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Supplemental Data

Gene Expression as Peripheral Biomarkers for Sporadic Alzheimer's Disease

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SUPPLEMENTARY PROCEDURE

Briefly, 1–3 μl cDNA (1:10 diluted final amount of 10-30 ng) and gene specific primers (probes, when assays with probes) were used in each reaction with total volume of 25 μl. For each assay (one gene expression analysis), the same amount of cDNA was used for analysis, high abundant genes were assayed with the lower amount of cDNA while the lower abundant genes were assayed with the higher amount of cDNA. For the QuantiTect assays, we used QuantiTect^{TM} Custome Assay (Qiagen Inc., Valencia, CA, USA) (Supplementary Table 2), which was added to QuantiTect (Probe/SYBR Green) PCR Master Mix (Qiagen Inc., Valencia, CA, USA). For the assays with designed primers, we used the iQ-SYBR Green Supermix (Bio-Rad, Hercules, California, USA).

Absence of DNA contamination was verified by amplifying the house-keeping gene, R18S. The products were visualized on gel-electrophoresis to observe no product. Minus reverse transcribed (RT) samples were tested simultaneously with experimental samples.

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Real-time PCR was performed in the iCycler iQ system (BioRad Co., Hercules, CA, USA). The amplifications were conducted on the real-time thermocycler:

For assays with primers the following program scheme was used: 1 cycle at 95°C for 3 min, 35–45 cycles at 95°C for 10 s and T-annealing for 45 s, 1 cycle at 95°C for 1 min, 1 cycle at 55°C for 1 min, 80 cycles beginning at 55°C and increasing each cycle by 0.5°C for melting point analysis. All PCR reactions were run in duplicate.

For assays using the QuantiTect assay, we used the PCR amplification scheme as indicated in the manual (Qiagen, Hilden, Germany).

SUPPLEMENTARY

All subjects underwent formal diagnostic procedure according to the NINCDS-ADRDA criteria [1]. Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) [2,3], MMSE [4], and Clinical Dementia Rating (CDR) [5] were administered to all subjects. The MMSE was used for following up the progression, while the other two were used for rough estimation of the diagnosis assessment. Further information was obtained by Unified Parkinson Scale [6]. Depressive and anxiety symptoms were assessed with Hamilton Depression Scale [7], Short Geriatric Depres-

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Table S2, continued

Gene (accession number)	Symbol	QuantiTect Probe/primer assay	Detection method	Product size (bp)	Cycle No.	Annealing Temperature (°C)	Reaction Efficiency (%)
glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A (GRINL1A), transcript variant 1 (NM_015532)	Gcom1	QuantiTect Primer Assay	SYBR	105	45	55	91.2
Glutamate receptor, ionotropic, Kainat 4 (NM_014619)	GRIK	QuantiTect Primer Assay	SYBR	135	45	55	96

Manual, according to the manual (Qiagen). **Bold**, house-keeping genes used for normalization.

Table S3

Descriptive statistics for the 33 gene expressions

Variable	N	Mean	Std Dev	Variable	N	Mean	Std Dev
CAT	178	0.10	0.16	**NGRN**	178	0.15	0.16
APP	178	0.17	0.21	**MBP**	178	0.33	0.23
OR10H3	178	0.09	0.13	**OPHN1**	178	0.05	0.05
TRPM6	178	0.12	0.17	**GFAP**	178	0.21	0.26
SNX2	178	0.15	0.21	**APBA3**	178	0.20	0.25
GSTM1	178	0.08	0.17	**Gcom1**	178	0.19	0.19
FTH1	178	0.20	0.25	**CNR2**	178	0.31	0.26
DBI	178	0.10	0.16	**GRIK**	178	0.12	0.15
KLF12	178	0.22	0.22	**IRS4**	178	0.03	0.11
HIST1H4I	178	0.09	0.15	**SYT1**	178	0.09	0.13
HIST1H3E	178	0.08	0.12	**VPS35**	178	0.28	0.21
LYST	178	0.16	0.16	**BACE1**	178	0.05	0.13
TUB2A	178	0.08	0.24	**IDE**	178	0.29	0.24
COG2	178	0.17	0.20	**VPS41**	178	0.19	0.18
SYN2	178	0.18	0.23	**IGF-1R**	178	0.27	0.25
HMOX1	178	0.20	0.22	**TF**	178	0.20	0.20
				GBP10	178	0.08	0.17

The mean values and standard deviations are calculated over all time points and patients.
The analyses were repeated twice; once age was included as continuous variable and once age was subdivided in eight groups. The resulting p-values of the genes did not differ in both methods.

sion Scale [8] and State Trait Anxiety Inventory (X1, X2) [9]. These scales were used in order to rule out subjects with sever neurologic of psychiatric disorders (data not shown). In addition, detailed information on medication and smoking habits were collected.

Continuous decrease of cognitive and mnestic functions is typical in Alzheimer's disease, as can be seen in most of the subjects. Subject A6 and subject A11 showed at assessment period-III and subject A17 showed at assessment period-II unspecific symptoms of physical diseases. Subject A9 underwent surgery some days before assessment. At assessment period-I and period-II, the same 10 subjects took anticholinergic drugs but only two of them have increased dosage. At assessment period-II, an additional four subjects that were assessed the first time took anticholinergic drugs. At assessment period-III, again, 14 subjects took anticholinergic drugs while two discontinued anticholinergic medication, two started medication, and one decreased dosage. At assessment period-IV, one of these subjects had discontinued anticholinergic medication, and one had decreased dosage. At all four assessment periods, all the subjects underwent pharmacological treatment with the exception of two healthy controls.

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Table S1

Subjects MMSE scores information at all recruitment time points

Subject Code	Age (Years)	Gender Female = 0 Male = 1	Diagnosis[a] 0 = Control 2 = Demented	MMSE (Score) Basis	MMSE (Score) second	MMSE (Score) third	MMSE (Score) Forth
A1	58	1	2	17*	15*	13*	14*
A2	84	0	2	23	23	23	–
A3	80	0	2	23*	22*	18	17
A4	82	0	2	18*	13*	10*	10*
A5	77	1	2	11	12	–	–
A6	61	1	2	24*	24*	9*	22*
A8	75	1	2	15*	15*	8*	5*
A9	74	0	2	14	9	22	5
A10	76	0	2	24*	24*	24*	25*
A11	66	1	2	23*	25*	13*	24*
A12	90.5	0	2	–	14	–	–
A13	65.5	0	2	–	20	–	–
A14	54.5	0	2	–	21*	22*	20*
A15	67.5	0	2	–	25	25	–
A16	62.5	0	2	–	21	18	21
A17	69.5	0	2	–	11*	–	24*
A19	64.7	1	0	–	–	27	29
A20	82.7	1	2	–	–	4*	–
A21	66	0	0	30	30	28	28
A22	64	1	0	29*	29*	28*	29*
A23	57	0	0	30	30	30	30
A24	60	1	0	28*	28*	25*	24*
A25	69	1	0	29	–	29	29
A26	83	1	0	29	29	–	–
A27	68	0	0	28	29	30	30
A28	77	0	0	30	30	30	30
A29	57	0	0	30	30	30	30
A30	61	1	0	29	29	28*	29*
A31	74	0	0	30*	30*	30	30
A32	77	0	0	30	30	30	30
A33	79	1	0	30	29	30	30
A34	63	1	0	30	30	30	30
A35	65	0	0	30	–	30	30
A36	67	1	0	30	–	30	–
A37	80	0	0	28	29	30	29
A38	65	0	0	30	30	30	30
A39	69	1	0	30	30	30	30
A40	82	1	0	29	30	30	29
A41	59.5	0	0	–	28	30*	29*
A42	59.5	1	0	–	30	30	30
A43	64	1	0	30	30	30	30
A44	58	0	0	30	30	–	30
A45	95	0	0	29	29	29	30
A46	79	0	0	30	30	30	30
A47	74	0	0	30	30	30	30
A48	75	1	0	30	30	–	
A49	65	1	0	30	30	30	30
A50	66	0	0	30	30	30	30
A51	77.5	0	0	–	27*	27*	25*
A52	66	0	0	30	30	30	30
A53	76.7	0	0	–	–	27*	–
A55	58.7	1	2	–	–	24	26

[a]Diagnosis: at baseline (first recruitment) according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.

Abbreviations: MMSE, Mini-Mental State Examination; –, no information and no blood withdrawal due to subjects refusal/death.

* Medication with anticholinergic drugs.

Table S2
Assays details for real time quantitative PCR

Gene (accession number)	Symbol	QuantiTect Probe/primer assay	Detection method	Product size (bp)	Cycle No.	Annealing Temperature (°C)	Reaction Efficiency (%)
18s ribosomal (V01270)	**R18S**	QuantiTect Gene Expression Assay (Probe)	FAM	150	36	manual	95.0
$\beta-$Actin (NM_001101)	**ACTB**	QuantiTect Gene Expression Assay (Probe)	FAM	150	40	manual	87.9
aminolevulinate delta synthase 1 (NM_000688)	**ALAS1**	QuantiTect Gene Expression Assay (Probe)	FAM	100	40	manual	80.7
glyceraldehydes-3-phosphate dehydrogenase (NM_002046)	**GAPDH**	QuantiTect Gene Expression Assay (Probe)	FAM	130	35	manual	89.0
Ribosomal protein L13a (NM_012423)	**RPL13A**	QuantiTect Gene Expression Assay (Probe)	FAM	100	35	manual	99.5
peptidylprolyl isomerase A (cyclophilin A) (NM_021130)	**PPIA**	QuantiTect Gene Expression Assay (Probe)	FAM	150	40	manual	99.9
Tubulin β 2A (NM_001069)	TUB2A	CACCTTCATCGGCAACAG TTCCACATCATTACATCAACAG	SYBR	375	40	61	97
Diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein) (NM_020548)	DBI	GAGCTGAAAGGGACTTCCAA TTAGAGCCGTATGGTGAGCA	SYBR	226	40	63	98
Synaptotagmin I (NM_005639)	SYT1	QuantiTect Primer Assay	SYBR	129	35	manual	95.2
Synapsin II (AW139618)	SYN2	CATACTGCTGTCATAGTG GTCATACCTGTGTTACTG	SYBR	223	40	60	92.8
component of oligomeric golgi complex 2 (NM_007357)	COG2	TCCTCACCATTGCCGCCTTC CCACACTGCCGTTCCAATCTTC	SYBR	228	40	61	96.4
vacuolar protein sorting 35 (NM_018206)	VPS35	QuantiTect Primer Assay	SYBR	127	40	manual	96.1
vacuolar protein sorting 41 (NM_014396)	VPS41	QuantiTect Primer Assay	SYBR	124	40	manual	90.2
olfactory receptor, family 10, subfamily H, member 3 (NM_013938)	OR10H3	ATGCCTGGTCAGAAC CAGATGATGTCTTGCTC	SYBR	144	40	58	98.8
Catalase (NM_001752)	CAT	GCCTGGGACCCAATTATCT GAATCTCCGCACTT	SYBR	203	40	62	98.7
Transient receptor potential cation channel, subfamily M, member 6 (AF350881)	TRPM6	GCACTC GCACTTCATCCTGTC CGGCACGCCTTGTCTTGAG	SYBR	130	40	61	96.6
Cannabinoid receptor 2 (NM 001841)	CNR2	QuantiTect Primer Assay	SYBR	87	40	manual	95
AβPP (A4) (BC004369)	APP	GATGCGGAGGAGGATGAC TCTGTGGCTTCTTCGTAGG	SYBR	203	35	61	99.9

Table S2, continued

Gene (accession number)	Symbol	QuantiTect Probe/primer assay	Detection method	Product size (bp)	Cycle No.	Annealing Temperature (°C)	Reaction Efficiency (%)
Sorting nexin II (NM_003100)	SNX2	AGAAGACTCATCATCCACTG AGACCAAGGCTTCAACAC	SYBR	326	40	52	88.7
AβPP binding, family A, member 3 (AI141541)	APBA3	CAGGCAAGGGATGAGGTG CTTGAGATCAATGGGCAGAG	SYBR	170	40	67	91.8
$\beta-$site AβPP-cleaving enzyme 1 (NM_012104)	BACE1	QuantiTect Primer Assay	SYBR	146	40	manual	95.3
Insulin degrading enzyme (NM_004969)	IDE	QuantiTect Primer Assay	SYBR	126	40	manual	91.1
Insulin-like growth factor 1 receptor (NM_000875)	IGF-1R	QuantiTect Primer Assay	SYBR	106	40	manual	98.5
Growth factor receptor-bound protein 10 (NM_005311)	GBP10	QuantiTect Primer Assay	SYBR	127	45	55	91
Insulin receptor substrate 4 (NM 003604)	IRS4	QuantiTect Primer Assay	SYBR	80	40	manual	80.8
Transferrin (NM_001063)	TF	QuantiTect Primer Assay	SYBR	190	40	manual	96.8
ferritin, heavy polypeptide 1 (NM_002032)	FTH1	CTGGAGCTCTACGCCTCCTA CACACTCCATTGCATTCAGC	SYBR	232	30	64	88.2
glutathione S-transferase M1, transcript variant 1 (X08020)	GSTM1	CCTCCTCGTTCCTTTCTCCT ACCAGTCAATGCTGCTCCTT	SYBR	285	40	63	88.2
Heme oxygenase (decycling) 1 (NM_002133)	HMOX1	GCCAGGTGACCCGAGACG GGAAGTAGACAGGGGCGAAGAC	SYBR	120	40	60	99
Glial fibrillary acidic protein (NM_002055)	GFAP	QuantiTect Primer Assay	SYBR	96	40	manual	91
Neugrin, neurite outgrowth associated (AF225423)	NGRN	CATGGAGCAGATACGGTATTTAC TCTTCGGATCACATCATCAGTGC	SYBR	100	45	63	94
Histone cluster 1, H3e (NM_003532)	H1ST1H3E	GGTGCGAGAAATAGCTCAGG GGGCAAGCTGGATGTCTTTA	SYBR	173	40	63	97.6
Histone cluster 1, H4i (NM_003542)	HIST1H4I	ACTTGCAAACACCCTTCCAC ATACAAACTTGGCGGACCTG	SYBR	172	40	64	99.2
Kruppel-like Factor 12 (NM_016285)	KLF12	CAGTCGGTGCCTGTTGTCTA AAGGTCACATTTGGCAGGTC	SYBR	179	38	64	85
Oligophrenin 1 (NM_002547)	OPHN1	QuantiTect Primer Assay	SYBR	100	45	55	97
Myelin Basis protein (M13577)	MBP	QuantiTect Primer Assay	SYBR	60	45	55	93
Lysosomal trafficking regulator (U84744)	LYST	ATTCCGAGGTTTCTGTGGTG GATCCTGGCTTCTGTTCAGC	SYBR	194	40	64	99.9