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Review

Alzheimer's Disease and the "Valley of Death": Not Enough Guidance from Human Brain Tissue?

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Abstract. Medical science is currently perceived as underperforming. This is because of the relatively slow recent rate of development of new disease treatments. This has been blamed on cultural, regulatory, and economic factors that generate a so-called "Valley of Death", hindering new drug candidates from being moved into clinical trials and eventually approved for use. We propose, however, that for neurodegenerative diseases, a relative decline of human brain tissue research is also a contributor. The present pharmacological agents for treating Alzheimer's disease (AD) were identified through direct examination of postmortem human brain tissue more than 30 years ago. Since that time the percentage of research grants awarded to human brain tissue-using projects has dropped precipitously and publication rates have stagnated. As human brain tissue research has played a central and often initiating role in identifying most of the targets that have gone to AD clinical trials, it is proposed that the rate of discovery of new targets has been curtailed. Additionally, the continued rejection of cortical biopsy as a diagnostic method for AD has most probably depressed the perceived effect sizes of new medications and contributed to the high Phase II clinical trial failure rates. Despite the relative lack of funding, human brain discovery research has continued to make important contributions to our understanding of neurodegenerative disease, and brain banks have played an essential role. It is likely that the pace of discovery will dramatically accelerate over the coming decades as increasingly powerful tools including genomics, epigenetics, transcriptomics, regulatory RNA, gene expression profiling, proteomics, and metabolomics are applied. To optimize the promise of these new technologies, however, it is critical that brain banks are rejuvenated by enhanced governmental and/or private support.

Keywords: Alzheimer's disease, autopsy, brain, clinical trials, human, neuropathology, postmortem

THE "VALLEY OF DEATH" AND HUMAN TISSUE-BASED RESEARCH

A previous issue of this journal appropriately celebrated a century of Alzheimer's disease (AD) research and simultaneously raised the question, "Where do we go from here?" Early in this second AD century, we have been presented with a challenge that is both disheartening and provocative. A nagging uneasiness with tangible progress, common across all biomedical research fields, has broken out into the open, forcing our collective gaze into what has been termed the "Valley of Death" [1]. Despite the stunning avalanche of data emanating from powerful new technology, the production rate of new effective medications has been steadily dropping. Neurodegenerative research is no exception. The major Food and Drug Association (FDA)-approved therapeutic agents for AD are based on work done three to four decades ago. As a result, our most urgent question has become, "Where have we gone wrong?"

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The "Valley of Death" has been variously portrayed as a chasm between biomedical researchers and patients, or between basic science researchers in academia and applied science researchers in the pharmaceutical industry, or between basic science ideas and the hurdles they must cross to regulatory approval. What is clear is that the translation of basic science discoveries to drugs with obvious real-world benefits is becoming less, rather than more frequent than any of us are comfortable with. Multiple reasons for the gap have been advanced, including a shift in National Institutes of Health research grants to PhDs rather than MDs, the increasingly complex barriers to FDA approval, the cost escalation of large clinical trials, the increasing dependence on for-profit corporate involvement, and the realization that many diseases are etiologically heterogeneous [2–4].

Another possible cause has received much less attention but may be much more important. In 2002, the National Dialogue on Cancer, convened to understand why the "war on cancer" was falling short of expectations, concluded that of the ten most important roadblocks to finding cures for cancer, the single most critical one was inadequate availability of "high-quality, highly characterized human tissues for translational research" (Carolyn Compton, Former Director, NCI Office of Biorepositories and Biospecimen Research). As a result, in 2003 the NCI published the National Biospecimen Network Blueprint and in 2005 formed the Office of Biorepositories and Biospecimen Research (OBBR) to stimulate and coordinate the development of tissue resources and capabilities. The need for better access to high quality tissue has been widely cited by other groups, including the NIH Blueprint for Neuroscience Research [5], the Genomics and Personalized Medicine Act of 2007, the Department of Health and Human Services' "Personalized Health Care Report" (2007), and the President's Council of Advisors on Science and Technology: Priorities for Personalized Medicine (2008).

This article presents the viewpoint that the stalemate in AD translational research may be at least partially attributable to a relative decline in human brain tissue-based research, not only due to poor availability of suitable tissue but also to funding declines and reduced publications emanating from human tissue-based research. Current FDA-approved therapies for AD are still largely restricted to cholinergic replacement, an approach that was suggested by human brain tissue studies in the 1970 s [6–9]. The only other approach, directed at blocking glutamatergic excitotoxicity (memantine/Namenda), was approved by the FDA in 2003 but the first description of excitotoxicity had been in 1957 [10] and its application to AD was first envisioned in the early 1980 s. Thirty years of ensuing research has really produced no new effective agents.

DECLINE IN FUNDING AND PUBLICATIONS USING HUMAN BRAIN TISSUE

We investigated whether or not there has been a decline in both the number of funded AD research projects and the number of AD publications that utilize human brain tissue. A search of the NIH RePORTER website database (http://projectreporter.nih.gov/reporter.cfm) was performed using "Alzheimer's" as a key word within the text of project titles, abstracts, and terms. The total number of awards was recorded and all awards with an abstract were examined to determine the

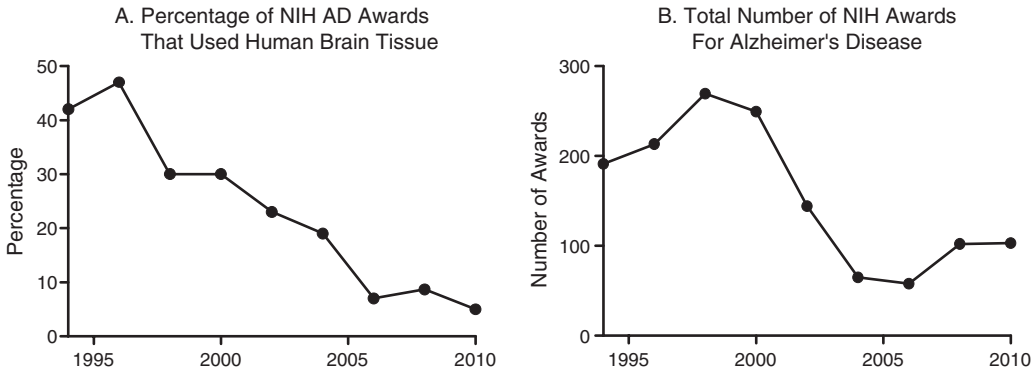

Fig. 1. Percentage of NIH awards for AD research that used human brain tissue (A) and total number of NIH awards for AD research (B) between 1994 and 2010.

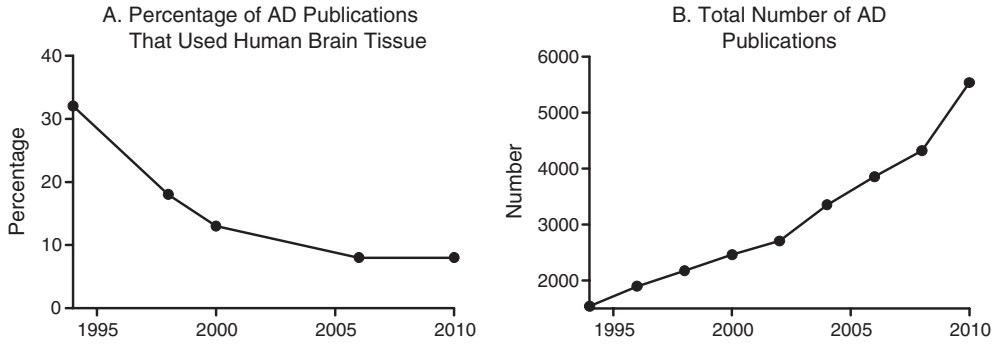

Fig. 2. Percentage of PubMed-listed AD publications that used human brain tissue (A) and total number of AD publications (B) between 1994 and 2010.

proportion that used human brain tissue. For years with more than 100 projects with abstracts, only the first 100 abstracts were examined to determine the proportion. Searches were done every two years over a time period extending from 1994 through 2010 and results are shown in Fig. 1. Over this time period, the proportion of awards that used human brain tissue peaked at 47% in 1996 and then steadily declined to only 5% in 2010 (Fig. 1a). As the total number of AD awards also declined (Fig. 1b), over time the absolute number of awards that have used human brain tissue has been considerably reduced.

The US National Library of Medicine was then searched for AD publications using their PubMed website (http://www.ncbi.nlm.nih.gov/pubmed). The percentage of publications that used human brain tissue was estimated by reading abstracts from the first 100 articles listed for each year. The only search term used other than year was "Alzheimer's". Only articles with an abstract were included and review articles were excluded. As for funding awards, searches were done over a time period extending from 1994 through 2010. The results, shown in Fig. 2, indicate that the percentage of AD publications that used human brain tissue in 2010 had dropped to one-quarter of what it was in 1994 (Fig. 2a) while in the same time period the total number of AD publications more than tripled (Fig. 2b). The estimated absolute number of AD publications using human brain tissue remained static, with 492 in 1994 and 443 in 2010.

WHAT IS THE EVIDENCE THAT HUMAN BRAIN TISSUE-BASED RESEARCH IS IMPORTANT?

Although it appears that human brain tissue-based AD research funding and publications have declined, how do we know that this has had any impact on AD translational research? We have pointed out that the major FDA-approved agents for the defining symptoms of AD (cognitive loss) act to replace the cortical cholinergic deficit that was discovered through direct examination of AD and control brains [7–9]. The only non-cholinergic FDA-approved agent, memantine, can be considered to have had contributing origins from animal, cell culture, and human studies. The animal studies, dating from 1951 [11, 12], demonstrated the neurotoxicity of glutamate and molecular analogues. The term "excitotoxicity" was coined by Olney in 1974 [10]. The idea of using excitotoxic animal models to model AD appears to have first been mentioned in the early 1980 s [13]. The first PubMed linkage of AD to glutamate and NMDA receptors was in 1986 when Geddes and Cotman described the localization of NMDA receptors to AD-susceptible hippocampal sectors [14, 15]. A 1985 cell culture study, meanwhile, had used glutamate to induce paired helical filaments [16].

What, however, has been the contribution of human brain tissue studies to approaches developed in subsequent years? We searched the NIH website (http://www.clinicaltrials.gov), for agents registered for AD clinical trials (Table 1). The therapeutic approaches have been loosely grouped into anti-amyloid, anti-tau, neurotransmitter modulation, anti-aging, vascular, and anti-inflammatory. Agents without a clear molecular mechanism are not listed. For each approach we searched the literature to determine whether the origin could be attributed to studies of human brain tissue, cell culture models, animal models, human molecular genetics, or epidemiology.

The most obvious approach to AD therapy has been to prevent or remove plaques and tangles. These were first linked to the clinical entity of AD by Alzheimer in 1906 [17] but a molecular strategy was not possible until Glenner and Wong isolated amyloid-β peptide

Table 1
Agents listed by the NIH website, http://www.clinicaltrials.gov, as registered for AD clinical trials. Agents are grouped into approaches by molecular mechanism. A literature search was done for each approach to determine whether the origin could be attributed to studies of human brain tissue (HBT), cell culture models (CLCT), animal models (ANML), human molecular genetics (HMG) or epidemiology (EPID). Dates of first conception and important original articles or reviews are listed

Approach or mechanism	Agents	Discovery method year and references
PLAQUES AND TANGLES		
Anti-amyloid		HBT 1984 [18–20]
	ACC-001	
Immunotherapy	Affitope AD01	
	Affitope AD02	
	AN1792	
	Bapineuzimab	
	CAD106	
	Gammagard (IVIg)	
	MABT5102A	
	Ponezumab	
	Solanezumab	
	B11B037	
	AAB003	
Gamma-secretase inhibitors	Besipirdine (BMS-708163)	
	LY450139 (Semagacestat)	
Alpha-secretase stimulation	EHT 0202 (Etazolate)	
	Epigallocatechin-Gallate	
	Acitretin	
Anti-amyloid, other	Alzhemed (tramiprosate)	
	Cerebrolysin	
	Clioquinil	
	Curcumin	
	ELN-D005 (scyllo-inositol)	
	Flurizan	
	Huperzine A	
	PBT-2	
	resveratrol	
	Rosiglitazone	
	Minocylcine	
	Zinc-cysteine	
Anti-tau		HBT 1986
Inhibition of tau phosphorylation	nicotinamide	
	lithium	
	TRX-0014	
NEUROTRANSMITTERS		
Acetylcholine		HBT 1976 [6–9]
Anti-cholinesterases	donepezil (Aricept)	
	Dimebon	
	Eptastigmine	
	Rivastigmine (Exelon)	
	galantamine (Reminyl)	
	Huperzine A	
	Metrifonate	
	Phenserine	
	physostigmine	
	propentophylline	
	Tacrine (Cognex)	
	ZT-1	
Muscarinic receptor agonists	xanomeline	
	AF 102B	
	AF 267B	
Nicotinic receptor agonists	AZD1446, TC-1734	
	EVP-6124	
	RO5313534	
	TC5619-238	
	MEM3454	
	AZD3480	
Nerve growth factor	CERE-110	
	Huperzine A	
	PRX-03140	
	NGF NSG0202	
Cholinergic, other	MKC-231	
	Nefiracetam	
	Tesofensine	
	PRX-03140	
	ST101	
Glutamate		
NMDA receptor antagonist	memantine (Namenda)	ANML 1951 [11]
		CLCT 1985 [16]
		HBT 1986 [14, 15]
	CX516 (Ampalex)	
$GABA_A$ receptor ligands	Suritozole	HBT 1980 s [28]
		CLCT 1997 [29]
		ANML 2003 [30]
	SG3742	
Histamine		
HT_3 receptor ligands	GSK 239512	CLCT 1988 [31]
	Dimebon	HBT 1998 [165, 166]
		ANML 1996 [33, 34]
ANTI-AGING		Extant aging literature
Sex hormone replacement		
Female hormone replacement	estrogen (Premarin)	
	Estrogen/progesterone	
	Raloxifene	
Male hormone replacement	testosterone	
Luteinizing hormone modulation	Leuprolide	
Anti-oxidants	Alpha-tocopherol	Extant aging literature
	Curcumin	HBT [35]
	Melatonin	
	resveratrol	
	Vitamin C	
	lutein	

Table 1
(*Continued*)

Approach or mechanism	Agents	Discovery method year and references
RAGE inhibitors	PF-04494700	HBT CLCT ANML 1996 [167]
VASCULAR		HBT 1906 [17] HBT 1991 [38] HMG 1993 [39, 40] EPID 1990 s [49, 168]
Cholesterol-lowering agents	Atorvastatin (Lipitor)	
	Lovastatin	
	Pravastatin	
	Choline	
	Clofibric acid	
	Gemfibrozil	
	Niacin	
	Pitastatin	
Anti-hypertensive agents	carvedilol	
Platelet aggregation inhibitors	Cilostazol	
	Resveratrol	
Arteriolar vasodilatation	NCT01439555	
	Phosphodiesterase inhibitor type IV	
	Cilostazol	
Omega 3 fatty acids	Docosahexanoic acid	
Anti-diabetic	Rosiglitazone	
	Insulin	
	Oral hypoglycemics	
	IGF-1	
	Exenatide	
Glucose/energy	AC-1204	
	Acetyl-l-carnitine	
	Rosiglitazone	
	Ketasyn	
ANTI-INFLAMMATORY		HBT 1987 [51, 52]
	Dapsone	
	Etanercept	
	Ibuprofen	
	Naproxen	
	Rofecoxib	
	Thalidomide	
	Cyclophosphamide	
	Minocycline	
	Doxycyline	
	Rimampin	
	Prednisone	
	Interferon-beta	
	Interferon-alpha	
	acetominophen	
	celecoxib	
	Cycloxygenase inhibitors	
	Cycloxygenase-2 inhibitors	
	Indomethacin	
	Lornoxicam	
	Piroxicam	
	Reficoxib	
	resveratrol	
	Rosiglitazone	

from AD and Down syndrome cerebrovascular amyloid brain tissue in 1984 [18] and further critical steps toward solidifying the "amyloid hypothesis" included working out the entire amino acid sequence, localizing the gene to chromosome 21, developing antibodies, identifying causal mutations in early-onset AD families, discovering the enzymes responsible for cleavage, and creating transgenic mice bearing the causative mutations [19, 20]. The amyloid hypothesis clearly originated from human brain tissue studies but its subsequent elaboration vividly illustrates how a constant interchange between human brain tissue studies and those involving cell and animal culture models as well as molecular genetics leads to the rapid development of a seed idea.

The molecular dissection of tangles occurred almost concurrently with that of amyloid as tau presence and hyperphosphorylation within tangles was first described from human brain tissue studies by Grundke-Iqbal in 1986 [21]. As with the amyloid hypothesis, a synergistic attack using multiple methods quickly established a critical mass of evidence and raised the idea that the toxicity of abnormal protein aggregates might be a common cause of neurodegeneration [22, 23].

Neurotransmitter replacement was the first truly molecular-based approach to neurodegenerative disease, beginning with the discovery of the striatal dopaminergic deficit in Parkinson's disease brains by Birkmayer and Hornykiewicz in 1961 [6, 24] and then the cortical cholinergic deficit in AD by three groups in the United Kingdom in 1976 [6–9]. Subsequently, several other neurotransmitters have been scrutinized for a possible role.

While memantine was developed to block NMDA receptor-mediated glutamate toxicity, another glutamatergic approach has been to positively modulate AMPA receptors. This has been based on a combination of methods, including cell culture and hippocampal slice model work done in 1990 that showed long-term potentiation is induced by AMPA receptor agonists [25], followed by human brain tissue research in 1993 and 1994 showing decreased AMPA receptor subunit density in AD entorhinal area and neocortex [26, 27].

The prospects for GABA receptor modulation were originally not compelling as studies of human and control AD brain in the 1980 s showed only modest loss of GABAergic components [28]. However, in 1997 studies in hippocampal slices indicated that $GABA_A$ receptor inverse agonists enhance long term potentiation [29] and in 2003 were shown to increase

performance of treated mice on the Morris water maze [30].

Tacrine was shown to increase action potential duration of *in vitro* central histaminergic neurons in 1988 [31] and the HT_3 autoreceptor was shown to regulate the release of not only histamine but also acetylcholine (ACh), norepinephrine, and dopamine from presynaptic nerve endings. Initial reports in 1989 on histamine in AD and control brain were contradictory but later reports agreed on significant cortical depletion [32]. In 1996, *in vivo* microdialysis experiments in rat brain demonstrated HT_3 antagonist-mediated release of ACh in rat cerebral cortex [33] while rats receiving intracerebroventricular HT_3 antagonist had improved short-term memory [34].

A number of different approaches have first originated in their identification with the aging process and therefore credit for their application to AD therapy is not really possible to assign as it was natural and obvious from the extant aging literature to investigate these as possible causes of AD. These include sex hormone modulation, antioxidants, and RAGE inhibitors. The latter two of these approaches, however, received important support from human brain tissue studies [35, 36].

The linkage of AD to atherosclerosis, arteriosclerosis, and arteriolosclerosis was already being debated, based on human brain studies showing their coincidence, by Alzheimer and his contemporaries [17, 37], and the debate continues but has nonetheless resulted in several agents being given clinical trials. A series of negative clinicopathological studies in the middle of the twentieth century stifled this approach but the discovery in the early 1990 s of the association of the apolipoprotein E ε4 genotype with AD, followed by epidemiological identification of vascular AD risk factors, brought it sharply back into prominence. Human brain tissue studies can be awarded precedence in developing the apolipoprotein E ε4 connection with AD, with two publications in 1991. Diedrich and colleagues used differential screening of cDNA libraries from diseased and normal brains to show increased ApoE expression in AD and scrapie while in Japan, Namba and colleagues showed that ApoE is bound to amyloid plaques in both AD and kuru brains. Subsequently, Sparks and colleagues showed increased coronary artery stenosis in middle-aged subjects with brain amyloid plaques [38] and Roses and colleagues reported increased prevalence of the ε4 allele in familial and sporadic AD [39, 40]. This was followed up by multiple epidemiological studies reporting that many of the risk factors for cardiovascular disease were also risk factors for AD [41–50]. Several approaches to AD therapy, including the use of cholesterol-lowering drugs, anti-hypertensive agents, platelet aggregation inhibitors, anti-diabetic agents, arteriolar vasodilators, and glucose/metabolic agents can be logically traced to this resurgence of the vascular hypothesis.

The only other major approach not yet discussed is the inflammatory hypothesis. Although phagocytic glial cells had been known since Alzheimer's time to be situated near senile plaque amyloid cores, the virulence of the microglial reaction to amyloid plaques, as demonstrated with new immunohistochemical methods by McGeer and colleagues in 1987 [51] was a startling revelation. Subsequently, localization of many components of the immune response to AD brain tissue solidified the findings [52, 53] and led quickly to the first clinical trial of an anti-inflammatory agent [54] while epidemiological and molecular genetic associations have continued to come in [55, 56].

This brief review of the origins of experimental therapeutic approaches to AD undoubtedly has not given adequate credit to all the contributors but it is evident that human brain tissue-based research has played a central and often initiating role. It is hard to avoid the conclusion that the stagnation of such work over the last 20 years has imposed limitations on the generation of new ideas and new targets.

DETRIMENTAL EFFECTS OF THE DECISION NOT TO USE BRAIN TISSUE-BASED DIAGNOSIS FOR AD

It has been more than four decades since a consensus developed not to use cortical biopsy to diagnose AD [57]. This may be one of the most significant factors responsible for the failure to develop new therapeutics. Clinical trials must utilize sufficient numbers of subjects so that the possibility of a false negative or false positive result is minimized. Estimating the appropriate minimal subject number requires an initial assessment of the effect size of the medication and this is usually done with pilot studies (e.g., Phase II trials) where a relatively small number of subjects clinically diagnosed with AD receive the agent to be tested. For AD, the effect of the treatment on a measure of cognition, usually the Alzheimer's Disease Assessment Scale-cognitive subscale is used. Recently, biomarker and imaging measures have been proposed as surrogate measures. Regardless of the specific measure of treatment effect, analysis of the pilot data for treated and placebo groups gives an estimate of the medication's

effect size, most simply represented by the percentage of subjects that had a statistically or clinically significant response. The effect size then is used to calculate the minimal trial subject number needed to minimize both false positive and false negative results. A major problem for AD clinical trials has been that effect sizes are often low and therefore very large numbers of subjects are needed, making the trials very expensive and risky. Consequently, many Phase II trials do not proceed to definitive Phase III trials because of small effect size; small effect size is the most common reason for Phase II trial failures across medical fields [58].

Effect size in AD trials is very probably lower than it might be due to inclusion of subjects whose dementia is not due to AD. According to a recent study of data from all National Institute on Aging AD Centers [59], the sensitivity of the clinical diagnosis of AD, as compared to new consensus autopsy criteria [60], may be about 80%. If a similar level of accuracy exists for subject selection for AD clinical trials, then 20% of subjects entered into trials may not have AD but another dementing disorder. If the agent being tested in the pilot study is effective only in patients with AD, then there will be a 20% reduction in effect size, compared to what it would be if all the pilot study subjects really had AD. The consequences of this for the calculation of subject number for the definitive trial depend on the overall effect size achieved by the test agent in the pilot trial. For effect sizes over 50%, a 20% diagnostic error does not change the required subject number much, but if the effect size is lower than 50%, the 20% diagnostic error may double or even triple the required subject number, greatly increasing the cost of the trial. The significance of this may be appreciated by knowing that acetylcholinesterase inhibitors, which are the most commonly-used AD therapeutic agents, all have effect sizes that are much less than 50% [61–63].

Using cortical biopsy to more accurately select subjects for clinical trials could, for agents with selective benefit for AD, increase the effect size, reduce the number of subjects needed for a definitive clinical trial and therefore increase the number of agents chosen to go on to Phase III trials. Why then, have cortical biopsies not been used? The rationale has been that the risks outweigh the benefits as disease-modifying treatments for AD have not been available and most elderly subjects with idiopathic dementia will be treated for presumptive AD anyway [57]. The usage of cortical biopsy for clinical trial selection, however, provides a benefit that has not previously been considered, while serious complications are rare and could potentially be reduced by using needle biopsy rather than open brain biopsy [64, 65]. When faced, decades ago, with a similar predicament at a time when glioblastoma and other brain tumors had no effective treatment, neurosurgeons and oncologists readily accepted that a biopsy diagnosis was essential to guide clinical trials.

Furthermore, oncologists have become aware of disease heterogeneity that further reduces the effect size of medications. This heterogeneity, originating in molecular diversity within a given histologically-defined tumor type, means that not everyone with the same initial biopsy diagnosis responds the same way to a given agent, and, due to tumor cell genetic evolution, even the same patient's response may vary over time. Molecular and genetic diversity within what used to be thought of as homogeneous diseases has given rise to the concept of "personalized medicine" and the need to know not only the histological diagnosis but also the molecular tissue changes that might cause one patient to be drug-sensitive and another drug-resistant [66]. Identifying these changes requires diseased tissue. While AD and cancer are very different diseases, there has been an increasing realization, from many human brain tissue-based studies, that AD is also pathologically heterogeneous. Aside from having different stages dependent on topographical spread of the signature plaques and tangles or the severity of amyloid angiopathy [67, 68], there are also several subtypes including AD with Lewy bodies, itself subdivided into neocortical and amgydala-predominant forms [69, 70], AD with vascular lesions, with different lesion types including large infarcts, lacunar infarcts, microscopic infarcts, and leukoencephalopathy [71–74], AD with TDP-43 positive protein aggregates [75], AD with hippocampal sclerosis [76–78], and AD with argyrophilic grains [79–82]. Additionally, AD may co-exist with progressive supranuclear palsy and other neurodegenerative conditions. If patients at different amyloid or tangle stages of AD or with different AD subtypes differ in their responses to a test medication, then the effect size in clinical trials would be further reduced. It would be extremely useful to be able to subtype AD trial patients with cortical biopsy as a trial that initially was thought to be completely negative might be found, on closer examination, to have had a significant benefit for an identifiable patient subset. A recent study has demonstrated the capability of cortical biopsy to identify AD with cortical Lewy bodies and AD with TDP-43 positive pathology [83] and cortical biopsy with assessment for amyloid-β and phosphorylated tau has been shown to predict the probability of later progression to dementia [84]. A cortical biopsy could also demonstrate the density and morphological

types of plaques and whether tangles have spread into the cortex or are still confined to limbic areas, features which are likely to affect treatment response. Recent biomarker approaches to diagnosing AD more accurately [85–87] are a considerable improvement over previous clinical methods confined to functional and neuropsychological assessment, but will undoubtedly still lack sensitivity and specificity compared to biopsy and will be largely unable to detect AD pathological heterogeneity. Correlation of these biomarker methods with human brain tissue, autopsy [88–91], and biopsy findings [92] have already been used to a limited degree but more of these studies are critically needed.

WHAT CAN BE DONE TO STIMULATE HUMAN BRAIN TISSUE-BASED RESEARCH?

What is the cause of the marked reduction in AD research using human brain tissue? We postulated two reasons: 1) declining availability of suitable human brain tissue, and 2) declining success rate, relative to all proposals, of research proposals that use human brain tissue. We did not have access to data on the second of these factors so we examined the first.

We attempted to determine whether the number of functional brain banks and available human brain tissue has declined over the past 20 years. A PubMed search revealed two papers published in 1991 and 1994 that give a baseline for what was available at that time [93–95]. At the time of the survey published in 1995, 69 brain banks were listed for the US, Europe, and Canada, collectively holding more than 45,000 brains. A detailed breakdown of diagnoses was not given but 69 banks held AD brains and 57 held control brains. Thirty-nine banks kept frozen tissue (fixed tissue is generally universally available) while 10 did not and 18 did not have that information available. Unfortunately there were no such comprehensive surveys listed by PubMed after this last 1995 article. We therefore used an open internet search for brain banks to compile a contemporary listing of brain banks with AD and control tissue. BrainNet Europe is a consortium of 19 separate brain banks (http://www.brainnet-europe.org/). Their website lists many brain banks from around the world, of which 83 or more appear to have AD and control brain tissue. The International Brain Banking Network (IBBN) website (http://www.intbbn.org/registry-of-brain-banks.aspx) lists 87 brain banks that appear to have AD and control brain tissue; these are composed mainly of 51 US and 24 European brain banks. A listing of the number of brains banked is not given but as many of these banks are the same as those listed in the 1995 survey, it is reasonable to conclude that many thousands of AD and control brains must be available for research. Therefore it does not seem that there should be a lack of AD brain tissue, however, various sources suggest that there is a critical shortage of normal control brain tissue [96–128].

Although 45,000 brains would seem enough for an almost infinite amount of AD research, again the recent experience of the NCI with tissue procural is instructive. In 2005 the NCI announced a new initiative, the Cancer Genome Atlas, to catalogue all the genetic mutations associated with cancer. The pilot project would aim to do this for three types of cancer, glioblastoma, serous ovarian cancer, and squamous cell lung carcinoma. For all of these, the original plan was to sequence 1,500 samples, which were to be derived from dozens of tissue banks. Preliminary estimates from the tissue banks had indicated that each could provide at least 500 samples. Once collection was underway, however, the great majority of samples were found to be unsuitable, due to inadequate consent, not enough tissue, inadequate tissue quality, and other reasons. One bank had claimed to have more than 12,000 samples of glioblastoma but in the end only 18 of these were suitable. Eventually, barely 500 samples were obtained for the ovarian cancer, not even 500 for glioblastoma, and collection efforts for lung cancer were suspended due to the huge efforts involved with obtaining the samples (Carolyn Compton, Former Director, NCI Office of Biorepositories and Biospecimen Research). Until such a massive quality-control assessment is done on AD and control brain tissue, we will not really know how many of those held in tissue banks are suitable for modern molecular studies. A major difference between tissue banking for cancer and tissue banking for AD is that cancer tissue banking is almost entirely done from biopsies whereas AD tissue banking is almost entirely done from autopsies. While autopsy offers the ability to obtain large amounts of tissue, it also brings with it many confounding factors including the tendency for gradual changes in the final months of life due to chronic illness, more radical physiological alterations associated with the agonal period immediately preceding death, and deterioration associated with the postmortem interval (PMI). Although studies do not all agree, it appears that RNA integrity and measures of gene expression both decline with increasing PMI, although with substantial variability between individual transcripts [120].

Susceptible transcripts include some that are of interest to neurodegenerative research, including synaptophysin [121], hsp-70 [127], ADAM9, LPL, PRKCG, SERPINA3 [129], and alpha-synuclein [130]. Additionally, RNA integrity may be lost with repeated cycles of freezing and thawing [131, 132], a problem common to all tissue banking. As a result of these issues, inadequate RNA integrity may substantially reduce the number of banked brains that are suitable for gene expression research. Some programs have reported that only one-third to one-half of cases have RNA suitable for molecular research methods [133]. Moreover, it is apparent that deterioration of molecular entities after death varies widely depending on what is being measured. Highly volatile energy storage molecules such as ATP disappear within minutes [134, 135] and catecholamines drop precipitously within the first few hours [136]. Some intensely-studied proteins are reported to show degradation within the first 4–8 hours of death, including α-synuclein and sarkosyl-insoluble tau [137]. The postmortem integrity of post-translational protein modifications is largely unknown, although it has been reported that tau protein is dephosphorylated within 30 minutes after death [138, 139]. The advent of "metabolomics", offering the comprehensive study of small molecules, may be severely hampered by long PMI and/or agonal tissue deterioration. Rapid autopsy programs, currently very few in number [138, 139], may need to become much more commonplace.

Although we have no data on the success rate of NIH grant proposals that are primarily human tissue-based, anecdotal experience suggests that these are often rejected on grounds that they are "descriptive only", "are not hypothesis-driven", and any conclusions are "not testable". The need for a continual back-and-forth between human tissue and experimental models seems not to be appreciated as it was in the not-too-distant past. The evidence put forth here documents the major role that human tissue-based research has had in discovering new targets as well as in validating targets identified with other modalities. Perhaps there should be an NIH study section devoted to human tissue-based research and/or a specific subset of the NIH budget set aside for this.

THE FUTURE OF HUMAN BRAIN TISSUE-BASED AD RESEARCH

Despite the handicaps limiting human brain tissue-based research its future is bright, in part because of the legacy of its past. The linkage of ubiquitin to tangles and plaques [140, 141], Lewy bodies [142, 143], and frontotemporal lobar degeneration (FTLD) [144, 145] in the 1980 s and 1990 s was instrumental in leading to the more recent discovery of FTLD and/or motor neuron disease-associated mutations in progranulin [146, 147], CHMP2B [148], FUS [149], TDP-43 [150, 151], UBQLN2 [152], and C9ORF72 [153, 154] genes. The discovery of the abnormal phosphorylation of tau [21] has been repeated with α-synuclein [155] and then TDP-43 [156]. The value of "deep phenotyping" through neuropathological study has been appreciated by molecular geneticists, who have found that an accurate diagnosis of AD can greatly reduce the number of subjects needed for whole genome association studies, and such studies have already contributed handfuls of new targets at a time [157, 157–160]. Additionally, autopsy-referenced molecular genetic dissection of other neurodegenerative diseases have provided important comparisons [147, 161, 162]. Post-genomics studies, including those involving epigenetic factors, microRNA, transcriptomics, proteomics, and metabolomics, will all depend even more heavily on human brain tissue. It is essential that brain banks receive enhanced governmental or private support, or exploit user-pay systems more heavily. Once again, we may look to cancer research to lead the way, as the NCI has called for an increased understanding of tissue banking economics [163, 164] to help realize their potential.

DISCLOSURE STATEMENT

The author's disclosure is available online (http://www.j-alz.com/disclosures/view.php?id=1346).

REFERENCES

[1] Butler D (2008) Translational research: Crossing the valley of death. *Nature* **453**, 840-842.
[2] LoRusso PM, Schnipper LE, Stewart DJ, Boerner SA, Averbuch SD, Wolf W (2010) Translating clinical trials into meaningful outcomes. *Clin Cancer Res* **16**, 5951-5955.
[3] Booth CM (2010) Evaluating patient-centered outcomes in the randomized controlled trial and beyond: Informing the future with lessons from the past. *Clin Cancer Res* **16**, 5963-5971.
[4] Stewart DJ, Whitney SN, Kurzrock R (2010) Equipoise lost: Ethics, costs, and the regulation of cancer clinical research. *J Clin Onco* **28**, 2925-2935.
[5] Baughman RW, Farkas R, Guzman M, Huerta MF (2006) The National Institutes of Health Blueprint for Neuroscience Research. *J Neurosci* **26**, 10329-10331.

[6] McGeer PL, Eccles JC, McGeer EG (1987) *Molecular Neurobiology of the Mammalian Brain*, 2nd edition, Plenum Press, New York, pp. 261-262.
[7] Davies P, Maloney AJ (1976) Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2**, 1403.
[8] White P, Hiley CR, Goodhardt MJ, Carrasco LH, Keet JP, Williams IE, Bowen DM (1977) Neocortical cholinergic neurons in elderly people. *Lancet* **1**, 668-671.
[9] Perry EK, Tomlinson BE, Blessed G, Bergmann K, Gibson PH, Perry RH (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br Med J* **2**, 1457-1459.
[10] Olney JW (1982) The toxic effects of glutamate and related compounds in the retina and the brain. *Retina* **2**, 341-359.
[11] Okamoto s (1951) Epileptogenic action of glutamate directly applied into the brain of animals and inhibitory effect of proteins and emulsions on its action. *J Physiol Soc Jpn* **13**, 555-562.
[12] McGeer PL, Eccles JC, McGeer EG (1987) Putative excitatory neurons: Glutamate and aspartate. In *Molecular Neurobiology of the Mammalian Brain*. McGeer PL, Eccles JC, McGeer EG, eds. Plenum Press, New York, pp. 175-196.
[13] McGeer EG, McGeer PL (1985) Neurotoxin-induced animal models of human diseases. In *Neurotoxicology*, Blum K, Manzo L, eds. Marcel Dekker, New York and Basel, pp. 515-533.
[14] Geddes JW, Cotman CW (1986) Plasticity in hippocampal excitatory amino acid receptors in Alzheimer's disease. *Neurosci Res* **3**, 672-678.
[15] Geddes JW, Chang-Chui H, Cooper SM, Lott IT, Cotman CW (1986) Density and distribution of NMDA receptors in the human hippocampus in Alzheimer's disease. *Brain Res* **399**, 156-161.
[16] De BU, McLachlan DR (1985) Controlled induction of paired helical filaments of the Alzheimer type in cultured human neurons, by glutamate and aspartate. *J Neurol Sci* **68**, 105-118.
[17] Beach TG (1987) The history of Alzheimer's disease: Three debates. *J Hist Med Allied Sci* **42**, 327-349.
[18] Glenner GG, Wong CW (1984) Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun* **120**, 885-890.
[19] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* **297**, 353-356.
[20] Sommer B (2002) Alzheimer's disease and the amyloid cascade hypothesis: Ten years on. *Curr Opin Pharmacol* **2**, 87-92.
[21] Iqbal K, Novak M (2006) From tangles to tau protein. *Bratisl Lek Listy* **107**, 341-342.
[22] Taylor JP, Hardy J, Fischbeck KH (2002) Toxic proteins in neurodegenerative disease. *Science* **296**, 1991-1995.
[23] Trojanowski JQ, Lee VM (2000) "Fatal attractions" of proteins. A comprehensive hypothetical mechanism underlying Alzheimer's disease and other neurodegenerative disorders. *Ann N Y Acad Sci* **924**, 62-67.
[24] Hornykiewicz O (1998) Biochemical aspects of Parkinson's disease. *Neurology* **51**, S2-S9.
[25] Ito I, Tanabe S, Kohda A, Sugiyama H (1990) Allosteric potentiation of quisqualate receptors by a nootropic drug aniracetam. *J Physiol* **424**, 533-543.
[26] Armstrong DM, Ikonomovic MD, Sheffield R, Wenthold RJ (1994) AMPA-selective glutamate receptor subtype immunoreactivity in the entorhinal cortex of non-demented elderly and patients with Alzheimer's disease. *Brain Res* **639**, 207-216.
[27] Carlson MD, Penney JB Jr, Young AB (1993) NMDA, AMPA, and benzodiazepine binding site changes in Alzheimer's disease visual cortex. *Neurobiol Aging* **14**, 343-352.
[28] Rissman RA, Mobley WC (2011) Implications for treatment: GABAA receptors in aging, Down syndrome and Alzheimer's disease. *J Neurochem* **117**, 613-622.
[29] Seabrook GR, Easter A, Dawson GR, Bowery BJ (1997) Modulation of long-term potentiation in CA1 region of mouse hippocampal brain slices by GABAA receptor benzodiazepine site ligands. *Neuropharmacology* **36**, 823-830.
[30] Chambers MS, Atack JR, Broughton HB, Collinson N, Cook S, Dawson GR, Hobbs SC, Marshall G, Maubach KA, Pillai GV, Reeve AJ, MacLeod AM (2003) Identification of a novel, selective GABA(A) alpha5 receptor inverse agonist which enhances cognition. *J Med Chem* **46**, 2227-2240.
[31] Reiner PB, McGeer EG (1988) THA increases action potential duration of central histamine neurons *in vitro*. *Eur J Pharmacol* **155**, 265-270.
[32] Schneider C, Risser D, Kirchner L, Kitzmuller E, Cairns N, Prast H, Singewald N, Lubec G (1997) Similar deficits of central histaminergic system in patients with Down syndrome and Alzheimer disease. *Neurosci Lett* **222**, 183-186.
[33] Blandina P, Giorgetti M, Bartolini L, Cecchi M, Timmerman H, Leurs R, Pepeu G, Giovannini MG (1996) Inhibition of cortical acetylcholine release and cognitive performance by histamine H3 receptor activation in rats. *Br J Pharmacol* **119**, 1656-1664.
[34] Prast H, Argyriou A, Philippu A (1996) Histaminergic neurons facilitate social memory in rats. *Brain Res* **734**, 316-318.
[35] Perry G, Nunomura A, Hirai K, Zhu X, Perez M, Avila J, Castellani RJ, Atwood CS, Aliev G, Sayre LM, Takeda A, Smith MA (2002) Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? *Free Radic Biol Med* **33**, 1475-1479.
[36] Yan SD, Stern D, Kane MD, Kuo YM, Lampert HC, Roher AE (1998) RAGE-AB Interactions in the Pathophysiology of Alzheimer's Disease. *Restor Neurol Neurosci* **12**, 167-173.
[37] Roher AE, Kokjohn TA, Beach TG (2006) An association with great implications: Vascular pathology and Alzheimer disease. *Alzheimer Dis Assoc Disord* **20**, 73-75.
[38] Sparks DL, Liu H, Scheff SW, Coyne CM, Hunsaker JC, III (1993) Temporal sequence of plaque formation in the cerebral cortex of non-demented individuals. *J Neuropathol Exp Neurol* **52**, 135-142.
[39] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921-923.
[40] Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, Schmechel D, Saunders AM, Goldgaber D, Roses AD (1993) Binding of human apolipoprotein E to synthetic amyloid beta peptide: Isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* **90**, 8098-8102.

[41] Ott A, Slooter AJ, Hofman A, van Harskamp F, Witteman JC, Van Broeckhoven C, van Duijn CM, Breteler MM (1998) Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: The Rotterdam Study. *Lancet* **351**, 1840-1843.

[42] Fujishima M, Tsuchihashi T (1999) Hypertension and dementia. *Clin Exp Hypertens* **21**, 927-935.

[43] Merchant C, Tang MX, Albert S, Manly J, Stern Y, Mayeux R (1999) The influence of smoking on the risk of Alzheimer's disease. *Neurology* **52**, 1408-1412.

[44] Launer LJ, Ross GW, Petrovitch H, Masaki K, Foley D, White LR, Havlik RJ (2000) Midlife blood pressure and dementia: The Honolulu-Asia aging study. *Neurobiol Aging* **21**, 49-55.

[45] McIlroy SP, Dynan KB, Lawson JT, Patterson CC, Passmore AP (2002) Moderately elevated plasma homocysteine, methylenetetrahydrofolate reductase genotype, and risk for stroke, vascular dementia, and Alzheimer disease in Northern Ireland. *Stroke* **33**, 2351-2356.

[46] Knopman D, Boland LL, Mosley T, Howard G, Liao D, Szklo M, McGovern P, Folsom AR (2001) Cardiovascular risk factors and cognitive decline in middle-aged adults. *Neurology* **56**, 42-48.

[47] Peila R, Rodriguez BL, Launer LJ (2002) Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes* **51**, 1256-1262.

[48] Kivipelto M, Helkala EL, Laakso MP, Hanninen T, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J, Nissinen A (2001) Midlife vascular risk factors and Alzheimer's disease in later life: Longitudinal, population based study. *BMJ* **322**, 1447-1451.

[49] Hofman A, Ott A, Breteler MM, Bots ML, Slooter AJ, van Harskamp F, van Duijn CN, Van Broeckhoven C, Grobbee DE (1997) Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* **349**, 151-154.

[50] Skoog I, Lernfelt B, Landahl S, Palmertz B, Andreasson LA, Nilsson L, Persson G, Oden A, Svanborg A (1996) 15-year longitudinal study of blood pressure and dementia. *Lancet* **347**, 1141-1145.

[51] McGeer PL, Itagaki S, Tago H, McGeer EG (1987) Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. *Neurosci Lett* **79**, 195-200.

[52] McGeer PL, McGeer EG (1998) Mechanisms of cell death in Alzheimer disease–immunopathology. *J Neural Transm Suppl* **54**, 159-166.

[53] McGeer PL, Rogers J (1992) Anti-inflammatory agents as a therapeutic approach to Alzheimer's disease. *Neurology* **42**, 447-449.

[54] Rogers J, Kirby LC, Hempelman SR, Berry DL, McGeer PL, Kaszniak AW, Zalinski J, Cofield M, Mansukhani L, Willson P (1993) Clinical trial of indomethacin in Alzheimer's disease. *Neurology* **43**, 1609-1611.

[55] Lambert JC, Amouyel P (2011) Genetics of Alzheimer's disease: New evidences for an old hypothesis? *Curr Opin Genet Dev* **21**, 295-301.

[56] Eikelenboom P, Veerhuis R, van EE, Hoozemans JJ, Rozemuller AJ, van Gool WA (2011) The early involvement of the innate immunity in the pathogenesis of late-onset Alzheimer's disease: Neuropathological, epidemiological and genetic evidence. *Curr Alzheimer Res* **8**, 142-150.

[57] Warren JD, Schott JM, Fox NC, Thom M, Revesz T, Holton JL, Scaravilli F, Thomas DG, Plant GT, Rudge P, Rossor MN (2005) Brain biopsy in dementia. *Brain* **128**, 2016-2025.

[58] Arrowsmith J (2011) Trial watch: Phase II failures: 2008-2010. *Nat Rev Drug Discov* **10**, 328-329.

[59] Beach TG, Monsell SE, Phillips LE, Kukull W (2012) Accuracy of the clinical diagnosis of Alzheimer's disease at National Institute on Aging Alzheimer's Disease Centers, 2005-2010. *J Neuropathol Exp Neurol* **71**, 266-273.

[60] Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Schneider JA, Thal DR, Trojanowski JQ, Vinters HV, Hyman BT (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: A practical approach. *Acta Neuropathol* **123**, 1-11.

[61] Lanctot KL, Herrmann N, Yau KK, Khan LR, Liu BA, LouLou MM, Einarson TR (2003) Efficacy and safety of cholinesterase inhibitors in Alzheimer's disease: A meta-analysis. *CMAJ* **169**, 557-564.

[62] Hansen RA, Gartlehner G, Lohr KN, Kaufer DI (2007) Functional outcomes of drug treatment in Alzheimer's disease: A systematic review and meta-analysis. *Drugs Aging* **24**, 155-167.

[63] Rockwood K (2004) Size of the treatment effect on cognition of cholinesterase inhibition in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* **75**, 677-685.

[64] Hall WA (1998) The safety and efficacy of stereotactic biopsy for intracranial lesions. *Cancer* **82**, 1749-1755.

[65] Dammers R, Schouten JW, Haitsma IK, Vincent AJ, Kros JM, Dirven CM (2010) Towards improving the safety and diagnostic yield of stereotactic biopsy in a single centre. *Acta Neurochir (Wien)* **152**, 1915-1921.

[66] Vitucci M, Hayes DN, Miller CR (2011) Gene expression profiling of gliomas: Merging genomic and histopathological classification for personalised therapy. *Br J Cancer* **104**, 545-553.

[67] Armstrong RA, Wood L (1994) The identification of pathological subtypes of Alzheimer's disease using cluster analysis. *Acta Neuropathol* **88**, 60-66.

[68] Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol (Berl)* **82**, 239-259.

[69] Beach TG, Adler CH, Lue L, Sue LI, Bachalakuri J, Henry-Watson J, Sasse J, Boyer S, Shirohi S, Brooks R, Eschbacher J, White CL, III, Akiyama H, Caviness J, Shill HA, Connor DJ, Sabbagh MN, Walker DG (2009) Unified staging system for Lewy body disorders: Correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. *Acta Neuropathol* **117**, 613-634.

[70] Uchikado H, Lin WL, DeLucia MW, Dickson DW (2006) Alzheimer disease with amygdala Lewy bodies: A distinct form of alpha-synucleinopathy. *J Neuropathol Exp Neurol* **65**, 685-697.

[71] Zekry D, Duyckaerts C, Moulias R, Belmin J, Geoffre C, Herrmann F, Hauw JJ (2002) Degenerative and vascular lesions of the brain have synergistic effects in dementia of the elderly. *Acta Neuropathol (Berl)* **103**, 481-487.

[72] Schneider JA, Bennett DA (2010) Where vascular meets neurodegenerative disease. *Stroke* **41**, S144-S146.

[73] Jellinger KA (2008) The pathology of "vascular dementia": A critical update. *J Alzheimers Dis* **14**, 107-123.

[74] Strozyk D, Dickson DW, Lipton RB, Katz M, Derby CA, Lee S, Wang C, Verghese J (2010) Contribution of vascular pathology to the clinical expression of dementia. *Neurobiol Aging* **31**, 1710-1720.

[75] Wilson AC, Dugger BN, Dickson DW, Wang DS (2011) TDP-43 in aging and Alzheimer's disease - a review. *Int J Clin Exp Pathol* **4**, 147-155.

[76] Probst A, Taylor KI, Tolnay M (2007) Hippocampal sclerosis dementia: A reappraisal. *Acta Neuropathol* **114**, 335-345.

[77] Zarow C, Sitzer TE, Chui HC (2008) Understanding hippocampal sclerosis in the elderly: Epidemiology, characterization, and diagnostic issues. *Curr Neurol Neurosci Rep* **8**, 363-370.

[78] Beach TG, Sue L, Scott S, Layne K, Newell A, Walker D, Baker M, Sahara N, Yen SH, Hutton M, Caselli R, Adler C, Connor D, Sabbagh M (2003) Hippocampal sclerosis dementia with tauopathy. *Brain Pathol* **13**, 263-278.

[79] Braak H, Braak E (1998) Argyrophilic grain disease: Frequency of occurrence in different age categories and neuropathological diagnostic criteria. *J Neural Transm* **105**, 801-819.

[80] Martinez-Lage P, Munoz DG (1997) Prevalence and disease associations of argyrophilic grains of Braak. *J Neuropathol Exp Neurol* **56**, 157-164.

[81] Togo T, Cookson N, Dickson DW (2002) Argyrophilic grain disease: Neuropathology, frequency in a dementia brain bank and lack of relationship with apolipoprotein E. *Brain Pathol* **12**, 45-52.

[82] Sabbagh MN, Sandhu SS, Farlow MR, Vedders L, Shill HA, Caviness JN, Connor DJ, Sue L, Adler CH, Beach TG (2009) Correlation of clinical features with argyrophilic grains at autopsy. *Alzheimer Dis Assoc Disord* **23**, 229-233.

[83] Venneti S, Robinson JL, Roy S, White MT, Baccon J, Xie SX, Trojanowski JQ (2011) Simulated brain biopsy for diagnosing neurodegeneration using autopsy-confirmed cases. *Acta Neuropathol* **122**, 737-745.

[84] Leinonen V, Koivisto AM, Savolainen S, Rummukainen J, Tamminen JN, Tillgren T, Vainikka S, Pyykko OT, Molsa J, Fraunberg M, Pirttila T, Jaaskelainen JE, Soininen H, Rinne J, Alafuzoff I (2010) Amyloid and tau proteins in cortical brain biopsy and Alzheimer's disease. *Ann Neurol* **68**, 446-453.

[85] Reiman EM, McKhann GM, Albert MS, Sperling RA, Petersen RC, Blacker D (2011) Clinical impact of updated diagnostic and research criteria for Alzheimer's disease. *J Clin Psychiatry* **72**, e37.

[86] Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* **9**, 119-128.

[87] DeKosky ST, Carrillo MC, Phelps C, Knopman D, Petersen RC, Frank R, Schenk D, Masterman D, Siemers ER, Cedarbaum JM, Gold M, Miller DS, Morimoto BH, Khachaturian AS, Mohs RC (2011) Revision of the criteria for Alzheimer's disease: A symposium. *Alzheimers Dement* **7**, e1-e12.

[88] Lister-James J, Pontecorvo MJ, Clark C, Joshi AD, Mintun MA, Zhang W, Lim N, Zhuang Z, Golding G, Choi SR, Benedum TE, Kennedy P, Hefti F, Carpenter AP, Kung HF, Skovronsky DM (2011) Florbetapir f-18: A histopathologically validated Beta-amyloid positron emission tomography imaging agent. *Semin Nucl Med* **41**, 300-304.

[89] Lockhart A, Lamb JR, Osredkar T, Sue LI, Joyce JN, Ye L, Libri V, Leppert D, Beach TG (2007) PIB is a non-specific imaging marker of amyloid-beta (Abeta) peptide-related cerebral amyloidosis. *Brain* **130**, 2607-2615.

[90] Thompson PW, Ye L, Morgenstern JL, Sue L, Beach TG, Judd DJ, Shipley NJ, Libri V, Lockhart A (2009) Interaction of the amyloid imaging tracer FDDNP with hallmark Alzheimer's disease pathologies. *J Neurochem* **109**, 623-630.

[91] Clark CM, Schneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA, Pontecorvo MJ, Hefti F, Carpenter AP, Flitter ML, Krautkramer MJ, Kung HF, Coleman RE, Doraiswamy PM, Fleisher AS, Sabbagh MN, Sadowsky CH, Reiman EP, Zehntner SP, Skovronsky DM (2011) Use of florbetapir-PET for imaging beta-amyloid pathology. *JAMA* **305**, 275-283.

[92] Leinonen V, Alafuzoff I, Aalto S, Suotunen T, Savolainen S, Nagren K, Tapiola T, Pirttila T, Rinne J, Jaaskelainen JE, Soininen H, Rinne JO (2008) Assessment of beta-amyloid in a frontal cortical brain biopsy specimen and by positron emission tomography with carbon 11-labeled Pittsburgh Compound B. *Arch Neurol* **65**, 1304-1309.

[93] Bell JE, Alafuzoff I, Al-Sarraj S, Arzberger T, Bogdanovic N, Budka H, Dexter DT, Falkai P, Ferrer I, Gelpi E, Gentleman SM, Giaccone G, Huitinga I, Ironside JW, Kliloueva N, Kovacs GG, Meyronet D, Palkovits M, Parchi P, Patsouris E, Reynolds R, Riederer P, Roggendorf W, Seilhean D, Schmitt A, Schmitz P, Streichenberger N, Schwalber A, Kretzschmar H (2008) Management of a twenty-first century brain bank: Experience in the BrainNet Europe consortium. *Acta Neuropathol* **115**, 497-507.

[94] Duyckaerts C, Sazdovitch V, Seilhean D, Delaere P, Hauw JJ (1993) A brain bank in a neuropathology laboratory (with some emphasis on diagnostic criteria). *J Neural Transm Suppl* **39**, 107-118.

[95] (2009) A network of brain banks fit for the future. *Lancet Neurol* **8**, 691.

[96] Morrison MR, Griffin WS (1981) The isolation and *in vitro* translation of undegraded messenger RNAs from human postmortem brain. *Anal Biochem* **113**, 318-324.

[97] Perrett CW, Marchbanks RM, Whatley SA (1988) Characterisation of messenger RNA extracted post-mortem from the brains of schizophrenic, depressed and control subjects. *J Neurol Neurosurg Psychiatry* **51**, 325-331.

[98] Leonard S, Logel J, Luthman D, Casanova M, Kirch D, Freedman R (1993) Biological stability of mRNA isolated from human postmortem brain collections. *Biol Psychiatry* **33**, 456-466.

[99] Cummings TJ, Strum JC, Yoon LW, Szymanski MH, Hulette CM (2001) Recovery and expression of messenger RNA from postmortem human brain tissue. *Mod Pathol* **14**, 1157-1161.

[100] Yasojima K, McGeer EG, McGeer PL (2001) High stability of mRNAs postmortem and protocols for their assessment by RT-PCR. *Brain Res Brain Res Protoc* **8**, 212-218.

[101] Yasojima K, McGeer EG, McGeer PL (2001) Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain. *Brain Res* **919**, 115-121.

[102] Preece P, Cairns NJ (2003) Quantifying mRNA in postmortem human brain: Influence of gender, age at death, postmortem interval, brain pH, agonal state and inter-lobe mRNA variance. *Brain Res Mol Brain Res* **118**, 60-71.

[103] Li JZ, Vawter MP, Walsh DM, Tomita H, Evans SJ, Choudary PV, Lopez JF, Avelar A, Shokoohi V, Chung T, Mesarwi O, Jones EG, Watson SJ, Akil H, Bunney WE Jr, Myers RM (2004) Systematic changes in gene expression in postmortem human brains associated with tissue pH and terminal medical conditions. *Hum Mol Genet* **13**, 609-616.

[104] Tomita H, Vawter MP, Walsh DM, Evans SJ, Choudary PV, Li J, Overman KM, Atz ME, Myers RM, Jones EG, Watson SJ, Akil H, Bunney WE Jr (2004) Effect of agonal and postmortem factors on gene expression profile: Quality con-

trol in microarray analyses of postmortem human brain. *Biol Psychiatry* **55**, 346-352.
[105] Ervin JF, Heinzen EL, Cronin KD, Goldstein D, Szymanski MH, Burke JR, Welsh-Bohmer KA, Hulette CM (2007) Postmortem delay has minimal effect on brain RNA integrity. *J Neuropathol Exp Neurol* **66**, 1093-1099.
[106] Popova T, Mennerich D, Weith A, Quast K (2008) Effect of RNA quality on transcript intensity levels in microarray analysis of human post-mortem brain tissues. *BMC Genomics* **9**, 91.
[107] Durrenberger PF, Fernando S, Kashefi SN, Ferrer I, Hauw JJ, Seilhean D, Smith C, Walker R, Al-Sarraj S, Troakes C, Palkovits M, Kasztner M, Huitinga I, Arzberger T, Dexter DT, Kretzschmar H, Reynolds R (2010) Effects of antemortem and postmortem variables on human brain mRNA quality: A BrainNet Europe study. *J Neuropathol Exp Neurol* **69**, 70-81.
[108] Sajdel-Sulkowska EM, Majocha RE, Salim M, Zain SB, Marotta CA (1988) The postmortem Alzheimer brain is a source of structurally and functionally intact astrocytic messenger RNA. *J Neurosci Methods* **23**, 173-179.
[109] Gilmore JH, Lawler CP, Eaton AM, Mailman RB (1993) Postmortem stability of dopamine D1 receptor mRNA and D1 receptors. *Brain Res Mol Brain Res* **18**, 290-296.
[110] Kingsbury AE, Foster OJ, Nisbet AP, Cairns N, Bray L, Eve DJ, Lees AJ, Marsden CD (1995) Tissue pH as an indicator of mRNA preservation in human post-mortem brain. *Brain Res Mol Brain Res* **28**, 311-318.
[111] Johnston NL, Cervenak J, Shore AD, Torrey EF, Yolken RH (1997) Multivariate analysis of RNA levels from postmortem human brains as measured by three different methods of RT-PCR. Stanley Neuropathology Consortium. *J Neurosci Methods* **77**, 83-92.
[112] Mathern GW, Pretorius JK, Kornblum HI, Mendoza D, Lozada A, Leite JP, Chimelli L, Born DE, Fried I, Sakamoto AC, Assirati JA, Peacock WJ, Ojemann GA, Adelson PD (1998) Altered hippocampal kainate-receptor mRNA levels in temporal lobe epilepsy patients. *Neurobiol Dis* **5**, 151-176.
[113] Schramm M, Falkai P, Tepest R, Schneider-Axmann T, Przkora R, Waha A, Pietsch T, Bonte W, Bayer TA (1999) Stability of RNA transcripts in post-mortem psychiatric brains. *J Neural Transm* **106**, 329-335.
[114] Miller CL, Diglisic S, Leister F, Webster M, Yolken RH (2004) Evaluating RNA status for RT-PCR in extracts of postmortem human brain tissue. *Biotechniques* **36**, 628-633.
[115] Johnson SA, Morgan DG, Finch CE (1986) Extensive postmortem stability of RNA from rat and human brain. *J Neurosci Res* **16**, 267-280.
[116] Lukiw WJ, Wong L, McLachlan DR (1990) Cytoskeletal messenger RNA stability in human neocortex: Studies in normal aging and in Alzheimer's disease. *Int J Neurosci* **55**, 81-88.
[117] Burke WJ, O'Malley KL, Chung HD, Harmon SK, Miller JP, Berg L (1991) Effect of pre- and postmortem variables on specific mRNA levels in human brain. *Brain Res Mol Brain Res* **11**, 37-41.
[118] Ragsdale DS, Miledi R (1991) Expressional potency of mRNAs encoding receptors and voltage-activated channels in the postmortem rat brain. *Proc Natl Acad Sci U S A* **88**, 1854-1858.
[119] Ross BM, Knowler JT, McCulloch J (1992) On the stability of messenger RNA and ribosomal RNA in the brains of control human subjects and patients with Alzheimer's disease. *J Neurochem* **58**, 1810-1819.
[120] Eastwood SL, Burnet PW, McDonald B, Clinton J, Harrison PJ (1994) Synaptophysin gene expression in human brain: A quantitative *in situ* hybridization and immunocytochemical study. *Neuroscience* **59**, 881-892.
[121] Pardue S, Zimmerman AL, Morrison-Bogorad M (1994) Selective postmortem degradation of inducible heat shock protein 70 (hsp70) mRNAs in rat brain. *Cell Mol Neurobiol* **14**, 341-357.
[122] Harrison PJ, Heath PR, Eastwood SL, Burnet PW, McDonald B, Pearson RC (1995) The relative importance of premortem acidosis and postmortem interval for human brain gene expression studies: Selective mRNA vulnerability and comparison with their encoded proteins. *Neurosci Lett* **200**, 151-154.
[123] Harrison PJ, Barton AJ, Procter AW, Bowen DM, Pearson RC (1994) The effects of Alzheimer's disease, other dementias, and premortem course on beta-amyloid precursor protein messenger RNA in frontal cortex. *J Neurochem* **62**, 635-644.
[124] Castensson A, Emilsson L, Preece P, Jazin EE (2000) High-resolution quantification of specific mRNA levels in human brain autopsies and biopsies. *Genome Res* **10**, 1219-1229.
[125] Bauer M, Gramlich I, Polzin S, Patzelt D (2003) Quantification of mRNA degradation as possible indicator of postmortem interval–a pilot study. *Leg Med (Tokyo)* **5**, 220-227.
[126] Barrachina M, Castano E, Ferrer I (2006) TaqMan PCR assay in the control of RNA normalization in human postmortem brain tissue. *Neurochem Int* **49**, 276-284.
[127] Birdsill AC, Walker DG, Lue L, Sue LI, Beach TG (2011) Postmortem interval effect on RNA and gene expression in human brain tissue. *Cell Tissue Bank* **12**, 311-318.
[128] Broniscer A, Baker JN, Baker SJ, Chi SN, Geyer JR, Morris EB, Gajjar A (2010) Prospective collection of tissue samples at autopsy in children with diffuse intrinsic pontine glioma. *Cancer* **116**, 4632-4637.
[129] Botling J, Edlund K, Segersten U, Tahmasebpoor S, Engstrom M, Sundstrom M, Malmstrom PU, Micke P (2009) Impact of thawing on RNA integrity and gene expression analysis in fresh frozen tissue. *Diagn Mol Pathol* **18**, 44-52.
[130] Dumitriu A, Moser C, Hadzi TC, Williamson SL, Pacheco CD, Hendricks AE, Latourelle JC, Wilk JB, DeStefano AL, Myers RH (2012) Post-mortem interval influences α-synuclein expression in Parkinson disease brain. *Parkinsons Dis* **2012**, 614212.
[131] Vanderburg CR, Pfanni R, Tian D, Kiehl T-R, Hsi T, Hedley-Whyte ET, Frosch MP (2005) Factors influencing postmortem RNA integrity in human brain. *J Neuropathol Exp Neurol* **64**, 443-443.
[132] Vonsattel JP, Aizawa H, Ge P, DiFiglia M, McKee AC, MacDonald M, Gusella JF, Landwehrmeyer GB, Bird ED, Richardson EP Jr (1995) An improved approach to prepare human brains for research. *J Neuropathol Exp Neurol* **54**, 42-56.
[133] Ravid R, Van Zwieten EJ, Swaab DF (1992) Brain banking and the human hypothalamus–factors to match for, pitfalls and potentials. *Prog Brain Res* **93**, 83-95.
[134] Spokes EG (1979) An analysis of factors influencing measurements of dopamine, noradrenaline, glutamate decarboxylase and choline acetylase in human post-mortem brain tissue. *Brain* **102**, 333-346.
[135] Spokes EG, Koch DJ (1978) Post-mortem stability of dopamine, glutamate decarboxylase and choline acetyltransferase in the mouse brain under conditions simulating

the handling of human autopsy material. *J Neurochem* **31**, 381-383.

[136] Ferrer I, Santpere G, Arzberger T, Bell J, Blanco R, Boluda S, Budka H, Carmona M, Giaccone G, Krebs B, Limido L, Parchi P, Puig B, Strammiello R, Strobel T, Kretzschmar H (2007) Brain protein preservation largely depends on the postmortem storage temperature: Implications for study of proteins in human neurologic diseases and management of brain banks: A Brain Net Europe Study. *J Neuropathol Exp Neurol* **66**, 35-46.

[137] Gartner U, Janke C, Holzer M, Vanmechelen E, Arendt T (1998) Postmortem changes in the phosphorylation state of tau-protein in the rat brain. *Neurobiol Aging* **19**, 535-543.

[138] Beach TG, Sue LI, Walker DG, Roher AE, Lue L, Vedders L, Connor DJ, Sabbagh MN, Rogers J (2008) The Sun Health Research Institute Brain Donation Program: Description and experience, 1987-2007. *Cell Tissue Bank* **9**, 229-245.

[139] Hulette CM, Welsh-Bohmer KA, Crain B, Szymanski MH, Sinclaire NO, Roses AD (1997) Rapid brain autopsy. The Joseph and Kathleen Bryan Alzheimer's Disease Research Center experience. *Arch Pathol Lab Med* **121**, 615-618.

[140] Mori H, Kondo J, Ihara Y (1987) Ubiquitin is a component of paired helical filaments in Alzheimer's disease. *Science* **235**, 1641-1644.

[141] Perry G, Friedman R, Shaw G, Chau V (1987) Ubiquitin is detected in neurofibrillary tangles and senile plaque neurites of Alzheimer disease brains. *Proc Natl Acad Sci U S A* **84**, 3033-3036.

[142] Kuzuhara S, Mori H, Izumiyama N, Yoshimura M, Ihara Y (1988) Lewy bodies are ubiquitinated. A light and electron microscopic immunocytochemical study. *Acta Neuropathol* **75**, 345-353.

[143] Lowe J, Blanchard A, Morrell K, Lennox G, Reynolds L, Billett M, Landon M, Mayer RJ (1988) Ubiquitin is a common factor in intermediate filament inclusion bodies of diverse type in man, including those of Parkinson's disease, Pick's disease, and Alzheimer's disease, as well as Rosenthal fibres in cerebellar astrocytomas, cytoplasmic bodies in muscle, and mallory bodies in alcoholic liver disease. *J Pathol* **155**, 9-15.

[144] Tolnay M, Probst A (1995) Frontal lobe degeneration: Novel ubiquitin-immunoreactive neurites within frontotemporal cortex. *Neuropathol Appl Neurobiol* **21**, 492-497.

[145] Cooper PN, Jackson M, Lennox G, Lowe J, Mann DM (1995) Tau, ubiquitin, and alpha B-crystallin immunohistochemistry define the principal causes of degenerative frontotemporal dementia. *Arch Neurol* **52**, 1011-1015.

[146] Gass J, Cannon A, Mackenzie IR, Boeve B, Baker M, Adamson J, Crook R, Melquist S, Kuntz K, Petersen R, Josephs K, Pickering-Brown SM, Graff-Radford N, Uitti R, Dickson D, Wszolek Z, Gonzalez J, Beach TG, Bigio E, Johnson N, Weintraub S, Mesulam M, White CL III, Woodruff B, Caselli R, Hsiung GY, Feldman H, Knopman D, Hutton M, Rademakers R (2006) Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. *Hum Mol Genet* **15**, 2988-3001.

[147] Rademakers R, Baker M, Gass J, Adamson J, Huey ED, Momeni P, Spina S, Coppola G, Karydas AM, Stewart H, Johnson N, Hsiung GY, Kelley B, Kuntz K, Steinbart E, Wood EM, Yu CE, Josephs K, Sorenson E, Womack KB, Weintraub S, Pickering-Brown SM, Schofield PR, Brooks WS, Van Deerlin VM, Snowden J, Clark CM, Kertesz A, Boylan K, Ghetti B, Neary D, Schellenberg GD, Beach TG, Mesulam M, Mann D, Grafman J, Mackenzie IR, Feldman H, Bird T, Petersen R, Knopman D, Boeve B, Geschwind DH, Miller B, Wszolek Z, Lippa C, Bigio EH, Dickson D, Graff-Radford N, Hutton M (2007) Phenotypic variability associated with progranulin haploinsufficiency in patients with the common 1477C–>T (Arg493X) mutation: An international initiative. *Lancet Neurol* **6**, 857-868.

[148] Parkinson N, Ince PG, Smith MO, Highley R, Skibinski G, Andersen PM, Morrison KE, Pall HS, Hardiman O, Collinge J, Shaw PJ, Fisher EM (2006) ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). *Neurology* **67**, 1074-1077.

[149] Baumer D, Hilton D, Paine SM, Turner MR, Lowe J, Talbot K, Ansorge O (2010) Juvenile ALS with basophilic inclusions is a FUS proteinopathy with FUS mutations. *Neurology* **75**, 611-618.

[150] Borroni B, Bonvicini C, Alberici A, Buratti E, Agosti C, Archetti S, Papetti A, Stuani C, Di LM, Gennarelli M, Padovani A (2009) Mutation within TARDBP leads to frontotemporal dementia without motor neuron disease. *Hum Mutat* **30**, E974-E983.

[151] Kovacs GG, Murrell JR, Horvath S, Haraszti L, Majtenyi K, Molnar MJ, Budka H, Ghetti B, Spina S (2009) TARDBP variation associated with frontotemporal dementia, supranuclear gaze palsy, and chorea. *Mov Disord* **24**, 1843-1847.

[152] Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, Yang Y, Fecto F, Shi Y, Zhai H, Jiang H, Hirano M, Rampersaud E, Jansen GH, Donkervoort S, Bigio EH, Brooks BR, Ajroud K, Sufit RL, Haines JL, Mugnaini E, Pericak-Vance MA, Siddique T (2011) Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature* **477**, 211-215.

[153] Stewart H, Rutherford NJ, Briemberg H, Krieger C, Cashman N, Fabros M, Baker M, Fok A, jesus-Hernandez M, Eisen A, Rademakers R, Mackenzie IR (2012) Clinical and pathological features of amyotrophic lateral sclerosis caused by mutation in the C9ORF72 gene on chromosome 9p. *Acta Neuropathol* **123**, 409-417.

[154] Murray ME, jesus-Hernandez M, Rutherford NJ, Baker M, Duara R, Graff-Radford NR, Wszolek ZK, Ferman TJ, Josephs KA, Boylan KB, Rademakers R, Dickson DW (2011) Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in C9ORF72. *Acta Neuropathol* **122**, 673-690.

[155] Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K, Iwatsubo T (2002) alpha-Synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* **4**, 160-164.

[156] Hasegawa M, Arai T, Nonaka T, Kametani F, Yoshida M, Hashizume Y, Beach TG, Buratti E, Baralle F, Morita M, Nakano I, Oda T, Tsuchiya K, Akiyama H (2008) Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol* **64**, 60-70.

[157] Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, Bird TD, Boeve BF, Graff-Radford NR, De Jager PL, Evans D, Schneider JA, Carrasquillo MM, Ertekin-Taner N, Younkin SG, Cruchaga C, Kauwe JS, Nowotny P, Kramer P, Hardy J, Huentelman MJ, Myers AJ, Barmada MM, Demirci FY, Baldwin CT, Green RC, Rogaeva E, St George-Hyslop P, Arnold SE, Barber R, Beach T, Bigio EH, Boxer A, Burke JR, Cairns NJ, Carlson CS, Carney RM, Carroll SL, Chui HC, Clark DG, Corneveaux J, Cotman CW, Cummings JL, DeCarli C, DeKosky ST, az-Arrastia R, Dick M, Dickson DW, Ellis WG, Faber KM, Fallon KB, Farlow MR, Ferris S, Frosch MP, Galasko DR, Ganguli M, Gearing M, Geschwind DH, Ghetti B, Gilbert JR, Gilman S, Giordani B, Glass JD,

Growdon JH, Hamilton RL, Harrell LE, Head E, Honig LS, Hulette CM, Hyman BT, Jicha GA, Jin LW, Johnson N, Karlawish J, Karydas A, Kaye JA, Kim R, Koo EH, Kowall NW, Lah JJ, Levey AI, Lieberman AP, Lopez OL, Mack WJ, Marson DC, Martiniuk F, Mash DC, Masliah E, McCurry SM, McDavid AN, McKee AC, Mesulam M, Miller BL, Miller CA, Miller JW, Parisi JE, Perl DP, Peskind E, Petersen RC, Poon WW, Quinn JF, Rajbhandary RA, Raskind M, Reisberg B, Ringman JM, Roberson ED, Rosenberg RN, Sano M, Schneider LS, Seeley W, Shelanski ML, Slifer MA, Smith CD, Sonnen JA, Spina S, Stern RA, Tanzi RE, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Bohmer KA, Williamson J, Woltjer RL, Cantwell LB, Dombroski BA, Beekly D, Lunetta KL, Martin ER, Kamboh MI, Saykin AJ, Reiman EM, Bennett DA, Morris JC, Montine TJ, Goate AM, Blacker D, Tsuang DW, Hakonarson H, Foroud TM, Haines JL, Mayeux R, Pericak-Vance MA, Farrer LA, Schellenberg GD (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet* **43**, 436-441.

[158] Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Lince DH, Zismann VL, Beach TG, Leung D, Bryden L, Halperin RF, Marlowe L, Kaleem M, Walker DG, Ravid R, Heward CB, Rogers J, Papassotiropoulos A, Reiman EM, Hardy J, Stephan DA (2007) A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. *J Clin Psychiatry* **68**, 613-618.

[159] Reiman EM, Webster JA, Myers AJ, Hardy J, Dunckley T, Zismann VL, Joshipura KD, Pearson JV, Hu-Lince D, Huentelman MJ, Craig DW, Coon KD, Liang WS, Herbert RH, Beach T, Rohrer KC, Zhao AS, Leung D, Bryden L, Marlowe L, Kaleem M, Mastroeni D, Grover A, Heward CB, Ravid R, Rogers J, Hutton ML, Melquist S, Petersen RC, Alexander GE, Caselli RJ, Kukull W, Papassotiropoulos A, Stephan DA (2007) GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. *Neuron* **54**, 713-720.

[160] Webster J, Reiman EM, Zismann VL, Joshipura KD, Pearson JV, Hu-Lince D, Huentelman MJ, Craig DW, Coon KD, Beach T, Rohrer KC, Zhao AS, Leung D, Bryden L, Marlowe L, Kaleem M, Mastroeni D, Grover A, Rogers J, Heun R, Jessen F, Kolsch H, Heward CB, Ravid R, Hutton ML, Melquist S, Petersen RC, Caselli RJ, Papassotiropoulos A, Stephan DA, Hardy J, Myers A (2010) Whole genome association analysis shows that ACE is a risk factor for Alzheimer's disease and fails to replicate most candidates from Meta-analysis. *Int J Mol Epidemiol Genet* **1**, 19-30.

[161] Chen-Plotkin AS, Martinez-Lage M, Sleiman PM, Hu W, Greene R, Wood EM, Bing S, Grossman M, Schellenberg GD, Hatanpaa KJ, Weiner MF, White CL III, Brooks WS, Halliday GM, Kril JJ, Gearing M, Beach TG, Graff-Radford NR, Dickson DW, Rademakers R, Boeve BF, Pickering-Brown SM, Snowden J, van Swieten JC, Heutink P, Seelaar H, Murrell JR, Ghetti B, Spina S, Grafman J, Kaye JA, Woltjer RL, Mesulam M, Bigio E, Llado A, Miller BL, Alzualde A, Moreno F, Rohrer JD, Mackenzie IR, Feldman HH, Hamilton RL, Cruts M, Engelborghs S, De Deyn PP, Van BC, Bird TD, Cairns NJ, Goate A, Frosch MP, Riederer PF, Bogdanovic N, Lee VM, Trojanowski JQ, Van DV (2011) Genetic and clinical features of progranulin-associated frontotemporal lobar degeneration. *Arch Neurol* **68**, 488-497.

[162] Van Deerlin VM, Sleiman PM, Martinez-Lage M, Chen-Plotkin A, Wang LS, Graff-Radford NR, Dickson DW, Rademakers R, Boeve BF, Grossman M, Arnold SE, Mann DM, Pickering-Brown SM, Seelaar H, Heutink P, van Swieten JC, Murrell JR, Ghetti B, Spina S, Grafman J, Hodges J, Spillantini MG, Gilman S, Lieberman AP, Kaye JA, Woltjer RL, Bigio EH, Mesulam M, Al-Sarraj S, Troakes C, Rosenberg RN, White CL III, Ferrer I, Llado A, Neumann M, Kretzschmar HA, Hulette CM, Welsh-Bohmer KA, Miller BL, Alzualde A, Lopez de MA, McKee AC, Gearing M, Levey AI, Lah JJ, Hardy J, Rohrer JD, Lashley T, Mackenzie IR, Feldman HH, Hamilton RL, DeKosky ST, van der ZJ, Kumar-Singh S, Van BC, Mayeux R, Vonsattel JP, Troncoso JC, Kril JJ, Kwok JB, Halliday GM, Bird TD, Ince PG, Shaw PJ, Cairns NJ, Morris JC, McLean CA, DeCarli C, Ellis WG, Freeman SH, Frosch MP, Growdon JH, Perl DP, Sano M, Bennett DA, Schneider JA, Beach TG, Reiman EM, Woodruff BK, Cummings J, Vinters HV, Miller CA, Chui HC, Alafuzoff I, Hartikainen P, Seilhean D, Galasko D, Masliah E, Cotman CW, Tunon MT, Martinez MC, Munoz DG, Carroll SL, Marson D, Riederer PF, Bogdanovic N, Schellenberg GD, Hakonarson H, Trojanowski JQ, Lee VM (2010) Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nat Genet* **42**, 234-239.

[163] Rogers J, Carolin T, Vaught J, Compton C (2011) Biobankonomics: A taxonomy for evaluating the economic benefits of standardized centralized human biobanking for translational research. *J Natl Cancer Inst Monogr* **2011**, 32-38.

[164] Vaught J, Rogers J, Carolin T, Compton C (2011) Biobankonomics: Developing a sustainable business model approach for the formation of a human tissue biobank. *J Natl Cancer Inst Monogr* **2011**, 24-31.

[165] Panula P, Rinne J, Kuokkanen K, Eriksson KS, Sallmen T, Kalimo H, Relja M (1998) Neuronal histamine deficit in Alzheimer's disease. *Neuroscience* **82**, 993-997.

[166] Schneider C, Risser D, Kirchner L, Kitzmuller E, Cairns N, Prast H, Singewald N, Lubec G (1997) Similar deficits of central histaminergic system in patients with Down syndrome and Alzheimer disease. *Neurosci Lett* **222**, 183-186.

[167] Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D, Schmidt AM (1996) RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature* **382**, 685-691.

[168] Kosunen O, Talasniemi S, Lehtovirta M, Heinonen O, Helisalmi S, Mannermaa A, Paljarvi L, Ryynanen M, Riekkinen PJ Sr, Soininen H (1995) Relation of coronary atherosclerosis and apolipoprotein E genotypes in Alzheimer patients. *Stroke* **26**, 743-748.