Supplementary material

Brain transcriptome sequencing of a natural model of Alzheimer's disease

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Supplementary tables

Table S1. Accuracy of gene prediction. Accuracy of gene prediction on an *O. degus* "artificial scaffold" consisting of 238 concatenated *O. degus* test sequences (with 800 nucleotides of sequence between each of the gene models) using the ab initio programs geneid, AUGUSTUS and SNAP with their pre-existing Mammalian/H. sapiens parameter files (i.e. "mam/hs"). The accuracy of SGP2 (homology evidence-based prediction tool that used the genome of H. sapiens as reference) and that of Augustus (using RNASeq and transcript evidence i.e. "AUGUSTUS+hints") were also tested for accuracy on the same set of sequences. Geneid (geneid+introns) and SGP2 (SGP2+introns) using introns as external evidence were also evaluated. (SN & SP: sensitivity & specificity at nucleotide level; SNe & SPe: sensitivity & specificity at exon level; SNg & SPg: sensitivity & specificity at gene level).

Program**/param**	**SN**	**SP**	**SNe**	**SPe**	**SNg**	**SPg**
Geneid **man/hs**	0.83	0.75	0.65	0.69	0.09	0.06
Geneid+intron **mam/hs**	0.92	0.82	0.83	0.79	0.24	0.17
SGP2 **odegus / Hs**	0.90	0.82	0.77	0.73	0.12	0.08
SGP2+intron **odegus / mam/hs**	0.95	0.86	0.86	0.79	0.26	0.17
Augustus+hints **mam/hs**	0.87	0.94	0.81	0.90	0.33	0.35
Augustus **mam/hs**	0.81	0.84	0.68	0.75	0.06	0.07
SNAP **mam/hs**	0.83	0.45	0.57	0.32	0.03	0.01

Table S2. Weights used by EVM to create a consensus CDS model *O. degus*. The shortcuts interpretation: SPLAN2 uniprot90: SPLAN2 search against Uniprot90 proteins; SPALN2 uniprot-swissprot: SPALN2 against rodent uniprot/swissprot curated proteins; Exonerate uniprot-swissprot: exonerate against against rodent uniprot/swissprot curated proteins;

Type	Source	Weight
ABINITIO_PREDICTION	Augustus	1
ABINITIO_PREDICTION	AugustusHints	1.75
ABINITIO_PREDICTION	geneid	1
ABINITIO_PREDICTION	SGP2	1.25
ABINITIO_PREDICTION	geneid+introns	1.5
ABINITIO_PREDICTION	SGP2+introns	1.75
ABINITIO_PREDICTION	SNAP	0.3
PROTEIN	SPALN2 uniprot90	5
PROTEIN	SPALN2 uniprot-swissprot	4
PROTEIN	exonerate uniprot-swissprot	4
TRANSCRIPT	PASA	10

Table S3. Comparison between EVM-based and GNOMON-based protein coding gene annotation. Statistics for two protein-coding annotations for *O. degus.*

Annotation versions	***O.degus 2a (EVM-generated)***	***O.degus (ncbi GNOMON) -protein-coding only-***
Genome length (Mbases)	**2,995.89**	
number of scaffolds	**7,134**	
Number of protein-coding genes	**31,739**	**20,779**
Gene density (genes/Kbase)	**0.0106**	**0.007**
Number of protein-coding transcripts	**36,866**	**26,248**
Transcripts/gene (range) (% genes with more than 1 transcript)	**1.16 (SD 0.72) (1 – 32) (9.24%)**	**1.26 (SD 0.94) (1 – 31)(15%)**
Number of transcripts with UTRs	**10,648**	**-**
Number of proteins	**36,575**	**26,248**
Number of complete proteins (%)	**33,858 (92.57%)**	**-**
Number/(%) proteins with similarity to sequences in the NCBI NR database (E=10-2; min. identity=25%)	**35,475 (97%)**	**-**
Avg. length of proteins (range)	**461.96 aa. (SD 593.73) (25 – 34,458)**	**577.56 aa. (SD 641.03) (23 – 34,357)**
Avg. length of full-length proteins (range)	**478.57 aa. (SD 602.27) (25 – 34,458)**	**-**
Number of partial proteins (not starting with "M")	**1842 (5.04%)**	**259 (0.98%)**
Avg. length of partial proteins (not starting with "M")	**253.11 aa. (SD 431.8)**	**-**
Number of partial proteins (no terminal STOP codon)	**1589 (4.34%)**	**(can't determine as gnomon protein set has no clear STOP signal)**
Avg. length of partial proteins (no	**213.91 aa. (SD**	**-**

terminal STOP codon)	**350.85)**	
Number of partial proteins (not starting with an M -and- no terminal STOP codon)	**714 (1.95%)**	**-**
Avg. length of partial proteins (not starting with an M -and- no terminal STOP codon)	**158.83 aa. (SD 261.08)**	**-**
Number of partial proteins (not starting with an M -or- no terminal STOP codon)	**2,717 (7.43%)**	**-**
Avg. length of partial proteins (not starting with an M -or- no terminal STOP codon)	**254.96 aa. (SD 423.14)**	**-**
Number of protein-coding exons	**288,884**	**268,660**
Number of introns	**252,018**	**242,412**
Number of UTRs (spliced)	**19,003**	**-**
Number of single-exon genes	**10,114**	**3,156**
Number of multi-exonic transcripts (genes)	**26,752 (21,740)**	**23,092 (17,623)**
Exons/transcript (range) (excludes single-exon genes)	**10.42 (SD 10.50) (2 – 313)**	**11.49 (SD 10.35) (2 – 313)**
Introns/transcript (range)	**9.42 (SD 10.50) (1 – 312)**	**10.49 (SD 10.35) (1 – 312)**
“spliced” UTRs/transcript (range)	**1.785 (SD 0.74) (1 - 5)**	**-**
Avg. length of introns (range)	**5,998 (SD 19,994.1) (21 – 734,060)**	**5,613.03 (SD 19,909.6) (30 – 1,116,408)**
Avg. length of mono-exonic genes	**519.27 (SD 430.56)**	**872.88 (SD 618.70)**
Avg. length of exons (excludes mono-exonic genes)	**165.37 (SD 233.34)**	**161.25 (SD 230.37)**
Avg. length of first exons	**230.78 (SD 336.07)**	**-**
Avg. length of internal exons	**149.24 (SD 194.59)**	**-**
Avg. length of terminal exons	**235.72 (SD 352.41)**	**-**
Avg. length of CDS (range)	**1,392.9 (SD 1,782.42) (75 –**	**1,736.06 (SD 1,923.46)**

	103,074)	**(69 – 103,074)**
Avg. length of UTRs (range)	**653.40 (SD 942.07) (1 - 11,857)**	**-**
Avg. length of primary transcripts	**43,714.8 (SD 107,530)**	**56,055.1 (SD 117,349)**
G+C content exonic (mono-exonic genes)	**49.72% (SD 7.63%)**	**51.85% (SD 8.56%)**
G+C content exonic (excludes mono-exonic genes)	**52.53% (SD 7.47%)**	**53.52% (SD 7.42%)**
G+C content exonic (first exons)	**53.62% (SD 10.85%)**	**-**
G+C content exonic (internal exons)	**51.27% (SD 9.67%)**	**-**
G+C content exonic (terminal exons)	**53.59% (SD 10.84%)**	**-**
G+C content intronic	**45.05% (SD 11.54%)**	**45.45% (SD 11.61%)**
G+C content genomic	**40.16% (SD 5.63%)**	
G+C content UTRs	**53,76% (SD 5%)**	**-**

Table S4. Non-default parameters for RNA-seq mappings. Non-default parameters used during mapping step of pair-end reads of human brain AD subjects and control samples with STAR 2.4.0.1. First column refers to the name of the parameter, while the second to its value.

Parameter	**Value**
outSAMunmapped	Within
outFilterType	BySJout
outFilterMultimapNmax	20
outFilterMismatchNmax	999
outFilterMismatchNoverReadLmax	0.04
alignIntronMin	20
alignIntronMax	1000000
alignSJDBoverhangMin	1
readFilesCommand	zcat

Supplementary Table S5. Functional annotation statistics. Abbreviature KO – KEGG orthology groups.

Number of proteins/genes	36,575 / 31,739
Annotated proteins/genes	**34,571 (94.5%) / 30,336 (95.5%)**
Proteins with Interpro signatures	33,800 (92.4%)
Proteins with Blast2GO or KEGG definition	23,936 (65.4%)
Proteins with Blast2GO definition	16,737 (45.7%)
Proteins with KEGG definition	14,756 (40.3%)
Proteins assigned to KO groups	14,879 (40.6%)
Proteins with GO terms association	28,988 (79.2%)
Conserved domains signatures	31,874 (87.1%)
Conserved features signatures	15,017 (41%)

Supplementary Table S6. GO term annotation. **A**. Number of GO terms associated to each ontology **B**. Top 10 GO terms more frequently associated to proteins grouped by GO term type.

A.

Term type	**Number of proteins**
Biological process	22,200
Cellular component	20,110
Molecular function	27,220
All	**29,847**

B

GO term id	GO term description	# Proteins
	Biological process	
GO:0006412	translation	2938
GO:0006355	regulation of transcription, DNA-templated	1865
GO:0007186	G-protein coupled receptor signaling pathway	1505
GO:0006414	translational elongation	1073
GO:0055114	oxidation-reduction process	1016
GO:0006413	translational initiation	904
GO:0007165	signal transduction	902
GO:0006468	protein phosphorylation	882
GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	830
GO:0006614	SRP-dependent cotranslational protein targeting to membrane	823
	Molecular function	
GO:0005515	protein binding	6905
GO:0003735	structural constituent of ribosome	3023
GO:0005524	ATP binding	2431
GO:0003676	nucleic acid binding	2166
GO:0008270	zinc ion binding	1982
GO:0000166	nucleotide binding	1785
GO:0003677	DNA binding	1755
GO:0046872	metal ion binding	1706
GO:0004930	G-protein coupled receptor activity	1311
GO:0003723	RNA binding	1280
	Cellular component	
GO:0005622	intracellular	3658
GO:0005634	nucleus	3517
GO:0016021	integral component of membrane	3502
GO:0005840	ribosome	2927

GO:0005737	cytoplasm	2338
GO:0016020	membrane	2165
GO:0005829	cytosol	1641
GO:0005886	plasma membrane	1487
GO:0005730	nucleolus	1429
GO:0022625	cytosolic large ribosomal subunit	1007

Table S7. GO-terms enrichment for differentially expressed genes in *O. degus*. Biological processes overrepresented by up- and down-regulated genes identified in *O. degus* brain samples. GO terms shown are those significantly overrepresented (pvalue < 0.05) by genes showing differential expression between AD-like subjects and controls. Categories are sorted by p-value.

TABLE IN ADDITIONAL FILE 2

Table S8. GO terms enrichment for differentially expressed genes in human samples. Biological processes overrepresented by up- and down-regulated genes identified in human brain samples. GO terms shown are those significantly overrepresented (pvalue < 0.05) by genes showing differential expression between AD subjects and controls. Categories are sorted by p-value.

TABLE IN ADDITIONAL FILE 2

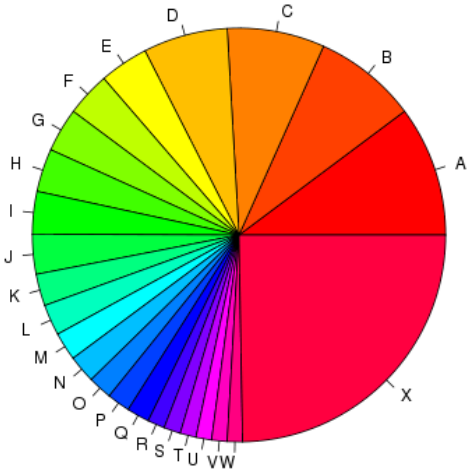
Table S9. Genes differentially expressed between human samples. The complete list of 2963 human genes displaying differential expression between AD subjects and controls. Genes are ranked by FDR (FDR < 0.05).

TABLE IN ADDITIONAL FILE 2

Table S10. Pairwise comparisons to measure the gene expression level differences between AD-like degus and controls.

TABLE IN ADDITIONAL FILE 2

Supplementary figures



A metabolism , 10.21 %
B binding , 8.08 %
C cell , 7.62 %
D intracellular , 6.61 %
E biosynthesis , 3.83 %
F protein_metabolism , 3.46 %
G nucleic_acid_metabolism , 3.43 %
H catalytic_activity , 3.37 %
I protein_binding , 3.33 %
J cytoplasm , 3.11 %
K cell_organization , 2.49 %
L cell_communication , 2.42 %
M development , 2.2 %
N signal_transduction , 2.16 %
O transport , 1.87 %
P nucleic_acid_binding , 1.8 %
Q nucleus , 1.77 %
R protein_biosynthesis , 1.34 %
S cell_differentiation , 1.31 %
T hydrolase_activity , 1.25 %
U transferase_activity , 1.22 %
V nucleotide_binding , 1.21 %
W protein_modification , 1.19 %
X Other , 24.72 %

Figure S1. Gene ontology mapping of O*. degus* genes. The GO terms were mapped into the general GO slims without top level categories – biological process, molecular function, cellular component.

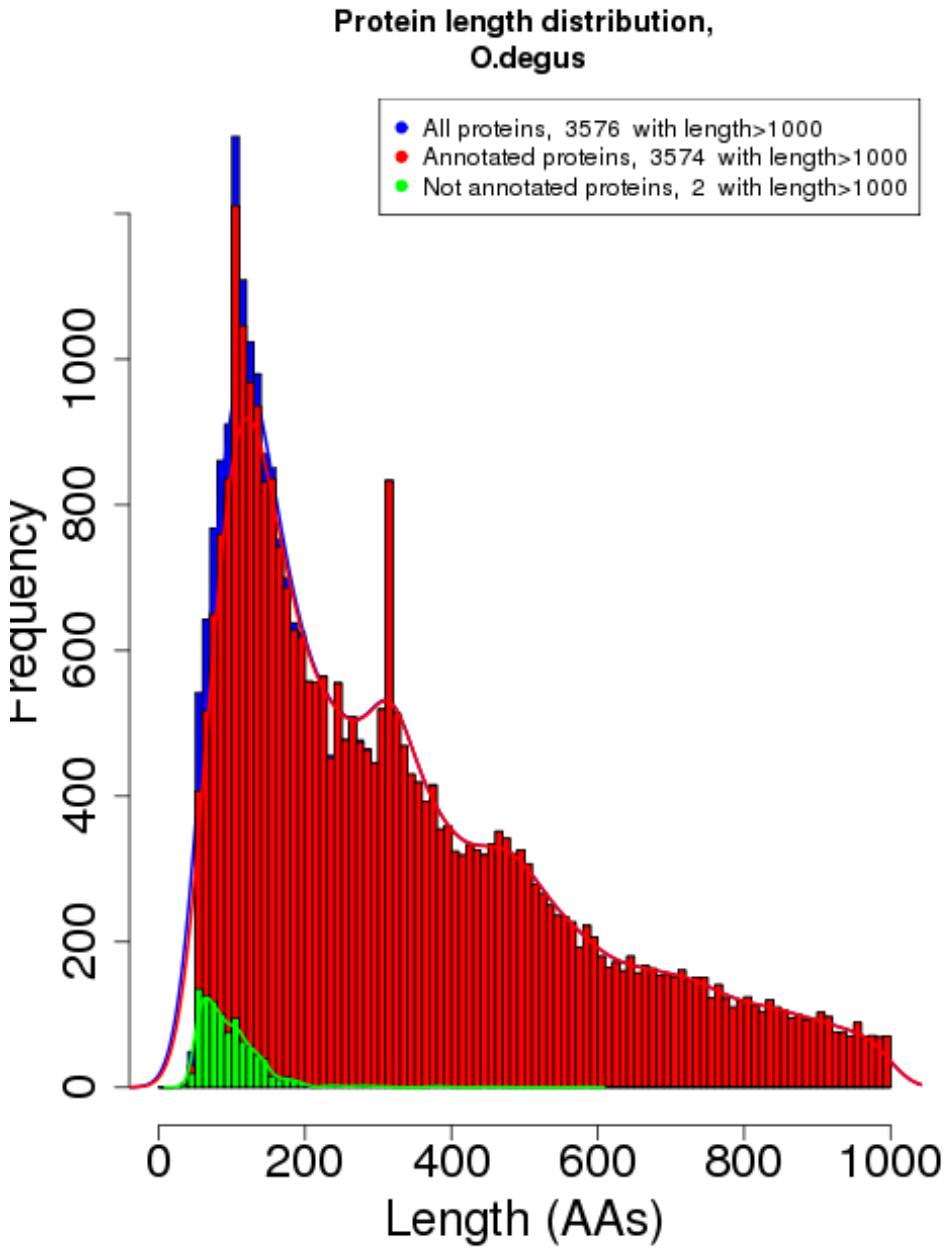


Figure S2. **Distribution of functionally annotated and non-annotated proteins.** Number of annotated and non-annotated sequences in relation to their length. The blue color correspond to all proteins, red – annotated proteins and green to the non-annotated proteins.