

COMPARISON BETWEEN GENES OF ALZHEIMER'S DISEASE AND BRAIN TISSUE OF OTHERS

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ALZHEIMER KÓROS BETEGEK GÉNJEINEK ÖSSZEHASOLÍTÁSA MÁS AGYSZÖVETI MINTÁKKAL

14 donor agyi szövetmintáját vizsgáltuk, ezeket esetenként 44 különböző anatómiai régióból választottuk. Igyekeztünk – az azonos régiók pontos megválasztása miatt, mélyfagyasztott mintavétellel párhuzamosan formalin-fixált parafinba ágyazott kontroll anyagot gyűjteni. A mélyfagyasztott állapotban való szállítást követően történtek az RNA vizsgálatok, melyek eredményei csaknem fele magas kvalitásúak voltak. Az Alzheimer kórban elhaltak agyszöveti mintáit ezüstözés módszerével hasonlítottuk össze más (szkizofrén, MID és normál) kontrollok agyszöveti mintáival.

KULCSSZAVAK: Alzheimer kór, agyszöveti minták, RNA, módszerek

SUMMARY

We have examined samples from 14 donors that were prepared from 44 different anatomical regions of the brain. These samples were prepared both as frozen and formalin-fixed, paraffin embedded. There were 310 frozen samples and 367 fixed samples. A total of 265 of the frozen samples were tested for RNA quality. A very high quality was obtained with 122 of these frozen samples (44%). The sample numbers were not large enough to draw conclusions about RNA quality for individual brain regions. Silver staining was performed on some samples and 5 of 7 samples tested from one donor diagnosed with Alzheimer's like dementia showed evidence of Alzheimer's disease by this method.

KEYWORDS: Alzheimer's disease, brain samples, RNA, methods

We have excellent knowledge about the brain's biological changes of Alzheimer's disease, but only a little about the gene and also the therapy.

There are reports suggesting that homozygosity for codon 129 (methionine or valine allele) is connected with early-onset Alzheimer's disease, but not late onset (Collinge 1999, Dermant et al. 2003). A small, but significant acceleration in cognitive decline in late onset Alzheimer's disease patients is homozygous for valine. Additionally, a link between V129V genotype and cognitive impairment in the elderly patients was demonstrated (Del Bo et al. 2003).

Also epidemiological studies indicate familial clustering of sporadic Alzheimer's disease (van Duijn et al. 1998).

In our studies we are investigating of some genes of the brain tissue, first of all from the

Alzheimer's disease patients. In this first article we are talking about the methods.

Samples and Methods

In collaboration we have been performing autopsies on the brains of deceased patients after fully informed consent by next of kin.

Table 1 shows the numbers of samples received and examined from each of 22 distinct regions. The total number is nearly double this if each hemisphere is considered separately. There were 371 fixed samples sent, but for technical reasons, 4 were not analyzed.

There were 299 samples received that were snap frozen. This number is less than the number of FFPE samples received because we received only FFPE samples from the first two donors (Table 2) and no frozen samples.

Table 1. Sample numbers by brain region

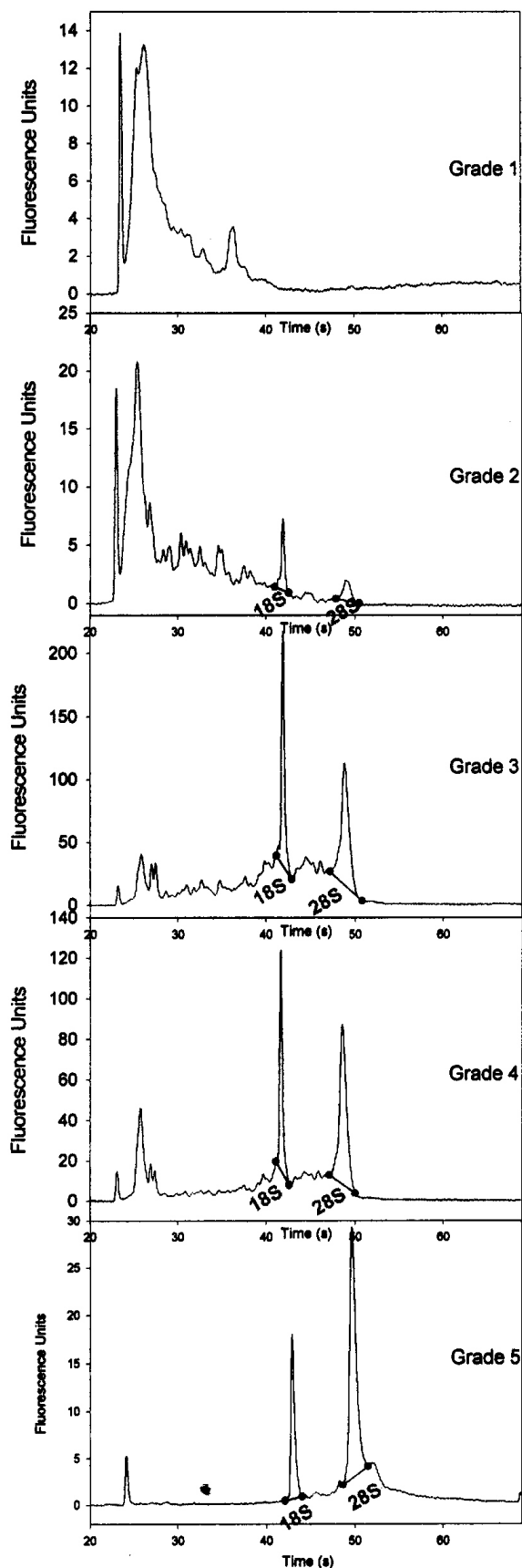
Brain Region	FFPE	Frozen	Total
Accumbens Area	24	20	44
Amygdala	18	17	35
Brodman's Area 10	20	16	36
Brodman's Area 9	18	12	30
Caudate Nucleus	16	13	29
Cerebellar Hemisphere	8	6	14
Cerebellum Caudate Nucleus	8	6	14
Cerebellum Hemisphere	16	14	30
Cingulum Bundle	24	20	44
Cortex Parietal Lobe	8	6	14
Cortex Prefrontal	4	4	8
Diencephalon Thalamus	8	6	14
Hippocampus	22	17	39
Nucleus Raphe	15	13	28
Orbital Gyrus	22	17	39
Parietal Lobe	16	13	29
Putamen	23	18	41
Substantia Nigra	24	20	44
Temporal Lobe	22	18	40
Thalamus	16	13	29
Ventral Tegmental Area	20	18	38
Vermis	15	12	27
Total	367	299	666

Table 2. Numbers of Fixed Samples per dono

DIN	Total	DIN	Total
OPI-001	32	OPI-002	32
OPI-003	29	OPI-004	32
OPI-005	32	OPI-006	32
OPI-007	32	OPI-008	32
OPI-009	32	OPI-010	29
OPI-011	26	OPI-012	31
Grand Total			371

The RNA grading system is based on the following criteria from the Agilent profile. If there is no measurable RNA, the grade is 0. For those samples with measurable RNA, the following criteria are each given 1 point for a total score between 1 and 5. Examples of grades 1-5 are shown in Fig. 1.

- The ratio of 28S to 18S peaks is equal to or greater than 1.3.
- The area under the 28S and 18S peaks combined is equal to or greater than 30% of the total area.
- The run time from the beginning of the 18S peak to the end of the peak is less than or equal to 4 seconds and the same for the sharpness of the 28S peak.
- There are no distinct peaks between the 28S and 18S peaks or between the 18S peak and the lower marker peak.
- The area under the low molecular weight peaks is less than the area under the 28S and 18S peaks.

Figure 1
Typical profiles from the Agilent
Bioanalyzer 2100

Grades 3, 4 and 5 are considered very high quality and can be used for many types of studies. RNA with Grade 2 may be used for a number of different molecular studies, particularly PCR analysis.

RNA Measurements

The overall summary of results of the RNA analyses is shown by grade in Table 3. There were 255 samples analyzed. This number is less than the total number of samples received (Table 1) because 40 samples were too small to assay. These „too small“ samples were primarily from following regions Nucleus Raphe, Ventral Tegmental Area, Substantia Nigra, and one Hippocampus L (Table 4), where the size of the anatomical structure is small. There were 4 samples that were not analyzed for other technical reasons.

Table 3
RNA Grade of Frozen Brain Samples

RNA Grade	Total	Percentage
0	2	1%
1	25	10%
2	116	45%
3	93	36%
4	19	7%
Grand Total	255	100%

Overall, 43% of the samples that were assayed had very high quality RNA with grades of 3 and 4. There were no samples with Grade 5, which is typical for postmortem samples because the time between death and preparation of the sample is on the order of several hours. The bulk of samples had RNA Grade 2, which is still useful for many investigations.

Table 4 shows the summary of RNA results by anatomical region. This table includes the samples that were too small to assay. Other than the size of the sample, the numbers of samples in each category from the different regions are too small for statistical analysis. Of the total of 292 samples, 38% had high quality RNA, the small samples may be expected to have a similar proportion with high quality as well.

The RNA results have also been analyzed by donor to determine if there are donor characteristics that may lead to poor RNA recoveries. The results are shown in Table 5. Donors OPI-003, OPI-006 and OPI-007 all had >75% of the samples with very high quality RNA. These are remarkably good for postmortem recoveries. Donors OPI-008, OPI-009 and OPI-012 had 15% of the samples or less with high quality RNA. In this

Table 4
RNA results for Frozen Brain Samples by Anatomical Region

	High quality	% High	Ordinary quality	% ordinary	Too small	% too small	Total
Accumbens Area	9	45	8	40	3	15	20
Amygdala	4	29	10	71	0	0	14
Brodmanns Area 10	8	50	8	50	0	0	16
Brodmanns Area 9	7	58	5	42	0	0	12
Caudate Nucleus	4	31	9	69	0	0	13
Cerebellar Hemisphere	2	33	4	67	0	0	6
Cerebellum Caudate Nucleus	3	50	3	50	0	0	6
Cerebellum Hemisphere	9	64	5	36	0	0	14
Cingulum Bundle	9	45	11	55	0	0	20
Cortex Parietal Lobe	2	50	2	50	0	0	4
Cortex Prefrontal	2	50	2	50	0	0	4
Diencephalon Thalamus	3	50	3	50	0	0	6
Hippocampus	4	24	10	59	3	18	17
Nucleus Raphe	0	0	10	48	11	52	21
Orbital Gyrus	7	78	2	22	0	0	9
Parietal Lobe	4	31	9	69	0	0	13
Putamen	11	61	7	39	0	0	18
Substantia Nigra	4	20	9	45	7	35	20
Temporal Lobe	10	56	8	44	0	0	18
Thalamus	3	25	9	75	0	0	12
Ventral Tegmental Area	0	0	1	6	16	94	17
Vermis	7	58	5	42	0	0	12
Grand Total	112	38	140	48	40	14	292

Table 5. RNA Results by Donor

Ex Spec ID	RNA Grade	Number	Percent age	Diagnosis	Cause of death
OPI-003	1	1	3%	Alzheimer's like dementia	Pneumonia
	2	2	7%		
	3	14	48%		
	4	12	41%		
OPI-004	1	10	38%	Alzheimer's disease	Myocardial infarction
	2	8	31%		
	3	8	31%		
OPI-005	1	1	4%	Alzheimer's like dementia	Pneumonia
	2	17	63%		
	3	9	33%		
OPI-006	2	4	18%	Multi-infarct dementia	Cardiomyopathy
	3	18	82%		
OPI-007	1	2	7%	Multi-infarct dementia	Coronary sclerosis
	2	4	14%		
	3	19	68%		
	4	3	11%		
OPI-008	1	7	27%	Paranoid Schizophrenia	Hypostatic pneumonia
	2	15	58%		
	3	4	15%		
OPI-009	0	2	9%	Multiple Infarct dementia	Cardiac arrest
	1	3	13%		
	2	18	78%		
OPI-010	1	1	2%	Paranoid Schizophrenia	Cardiac arrest
	2	9	21%		
	3	13	31%		
	4	1	2%		
OPI-011	2	16	70%	Alzheimer's disease	Pneumonia
	3	4	17%		
	4	3	13%		
OPI-012	2	23	85%	Normal	Pneumonia
	3	4	15%		

very small set of donors, no trends are evident in terms of diagnosis or cause of death.

In the next articles we shall present the detailed results and get the conclusions.

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