

Epigenetic silencing of *OR* and *TAS2R* genes expression in human orbitofrontal cortex at early stages of sporadic Alzheimer's disease

Cellular and Molecular Life Sciences

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Contents

Supplementary Table 1 - Detailed demographic and basic pathological information on orbitofrontal cortex specimens. PMI, *post-mortem* interval; NFT, neurofibrillary tangle; nd, no data available, M, male; F, female.

Supplementary Table 2 – List of primer sequences used for real time qPCR and H3K9me3 CHIP-qPCR analyses. Fw, forward primer; Rv, reverse primer; TSS, transcription start site.

Supplementary Table 3 and 4 – GO enrichment on biological process for the proteins identified in OFC common to Braak I and V (Supplementary Table 3) and unique to Braak V (Supplementary Table 4). GO enrichment analysis was performed with Panther software²⁰. *Homo sapiens* (all genes in database) was used as reference list for the enrichment analysis. Fold Enrichment of the genes observed in the uploaded list over the expected are shown. Raw p-values were determined by Fisher's exact test and False Discovery Rate (FDR) was calculated by the Benjamini-Hochberg procedure. In the tables, only terms with FDR < 0.05 are displayed.

Supplementary Table 1 - Detailed demographic and basic pathological information on orbitofrontal cortex specimens.

Case	Sex	Age at death (years)	PMI (hours)	Braak NFT Stage	APOE
1	M	64	3,5	0	33
2	M	63	2,8	0	33
3	M	72	nd	0	23
4	M	68	23	0	33
5	M	61	22,5	0	33
6	M	62	5	0	34
7	M	100	1,3	0	33
8	M	58	7	0	33
9	M	79	15,8	0	33
10	M	80	24	0	33
11	M	51	nd	0	nd
12	M	47	4,9	0	nd
13	M	46	15	0	nd
14	F	52	5,8	0	nd
15	F	81	4	0	33
16	F	64	5	0	23
17	F	75	6,2	0	33
18	F	55	8,5	0	33
19	F	86	7	0	nd
20	F	97	16,3	0	34
21	F	49	24	0	33
22	F	99	3	0	33
23	F	88	16,3	0	33
24	F	67	24	0	33
25	F	71	4	0	33
26	M	65	5,3	I	34
27	M	63	8,1	I	34
28	M	58	8,1	I	33
29	M	76	6,5	I	23
30	M	68	10,9	I	23
31	M	61	4,5	I	33
32	M	59	4,3	I	33
33	M	70	2,8	I	33
34	M	59	4,3	I	33
35	F	57	5	I	33
36	F	59	11,3	I	34
37	F	79	3,6	I	33
38	F	67	5,3	I	34
39	F	79	6,4	I	33
40	F	73	15,8	I	22
41	M	50	4,3	II	33
42	M	66	4,9	II	33
43	M	71	5,3	II	33
44	M	66	5,8	II	23
45	M	85	3,8	II	33
46	M	80	3,5	II	33
47	M	73	9,6	II	33
48	M	78	nd	II	23
49	F	59	4,3	II	33
50	F	65	nd	II	33
51	F	72	8,5	II	33
52	M	66	5,8	III	33
53	M	85	4,8	III	33
54	M	64	6	III	33
55	M	75	3,4	III	34
56	M	76	6	III	34
57	F	85	14,3	III	34
58	F	74	5,2	III	33
59	F	78	6	III	33
60	F	90	4	III	33
61	F	82	3,1	III	33
62	M	84	5	IV	33
63	M	74	4,8	IV	33
64	F	90	9,9	IV	33
65	F	88	4,1	IV	33
66	M	79	7,5	V	33
67	M	73	4,5	V	34
68	M	93	3	V	33
69	M	75	11,5	V	34
70	M	82	3,8	V	34
71	M	77	16	V	33
72	F	74	4,3	V	34
73	F	74	5,5	V	33
74	F	81	5,3	V	23

PMI, *post-mortem* interval; NFT, neurofibrillary tangle; nd, no data available, M, male; F, female.

Supplementary Table 2 – List of primer sequences used for real time qPCR and H3K9me3 ChIP-qPCR analyses.

Gene	Primer sequences (5' to 3')	Region of amplification (relative to TSS (+1))	Analysis
<i>Taste receptors, type 1</i>			
<i>TAS1R1</i>	Fw ATTTAAGCAACTGGCCTCCTTAGA Rv CCAGAATGGAGAGGGAACAGG	Exon 1	
<i>TAS1R2</i>	Fw CCTGGCACACCATCAACAAC Rv AGGACCACTCGTTATTCGGG	Exons 4-6	RT-qPCR
<i>TAS1R3</i>	Fw GCAGGGCTAAATCACCA Rv CTGAGGCGTTGCACTGAAGA	Exon 3	
<i>Taste receptors, type 2</i>			
<i>TAS2R14</i>	Fw GGTGCTGCTTCTTGACTT Rv TGCATCTTCTGCGATGTTCC	Exon 1 (+393 to +638)	RT-qPCR, ChIP-qPCR
	Fw TGTTTCCTTTCTCACCAGCTAC Rv AAATCTCTGTATTTCCCGAGACA	Proximal (-285 to -181)	ChIP-qPCR
<i>TAS2R5</i>	Fw CGGCTACTTGTGGCTGAA Rv TACCGAATGCTGCTGTTCCCT	Exon 1 (+338 to +479)	RT-qPCR, ChIP-qPCR
	Fw CCGGTGTCTGTGGTACTGTTT Rv CTCGTCCTCCGTAATCTGTCT	Proximal (-253 to -102)	ChIP-qPCR
<i>Olfactory receptors</i>			
<i>OR13A1</i>	Fw TGCTGAAGAAATGACTGGCTG Rv TGAGATCAAAGAATCGGGGCT	Exons 2-3	RT-qPCR
<i>OR1L8</i>	Fw CCATTCGCTTCAACCCCA Rv CACCCAGCATAGGAGATGGTC	Exon 5 (+146 to +293)	RT-qPCR, ChIP-qPCR
	Fw AAAACTCTCCCTCTGTCCCTCC Rv TTGTGGGATGGCTTGTTCACC	Proximal (-236 to -125)	ChIP-qPCR
<i>OR2H2</i>	Fw GGGCAAGTCTTTGGTCTCTTC Rv TTTGTGTGAGCCCATTTCTT	Exon 2 (+804 to +934)	RT-qPCR, ChIP-qPCR
	Fw TGTGAGTATCCATCTGCTGTCC Rv TCCCTGTTCTTCTGGTCAA	Proximal (-258 to -178)	ChIP-qPCR
<i>OR2K2</i>	Fw TGCCCATTTGACTGTGGTGA Rv AGCACTCCGTAAGCAACGA	Exon 2 (+810 to +926)	RT-qPCR, ChIP-qPCR
	Fw TCATATTTTCTCATTGCGTCACT Rv TCCTAAACCTATCCTTGGCACAC	Proximal (-268 to -195)	ChIP-qPCR
<i>OR7A17</i>	Fw CAGCTTACTCCTGGCTGTGA Rv TTCAGGGCAGCAATCATCCA	Exon 3	RT-qPCR
<i>Housekeeping genes</i>			
<i>GAPDH</i>	Fw TCACCAGGGCTGCTTTAACT Rv AGCATCGCCCCACTTGATTT	Exons 3-5	
<i>ACTB</i>	Fw CGCCGCCAGCTCACC Rv GCTCGATGGGGTACTTCAGG	Exons 1-3	RT-qPCR
<i>PGK1</i>	Fw GAATCACCGACCTCTCTCC Rv AAGGACTACCGACTTGGCTC	Exons 1-3	

Fw, forward primer; Rv, reverse primer; TSS, transcription start site.

Supplementary Table 3 – GO enrichment on biological process for the proteins identified in OFC common to Braak I and V.

GO number	GO term	Number of genes identified in OFC common to Braak I and V	Total number of genes in <i>Homo sapiens</i> reference list	Fold enrichment	raw P value	FDR
GO:0006342	chromatin silencing	4	51	> 100	3,19E-08	2,51E-04
GO:0016458	gene silencing	4	148	42,82	1,93E-06	2,17E-03
GO:0045814	negative regulation of gene expression, epigenetic	4	61	> 100	6,31E-08	2,48E-04
GO:0040029	regulation of gene expression, epigenetic	5	130	60,93	1,38E-08	2,17E-04
GO:0097549	chromatin organization involved in negative regulation of transcription	4	72	88,01	1,19E-07	3,12E-04
GO:0034401	chromatin organization involved in regulation of transcription	4	86	73,69	2,36E-07	4,64E-04
GO:0006325	chromatin organization	7	686	16,17	6,79E-08	2,13E-04
GO:0016043	cellular component organization	12	5298	3,59	8,41E-07	1,32E-03
GO:0071840	cellular component organization or biogenesis	12	5496	3,46	1,29E-06	1,84E-03
GO:0051276	chromosome organization	8	1021	12,41	3,87E-08	2,03E-04
GO:0006996	organelle organization	11	3360	5,19	1,25E-07	2,80E-04
GO:0006334	nucleosome assembly	4	101	62,74	4,39E-07	7,67E-04
GO:0065004	protein-DNA complex assembly	4	167	37,95	3,09E-06	3,03E-03
GO:0071824	protein-DNA complex subunit organization	4	208	30,47	7,24E-06	6,32E-03
GO:0031497	chromatin assembly	4	134	47,29	1,31E-06	1,72E-03
GO:0006323	DNA packaging	4	180	35,21	4,13E-06	3,82E-03
GO:0071103	DNA conformation change	4	269	23,56	1,96E-05	1,47E-02
GO:0006333	chromatin assembly or disassembly	4	154	41,15	2,25E-06	2,36E-03
GO:0034728	nucleosome organization	4	140	45,26	1,56E-06	1,88E-03
GO:0006338	chromatin remodeling	4	247	25,66	1,41E-05	1,17E-02
GO:0032200	telomere organization	3	82	57,96	1,88E-05	1,48E-02

GO enrichment analysis was performed with Panther software²⁰. *Homo sapiens* (all genes in database) was used as reference list for the enrichment analysis. Fold Enrichment of the genes observed in the uploaded list over the expected are shown. Raw p-values were determined by Fisher's exact test and False Discovery Rate (FDR) was calculated by the Benjamini-Hochberg procedure. In the tables, only terms with FDR < 0.05 are displayed.

Supplementary Table 4 – GO enrichment on biological process for the proteins identified in OFC unique to Braak V.

GO number	GO term	Number of genes identified in OFC unique to Braak V	Total number of genes in <i>Homo sapiens</i> reference list	Fold enrichment	raw P value	FDR
GO:0006342	chromatin silencing	5	51	91,78	3,13E-09	2,46E-05
GO:0016458	gene silencing	5	148	31,63	5,01E-07	7,16E-04
GO:0010629	negative regulation of gene expression	9	879	9,59	1,47E-07	2,89E-04
GO:0045814	negative regulation of gene expression, epigenetic	5	61	76,73	7,27E-09	3,81E-05
GO:0040029	regulation of gene expression, epigenetic	5	130	36,01	2,69E-07	4,70E-04
GO:0097549	chromatin organization involved in negative regulation of transcription	5	72	65,01	1,60E-08	6,27E-05
GO:0034401	chromatin organization involved in regulation of transcription	5	86	54,43	3,72E-08	1,17E-04
GO:0006325	chromatin organization	6	686	8,19	6,59E-05	4,31E-02
GO:0051276	chromosome organization	7	1021	6,42	6,62E-05	4,16E-02
GO:0002181	cytoplasmic translation	7	122	53,71	5,02E-11	7,90E-07
GO:0006412	translation	7	366	17,90	8,09E-08	2,12E-04
GO:0043043	peptide biosynthetic process	7	388	16,89	1,20E-07	2,68E-04
GO:0006518	peptide metabolic process	7	519	12,63	8,27E-07	1,00E-03
GO:0006807	nitrogen compound metabolic process	16	6360	2,36	6,63E-05	4,01E-02
GO:0043603	cellular amide metabolic process	7	780	8,40	1,19E-05	1,10E-02
GO:0034641	cellular nitrogen compound metabolic process	14	3193	4,10	4,36E-07	6,85E-04
GO:0043604	amide biosynthetic process	7	500	13,11	6,46E-07	8,46E-04
GO:1901566	organonitrogen compound biosynthetic process	8	1250	5,99	2,78E-05	2,08E-02
GO:0010467	gene expression	11	1992	5,17	1,81E-06	1,90E-03
GO:0034645	cellular macromolecule biosynthetic process	8	1368	5,47	5,30E-05	3,78E-02
GO:0009059	macromolecule biosynthetic process	8	1391	5,38	5,96E-05	4,07E-02
GO:0042273	ribosomal large subunit biogenesis	4	72	52,01	1,19E-06	1,33E-03
GO:0048024	regulation of mRNA splicing, via spliceosome	4	103	36,35	4,67E-06	4,58E-03
GO:0050684	regulation of mRNA processing	4	139	26,94	1,47E-05	1,22E-02
GO:1903311	regulation of mRNA metabolic process	5	291	16,08	1,27E-05	1,11E-02
GO:0043484	regulation of RNA splicing	4	150	24,96	1,97E-05	1,55E-02

GO enrichment analysis was performed with Panther software²⁰. *Homo sapiens* (all genes in database) was used as reference list for the enrichment analysis. Fold Enrichment of the genes observed in the uploaded list over the expected are shown. Raw p-values were determined by Fisher's exact test and False Discovery Rate (FDR) was calculated by the Benjamini-Hochberg procedure. In the tables, only terms with FDR < 0.05 are displayed.