGTGCTTAAGGTTTTC
52	HIST1H4C-R	AGTTCTCAGGAGACCGCGTA
53	MIF-L	AGAACCGCTCCTACAGCAAG
54	MIF-R	ATTTCTCCCCACCAGAAGGT
55	COX4I1-L	CAAGAGGATGCTGGACATGA
56	COX4I1-R	TTTGGCATAACAGGTTTCCAG
57	NDUFA3-L	GCCCTACAACTACCCAGTGC
58	NDUFA3-R	GGGGGTTGGTTTTCACATTT
59	RPL36-L	CGCCATGGAGTTACTGAAGG
60	RPL36-R	GGAGGGGCTCAGTCTTTCTT
61	GAPDH-L	CGACCACTTTGTCAAGCTCA
62	GAPDH-R	AGGGGTCTACATGGCAACTG
63	NDUFB8-L	TGATCCCTCCAAAGAACCAG
64	NDUFB8-R	TGAGGCACAAATAACACAGCA
65	LGALS1-L	ACGAATTCAAGTTCCCCAAC

66	LGALS1-R	GCTGGCTGATTTCAGTCAAAG
67	UBL5-L	TTAAGGACCACGTGTCTCTGG
68	UBL5-R	GGTGAGTTTTTACAAACAAGCATCT
69	COX7C-L	CCCTTCCTTGTAGTAAGACACCA
70	COX7C-R	AGTTTGATCCACTTCCAGAGG
71	PTMA-L	GATGGTTAAAAAGGCCAAAGA
72	PTMA-R	CATACATCAAACAGGCCAAAAA
73	TUBB2A-L	TCAATCGTGCATCCTTAGTGA
74	TUBB2A-R	GAGGCAAAACTGAGCACCAT
75	TMEM59L-L	CTAGGGCTTCCCCCTTCTC
76	TMEM59L-R	ACGCTTTATAGGGGCATGG
77	TYROBP-L	CCTGCACCTCATTCCAACTC
78	TYROBP-R	AGAGTATTGGGGAGCGGTCT
79	DYNLRB1-L	ACTTCCAGGTTTTGGAGCAA
80	DYNLRB1-R	AAGGGCGACAAAGTGAACTG
81	GNAS-L	TAAGGCGGCCTACAGAAAAA
82	GNAS-R	TTTGTTTGCTGCTGCTGTTT
83	TUBB3-R	GCAACTACGTGGGCGACT
84	TUBB3-L	CGAGGCACGTACTTGTGAGA
85	CAMK2B-R	GAGCCATCCTCACCACCAT
86	CAMK2B-L	TATTCGTCTGGGGCTTGACT
87	GPHN-R	TTCAACAGAAGATAGTTCCTCATCAC
88	GPHN-L	GGACTGGTGTAGAATGGATGC
89	PPP1R1B-R	CACACCACCTTCGCTGAAA
90	PPP1R1B-L	GAAGCTCCCCCAGCTCAT
91	PAX6-R	GGCACACACACATTAACACACTT
92	PAX6-L	GGTGTGTGAGAGCAATTCTCAG
93	TPM2-R	GGAGAAAACCATCGATGACC
94	TPM2-L	CAAGGTCTGGTGAATCTCGAC
95	ASCL1-R	CGACTTCACCAACTGGTTCTG
96	ASCL1-L	ATGCAGGTTGTGCGATCA

Supplementary Table 6: Lipids, see attached excel file

Supplementary Table 7: Cell type specific genes in Ensemble annotation (3).

Neurons	Astrocytes	Oligodendrocytes
ENSG00000132639	ENSG00000121742	ENSG00000234096
ENSG00000186297	ENSG00000182902	ENSG00000197971
ENSG00000125851	ENSG00000100033	ENSG00000128242
ENSG00000152784	ENSG00000141469	ENSG00000148180
ENSG00000182836	ENSG00000171885	ENSG00000101049
ENSG00000147676	ENSG00000080493	ENSG00000054690

ENSG00000081189	ENSG00000103740	ENSG00000164181
ENSG00000146469	ENSG00000164342	ENSG00000164303
ENSG00000101958	ENSG00000211448	ENSG00000065675
ENSG00000124140	ENSG00000198682	ENSG00000103089
ENSG00000144355	ENSG00000144908	ENSG00000135678
ENSG00000164600	ENSG00000119938	ENSG00000123560
ENSG00000165434	ENSG00000145681	ENSG00000065361
ENSG00000119125	ENSG00000163406	ENSG00000013297
ENSG00000186487	ENSG00000138696	ENSG00000064787
ENSG00000155926	ENSG00000109107	ENSG00000198753
ENSG00000122254	ENSG00000219607	ENSG00000184343
ENSG00000104435	ENSG00000153208	ENSG00000172005
ENSG00000147571	ENSG00000137561	ENSG00000126860
ENSG00000067715	ENSG00000101938	ENSG00000110944
ENSG00000135333	ENSG00000079215	ENSG00000105695
ENSG00000147246	ENSG00000162390	ENSG00000116711
ENSG00000119042	ENSG00000111783	ENSG00000134198
ENSG00000133636	ENSG00000148482	ENSG00000168314
ENSG00000091664	ENSG00000018625	ENSG00000180929
ENSG00000022355	ENSG00000100427	ENSG00000167641
ENSG00000113327	ENSG00000146070	
ENSG00000104327	ENSG00000117525	
ENSG00000171951	ENSG00000180340	
ENSG00000145934	ENSG00000110436	
ENSG00000174576	ENSG00000131095	
ENSG00000136999		
ENSG00000181656		
ENSG00000180616		

Supplementary Table 8: Cell type specific genes (4)

Astrocytes	**Microglia**	**Neurons**	**Oligodendrocytes**
ENSG00000103740.9	ENSG00000132965.9	ENSG00000070808.15	ENSG00000141338.13
ENSG00000135744.7	ENSG00000173372.16	ENSG00000187094.11	ENSG00000092529.22
ENSG00000171885.13	ENSG00000125730.16	ENSG00000101489.18	ENSG00000172508.10
ENSG00000127249.14	ENSG00000171860.4	ENSG00000089199.9	ENSG00000013297.10
ENSG00000138696.10	ENSG00000277632.1	ENSG00000122966.13	ENSG00000150656.14
ENSG00000068078.17	ENSG00000275302.1	ENSG00000237289.9	ENSG00000244274.7
ENSG00000131095.11	ENSG00000143119.12	ENSG00000106976.18	ENSG00000136960.12

ENSG00000152661.7	ENSG00000019582.14	ENSG00000022355.14	ENSG00000136541.14
ENSG00000121742.15	ENSG00000073737.16	ENSG00000145864.12	ENSG00000086205.16
ENSG00000170075.8	ENSG00000203747.9	ENSG00000113327.14	ENSG00000169562.9
ENSG00000008394.12	ENSG00000169508.6	ENSG00000184368.15	ENSG00000164161.9
ENSG00000100033.16	ENSG00000125538.11	ENSG00000214548.14	ENSG00000167755.13
ENSG00000164188.8	ENSG00000140678.16	ENSG00000021645.17	ENSG00000198121.13
ENSG00000124145.6	ENSG00000162511.7	ENSG00000171951.4	ENSG00000105695.14
ENSG00000144040.12	ENSG00000136167.13	ENSG00000132639.12	ENSG00000168314.17
ENSG00000182902.13	ENSG00000186818.12	ENSG00000163630.10	ENSG00000197430.10
ENSG00000148482.11	ENSG00000038945.14	ENSG00000154096.13	ENSG00000129538.13
ENSG00000080493.13	ENSG00000173391.8	ENSG00000166448.14	ENSG00000091513.14
ENSG00000139155.8	ENSG00000115956.9	ENSG00000144406.18	ENSG00000164124.10
	ENSG00000081237.18	ENSG00000163032.11	ENSG00000174607.10
	ENSG00000148908.14		

Supplementary Table 9: Permutation based P value for general gene differential expression for all SQUARE fields, as shown for field 'AC' in Figure 6.

	Permutation P value		
Primers	AD-Con	NDWP-Con	AD-NDWP
AC	0.03	0.03	0.18
AG	0.05	0.06	0.46
CA	0.07	0.08	0.2
GT	0.03	0.02	0.16
CT	0.09	0.08	0.28
GA	0.07	0.08	0.1
GC	0.02	0.02	0.06
GG	0.01	0.01	0.06
AA	0.01	0.07	0.08
AT	0.01	0.01	0.05
CC	0.02	0.03	0.11
CG	0.07	0.01	0.07

Supplementary Table 10: Full gene lists for each of the identified profiles. Attached as an excel file.

Supplementary Table 11: Association of genes of profile 3 with human diseases. Listed are genes that showed an AD-specific up-regulation, their association with disease and a targeting FDA-approved drug if exist.

	Gene	*Variant ending dinucleotide*	*Disease*	*Role*	*FDA-approved Drug*	*reference*
1	HLA-DRA	CC	Multiple sclerosis	Antigen presenting	Glatiramer Acetate	(5)
2	P2RY12	AG	Coronary artery disease, peripheral vascular disease, and cerebrovascular disease	Blood clotting	Clopidogrel	(6)
3	S100A4	CA	Metastatic disease	cell cycle progression and differentiation	Phenothiazines	(7)
4	TBXAS1	CC	Hemostasis, cardiovascular disease, and stroke	Lipid processing	Dipyridamole, Picotamide	(8)
5	SLC6A9	GA	Schizophrenia and cognitive disorders	NMDA potentiation	ASP2535 Bitopertin RG1678 Org 25935	(9)
6	LCAT	AC	LCAT deficiency syndromes, Alzheimer`s disease	Cholesterol processing	--	(10)
7	MT1F	CA	Parkinson	Metal storage, transport and detoxification	--	(11)
8	MT1G	CA	Parkinson	Metal storage, transport and detoxification	--	(12)
9	CLINT1	GA	Schizophrenia	Cell development	--	(13)
10	SPP1	AC	Autoimmune diseases, Multiple sclerosis, cancer, inflammatory diseases.	Immune functions	--	(14, 15)

Supplementary Table 12: Number of detected genes (RPKM>1) per patient, per SQUARE field. Rows are patients and columns are SQUARE fields.

		1	2	3	4	5	6	7	8	9	10	11	12
AD	1	6152	6729	6154	7224	9942	6284	4273	5176	6504	7879	5815	7500
	2	6961	6995	6251	7516	9611	6478	4549	5294	6698	7778	6204	7747
	3	6945	7107	6627	7388	9746	6221	4529	5037	6789	7983	6145	6059
	4	6811	6704	6530	7555	9664	6193	2877	4868	6250	7976	6246	8079
	5	6123	6417	5848	7628	9529	5953	4637	4639	6140	7481	5814	7654
	6	6956	7093	6308	7469	9927	6730	4542	4951	6091	7465	5699	6550
	7	6310	6217	5749	7176	9410	5910	4393	4695	6247	7203	5531	7122
	8	6770	6999	6436	7352	9675	6730	4451	5233	6761	7500	6114	7589
Con	9	6374	6456	5957	6785	9250	6039	3889	4726	6506	7182	5307	6633
	10	6820	6919	6083	6883	9270	5950	4367	4861	6763	7729	5266	6545
	11	5771	6029	5191	6705	9445	5654	4197	4565	6188	6804	5649	6986
	12	5660	7181	6486	7208	10160	7867	5148	5422	6939	8082	6243	6555
	13	6332	6962	6220	6468	9430	6576	3876	5126	6959	7805	5361	6657
	14	6103	6486	5507	7139	9583	5781	4467	4604	6150	7328	5632	7412
	15	6380	6414	5592	6486	9377	5557	3940	4277	6225	7869	5571	7090
	16	6511	6673	6217	7495	9710	6147	4238	4947	6788	8011	5732	7296
NDWP	17	6265	6450	5903	7247	9142	5907	4403	4718	6022	7212	5424	7478
	18	7153	7552	6945	7596	9822	6549	4382	5424	7282	8388	5926	7285
	19	6294	5943	5668	7300	9259	5648	4255	4416	6108	7468	5690	7321
	20	6268	6583	5753	7391	9732	6125	4635	4815	5896	7847	5733	7395
	21	7674	7822	6969	8201	10588	7826	5027	5787	7348	8095	6059	7434
	22	7274	7185	6793	7824	9945	6982	4755	5377	7258	8310	6185	7737
	23	7149	7073	6654	7206	9895	6774	4890	5274	6915	8485	5796	8190
	24	5676	6113	5303	6938	9863	6393	4630	4835	6651	7721	5964	6474

Supplementary discussion

Sequencing method bias

This study was based on two distinct sequencing techniques, both aimed at polyadenylated transcripts. The Illumina approach is a single primer-based method, which would amplify the most abundant polyadenylated transcript of any given gene, possibly causing a bias by selecting this more abundant variant while missing the less abundant ones; in comparison, the Lexogen approach involves multiple adenosine-initiated primers addressing all of the optional 3'-polyadenylated versions of such transcripts, which would detect such variabilities but might miss-represent the levels of the more abundant one.

APA and lipid changes

The connection between APA and lipid changes in our study has been driven by data. First, we detected apparent AD-associated changes in the expression levels of those genes known as involved in lipid processing, as is evident from the lipid-related target genes at the top of the list of AD-associated transcripts with altered APA (Table 1). This led us to directly explore the levels of the corresponding lipid molecules, which indeed identified group differences in their composition. However, due to technical limitations, the great majority of modified molecules cannot yet be chemically identified as individual lipids; and those 2400 identifiable lipids that could be individually identified failed to show statistically based correlation with the relevant transcript changes. Finding correlations between APA and specific lipid changes thus depends on progression in the technology of lipid profiling.

One-Way-ANOVA s*tatistical analysis*

We performed One-Way-ANOVA across the three NBB groups, with eight patients each. This is more than the minimum number required for One-Way-ANOVA (one more in a group than the number of groups). One-way ANOVA is considered a robust test against the normality assumption, since it tolerates violations to its normality assumption rather well (see the 'Laerd Statistics' web-site source for statistical guidelines for researchers). We further used the Shapiro-Wilk test for testing normality in our data, and found rather small deviations from normality.

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