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The Stanley Foundation brain collection and Neuropathology Consortium

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Abstract

The Stanley Foundation brain collection is an attempt to supplement existing brain collections for the purpose of promoting research on schizophrenia and bipolar disorder. Specimens are collected with the permission of the families in a standardized manner, with half of each specimen being frozen and half fixed in formalin. The Neuropathology Consortium is a subset of 60 specimens from the collection, well-matched groups of 15 each with diagnoses of schizophrenia, bipolar disorder, major depressive disorder without psychotic features, and normal controls. More than 75 000 sections and blocks from the Consortium have been sent to over 50 research groups worldwide to carry out a wide variety of assessments. These data will be integrated to provide a more complete picture of the neuropathology of these disorders. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The Stanley Foundation brain collection was begun in 1994 to promote research on schizophrenia and bipolar disorder. It was undertaken to supplement existing American brain collections because of a belief that the existing collections were insufficient to meet the growing needs of psychiatric researchers.

The Stanley Foundation brain collection is located in the Department of Psychiatry of the

Uniformed Services University of the Health Sciences (USUHS) in Bethesda, MD. From the brain collection's inception, the personnel associated with it have collaborated closely with, and initially shared resources with, personnel working with the NIMH brain collection in the Clinical Brain Disorders Branch under Drs. Joel Kleinman and Mary Herman.

The Stanley Foundation brain collection presently consists of over 300 specimens and is continuing to grow. Brains are obtained by pathologists in the offices of designated medical examiners in Maine, Minnesota, California, and Washington. The pathologists come to Bethesda to be trained in a standardized collection technique. All expenses

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associated with the collection are covered by the Theodore and Vada Stanley Foundation, and the specimens are made available without charge to qualified researchers worldwide.

2. Selection, clinical information, and diagnosis

Potential donors for the brain collection are identified by investigators in the offices of the designated medical examiners. Suicides, deaths occurring in group homes known to house individuals with psychiatric disorders, and deaths occurring in residences in which antipsychotic or mood stabilizing medications are found by the investigators are considered to be potential donors. Individuals over age 65 are excluded because of the increased likelihood of comorbid neurologic disorders. A pathologist then contacts the family of the deceased to make a preliminary diagnosis and requests permission for donation of the brain and for release of the deceased's medical records.

If permission is granted, the pathologist does a brief interview to ascertain information regarding the deceased's birth and development, education, job history, family history of severe psychiatric disorders, psychiatric hospitalizations, medication at time of death, drug and alcohol use, and an estimate of the person's awareness of their illness. Medical and psychiatric records are requested for known hospitalizations and outpatient treatments. Record requests continue to be made until sufficient information has been collected to make a clear diagnosis. In cases where medical records are insufficient to establish a working diagnosis, a psychiatrist contacts one or more family members by telephone to clarify the symptoms or course of the illness. In most cases, the available clinical information is not sufficient for the rating of cognitive deficits or for quantifying positive and negative symptoms.

All records are reviewed by one psychiatrist (EFT) and summarized in narrative form, and the information is entered into a computerized database by identifying number only. The database includes demographic data, family history, education, age of onset, total duration of hospitalizations, psychiatric diagnosis, cause of death,

medical diagnoses, medications at time of death, brain weight, interval between death and refrigeration of body, and interval between death and freezing of brain tissue [postmortem interval (PMI)]. An estimate of total lifetime antipsychotic medication (in fluphenazine milligram equivalents) is made (Torrey, 1988). Qualitative ratings (on a 1 to 5 scale) are entered for pregnancy and birth complications, developmental problems, global severity of illness, severity of drug and alcohol use (past and recent), awareness of illness, and whether or not the person was having an exacerbation of symptoms at the time of death. Smoking history is available on most cases collected since 1996. All assessments and coding have been done by a single individual (EFT).

Regarding family history of severe psychiatric disorders, it should be noted that the authors do not consider the information collected to be sufficient for genetic studies. The information available on family history consists only of questions asked by the pathologist, information available in the psychiatric records, and in some cases questions asked by the senior author. No attempt is made to obtain the psychiatric records of family members who are identified as possibly psychiatrically ill or to do a structured interview. Since these specimens are collected at the time of death, such probing would be intrusive.

For normal controls, a structured telephone interview with a first-degree family member is carried out in all cases. The interviewer requests information about birth and development, education, jobs, family history of mental illness, drug and alcohol use, smoking, medical problems, and medications and asks open-ended questions such as "Tell me about the personality of ____" and "Did ____ ever seek counseling or psychiatric help?" The reliability of this interview to rule out psychiatric diagnoses has not been ascertained.

When all the information has been collected, a DSM-IV psychiatric diagnosis is made independently by two senior psychiatrists. If there is disagreement between them, the records are given to a third senior psychiatrist, and a consensus diagnosis is arrived at. In almost all cases, information is not sufficient to make an axis II diagnosis. The DSM-IV diagnostic breakdown for all speci-

mens received to date is as follows: schizophrenia, 28%; bipolar disorder, 19%; major depressive disorder, 18%; psychotic disorder NOS or mood disorder NOS, 8%; other psychiatric diagnosis, 8%; normal control, 19%.

3. Processing of brain tissue

The medical examiners are trained to collect and process the brain tissue in a standardized manner. The cerebrum is hemisected, and one half is fixed in formalin while the other is cut into 1.5 cm thick coronal slices and frozen in a mixture of isopentane and dry ice. The cerebellum and brain stem are not hemisected and are completely frozen. Right and left brain hemispheres are randomly alternated for formalin fixing or freezing. The frozen half is then sent to our laboratory on dry ice by overnight delivery. All frozen tissue is stored at -70°C , with computerized temperature alarm and backup emergency generator. Each brain is examined by a certified neuropathologist to rule out Alzheimer's disease and other cerebral pathology.

All specimens are assessed for brain pH. Glyceraldehyde phosphate dehydrogenase (GAPdH, a housekeeping gene) mRNA level is measured with a reverse transcription polymerase chain reaction (RT-PCR). The mRNA is graded as A (excellent), B (good), C (fair), D (poor), or F (very poor). Studies have been carried out on 89 specimens from the Stanley Foundation brain collection to ascertain the relationship of pH, mRNA, and PMI. Consistent with previous studies (Harrison et al., 1991, 1995), it was found that the quality of mRNA deteriorates with conditions of agonal hypoxia (e.g. artificial ventilation) and that pH is a reasonably good measure of mRNA quality (i.e. the higher the pH, the better the mRNA), although there is a great degree of scatter in the data (Johnston et al., 1997).

From the above studies, it has become clear that pH as a measure of agonal state is the best single predictor of mRNA quality, not PMI, as has been widely assumed. Rapid death with a relatively long PMI usually yields better mRNA than a prolonged death with conditions of hypoxia

and a short PMI. Thus, in our collection, there are specimens with excellent mRNA with PMIs as long as 142 h (heart attack) and specimens with very poor mRNA with PMIs as short as 5 h (suicide by carbon monoxide). Other factors, such as interval between death and refrigeration of body and the ambient temperature during this interval, presumably also determine the relationship between mRNA and PMI.

This phenomenon is also supported on the protein level by a work in progress using two-dimensional gel electrophoresis to study protein in the frontal lobe. Of 217 proteins compared between 89 brains (including all Consortium brains plus 29 other individuals), five showed levels that correlated significantly ($P < 0.001$ or better) with pH, whereas only one protein showed the same significant association with PMI. Therefore, while PMI may be a consideration for certain susceptible proteins or mRNAs, it should no longer be the determining factor as to whether a tissue is suitable for postmortem study in these or other types of work.

4. Neuropathology Consortium

The Stanley Foundation Neuropathology Consortium is a selection of matched specimens from the brain collection. It contains 15 cases each from individuals with schizophrenia, bipolar disorder, major depressive disorder without psychotic features, and normal controls. These groups are matched as shown in Table 1 and were all collected between September 1994 and February 1997. Information on smoking history is available on some cases, but the groups are not matched for this factor. Table 2 contains data regarding cause of death, family history, presence of psychosis, lifetime exposure to antipsychotic drugs, and comorbid substance abuse.

Urine and blood toxicology screens were performed for subjects within the Consortium. However, these assays were performed according to the local practice of the medical examiners rather than a standardized protocol. Quantitative measurements of prescribed medications and of substances of abuse are available to researchers using Consortium tissue.

Table 1
Matched variables for the Stanley Foundation Neuropathology Consortium^a

	Schizophrenia	Bipolar disorder	Major depression	Normal controls
Age	44.2 (25–62)	42.3 (25–61)	46.4 (30–65)	48.1 (29–68)
Sex	9M, 6F	9M, 6F	9M, 6F	9M, 6F
Race	13C, 2A	14C, 1AA	15C	14C, 1AA
PMI (h)	33.7 (12–61)	32.5 (13–62)	27.5 (7–47)	23.7 (8–42)
mRNA yield	10A, 2B, 3C	13A, 2B	11A, 2B, 2C	12A, 2B, 1C
pH	6.1 (5.8–6.6)	6.2 (5.8–6.5)	6.2 (5.6–6.5)	6.3 (5.8–6.6)
Side of brain frozen	6R, 9L	8R, 7L	6R, 9L	7R, 8L

^a A = Asian, AA = African–American, C = Caucasian.

Table 2
Clinical characteristics of the Stanley Foundation Neuropathology Consortium

	Schizophrenia	Bipolar disorder	Major depression	Normal control
<i>Cause of death</i>				
Suicide	4	9	7	0
Cardiopulmonary	8	4	7	13
Accident	2	1	0	2
Other	1	1	1	0
<i>Family history of psychosis</i>				
First degree	3	4	1	0
Second degree	3	3	0	1
Family history not available	0	1	0	0
<i>History of psychosis</i>				
	15	11 with 4 without	0	0
<i>Antipsychotic exposure (mg)</i>				
	52 267 ± 62 062 1 never	20 827 ± 24 016 3 never 1 >20 years 1 several months	0	0
<i>Current alcohol/drug abuse or dependence</i>	3	4	3	0
<i>Past alcohol/drug abuse or dependence</i>	3	3	1	2

The 60 matched specimens that constitute the Neuropathology Consortium are made available without charge to research groups around the world. To date, more than 60 000 frozen sections, 11 000 fixed sections, 4000 frozen blocks and 1000 fixed blocks have been distributed to more than 50 groups in 10 countries in addition to the United States. The tissue is sent coded; when the researchers have completed their study, they send the results to the Stanley Foundation and simultaneously receive the code. The researchers are free to publish their results wherever they wish. However, they also agree that their findings for the 60 brain specimens can be integrated with findings from other researchers working on these specimens.

The regions of greatest interest, including the hippocampus and amygdala, striatum, prefrontal cortex, anterior cingulate, thalamus, and brainstem, are completely sectioned. Regions from the frozen hemisphere are sectioned at 14 µm thickness, and regions from the formalin-fixed hemisphere are embedded in paraffin and sectioned at 10 µm thickness. The formalin-fixed thalamus has been cryo-protected and sectioned at 60 µm. One section in every 100 is stained with a Nissl stain to establish the histological quality of the tissue and to determine the extent of individual nuclei within structures so that areas can be matched precisely when sections are sent to the various labs.

Studies currently in progress using these 60

specimens include cytoarchitectonics, neurotransmitters and receptors, neuropeptides, synthetic enzymes, neurotrophic factors, synaptic proteins, signal transduction pathways, and markers of inflammation and infection. In some cases, more than one laboratory is doing the same study; this will be especially valuable for studies in which past findings have been contradictory for specimens from different brain collections. A 2D protein electrophoresis has also been done on each of the consortium specimens and is available to researchers using them. Publications that have appeared to date from the Stanley Foundation Neuropathology Consortium are listed separately in the Bibliography of Stanley Foundation Neuropathology Consortium studies.

Researchers who wish to apply to use brain tissue from the Stanley Foundation Neuropathology Consortium should contact Dr. Torrey at the Stanley Foundation Research Programs, 5430 Grosvenor Lane, Suite 200, Bethesda, MD 20814, USA; tel.: +1-301-571-2078, fax: +1-301-571-0769, e-mail: ostmannl@stanleyresearch.org. Allocation of tissue is done by a selection committee that considers the availability of tissue, the hypothesis, and whether or not tissue has already been sent to multiple laboratories for the proposed work.

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