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PERSPECTIVE

Brains for Dementia Research: The Importance of Cohorts in Brain Banking

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Background: The Historical Importance of Studies of Human Brain in Dementia Research

The collection of brain and related tissue has a long history [1]. In terms of dementia, a more systematic examination of the relationship between brain pathology and clinical symptoms can be traced to Alois Alzheimer's group in Munich in the early 1900s [2]. The use of human brain tissue is essential to increase our understanding of dementia as it gives us the gold standard of disease pathogenesis and clues as to the molecular mechanisms that underpin the various diseases and conditions. From such human studies, experimental models can be interrogated against this standard and new treatment strategies can be discovered for these socially and economically devastating conditions.

Following reporting of the index case, it was not until the 1960s that more detailed neuropathological investigations of the brain in relation to dementia began. These studies were accompanied by efforts to introduce biochemical techniques to the understanding of how changes in the brain of certain individuals resulted in dementia. In particular, ultrastructural examination of biopsy samples using electron microscopy helped understand the detailed structure of plaques and tangles. One of the more significant biochemical discoveries was the observed, clinically relevant, alterations in the cholinergic system in people with Alzheimer's disease (AD) [3]. Following confirmation by us [4] and other groups, the clinical development of acetylcholinesterase inhibitors began, leading eventually to the licensing of drugs such as donepezil [5].

The use of human post-mortem material was also crucial in revealing the molecular basis of both plaques as Aβ [6] and tangles as tau [7]. Such tissue continues to provide important contributions to *in vivo* imaging of Aβ [8] and the effect of disease-modifying treatments such as vaccination against Aβ [9].

Current Directions in Brain Banking

Many countries have set up brain banks for dementia (and other) research and there is a growing consensus towards banking from longitudinal cohorts. Brain banking in China is at an early stage but, with the help of more established brain banks around the world, a plan is emerging [10]. This is timely as recent new evidence suggests that the number of people with dementia in China has been drastically underestimated [11].

There are some significant issues with the conventional widespread practice of accepting ad hoc donations to brain banks [12]. Firstly, these donations are often from people who died at the end-stage of their disease, secondly there is often little clinical information about the person during life and finally, very few such donations are from people without dementia. The latter are vital as a comparator group and to help identify the earliest changes on the path to dementia. Current thinking is that seeking brain donation consent from participants within existing longitudinal cohorts who are reaching the age at which dementia begins to become an issue is a good way forward. Such cohorts are likely to be very committed to research and may welcome the prospect of contributing one final "sample" as part of their involvement in the project. The most informative

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cohorts will have extensive clinical data (including measures of cognition, behavior, mood, and lifestyle as well as imaging and genetic profiles) and in some cases physiological samples. It is also likely that some participants will die before dementia or even early cognitive changes and will therefore contribute to the bank of brains from people without dementia.

In the UK, a number of initiatives have sought to match this thinking. For example, the Cognitive Function and Aging Study (CFAS, www.cfas.ac.uk/) added the option of brain donation and a proportion of participants consented. CFAS benefits from extensive and systematic antemortem information and has led to many valuable insights from the study of post-mortem brains [13]. Dementias Platform UK (DPUK, www.dementiasplatform.uk) is an overarching initiative that brings together more than 35 existing UK longitudinal cohorts that are ripe to be repurposed to accelerate the development of new treatments for dementia. As part of this project some of the cohorts are being approached to determine if they would be willing to consider brain donation. Furthermore, one of the cohorts, Brains for Dementia Research (BDR), was set up as a longitudinal study cohort including brain donation as the specific end point.

The BDR Cohort

BDR was set up in 2008 with funding from Alzheimer Brain Bank UK (ABBUK), a charity established by two leading UK dementia charities, the Alzheimer's Society and Alzheimer's Research UK. Over the last 10 years, BDR has received £7M of funding. BDR is a program of planned brain donation which provides high-quality post-mortem brain tissue and clinical data from both those with and without memory impairment to researchers working in the field of dementia with the aim of finding new treatments and a cure for dementia. BDR comprises a network of 6 leading dementia research centers based at the Universities of King's College London, Oxford, Bristol, Newcastle, Manchester, and Cardiff and builds on established brain banks in the first 5 named. In each collaborating center, recruitment/assessment teams work together with the staff of brain banks at their site. BDR has been adopted by the Clinical Research Network (funded by the National Institute of Health Research) and receives service support costs from the Medical Research Council (MRC) *via* the MRC UK Brain Bank Network. BDR was established as a Research Tissue Bank under the National Research Ethics Service, enabling tissue and data to be distributed to *bona fide* researchers in the UK following an approval process under devolved authority (08/H0704/128+5).

Recruitment methods included national and local press and radio and TV coverage, regular BDR features in charity newsletters and magazines aimed at an older readership, BDR posters and leaflets, collaborating memory clinics, word of mouth, advocate talks at carer and support groups and other organizations (e.g. University of the Third Age and the Woman's Institute). BDR has a dedicated website with links from those of the charities, MRC, and the Human Tissue Authority.

All participants were self-referring or were referred by a family member acting as a consultee where the participant lacked capacity. All participants with capacity on joining the study have a study partner and have identified a nominated representative who will act to facilitate brain donation. Where the participant lacked capacity, a consultee provided advice on ongoing participation and consent to donation on death. Practical aspects of recruitment of participants to brain donation and issues of consent have been reviewed elsewhere [14].

Between 2009 and January 2018, 3276 volunteers gave informed consent for brain donation or were recruited with the assistance of consultees, and a total of 9804 assessments were completed. This included 3128 baseline assessments representing 95.5% of participants. As would be expected from a highly motived, self-referring cohort, there have been only a small number of withdrawals overall (144 out of 2451, 5.9%). A more significant problem is failed donation (114 out of 825, 13.8%), where the family did not inform the project of the death of a participant or for logistical reasons the brain could not be removed during a reasonable period after death.

Between consent and baseline assessment, the attrition rate was 0.9% due to withdrawal and 13.8% due to no donation. Of 3276 consenting donors, 181 (5.5%) did not complete a baseline assessment.

The characteristics of the total cohort were a median age of 75 years, mostly female (1854, 59.9%), of white ethnicity (2593, 98.9%), educated for a median of 13 years (1834, 61.4%), and without cognitive impairment (2316, 74.8%). Participants in the surviving cohort also have similar characteristics, although reflecting cohort deaths, their median age was lower (73 years), predominantly female (63.3%), and fewer lived in residential care (5.2%).

Of the 8578 assessment visits entered into a comprehensive assessment database, 1512 (17.6%) related to deceased and donating participants. Based on cognitive scores at last assessment before death, the majority of donating participants (398, 65.2%) had dementia and a quarter (156, 25.6%) had no cognitive impairment. This group represents a major contribution to research studies of dementia; brain banks typically contain few control cases and those they do have, have very little clinical information.

The median number of assessments (interquartile range) for each donation was 2 (1–3) overall with an average of 2 (1–4) assessments for participants with dementia, 2.5 (2–4) for the Mild Cognitive Impairment group, and 2 (1–3) for those without cognitive impairment. Information indicating the presence of psychiatric symptoms (374, 61.3%), depression (307, 58.7%), and medication (465, 76.2%) was also available at last assessment for most participants who donated their brains.

Once the participant dies, a full neuropathological work-up is undertaken. This leads to a narrative description of the regional pathology within the brain together with standardized scoring for Braak tangle pathology [15, 16], Braak Lewy body score [17], Thal phase of Abeta pathology, Consortium to Establish a Registry of Alzheimer's Disease classification [16], extent, location, and classification of vascular pathology [18], and TDP43 status. Individual cases are searchable *via* the UK Brain Bank Network database and are classified according to a hierarchical tree structure based on the World Health Organization International Classification of Diseases, 10th Revision.

Our funders and our participants wish samples and data from BDR to be available to *bona fide* dementia researchers after suitable and ethical review. The process of requesting tissue and/or data is *via* an application form available through the BDR website http://brainsfordementiaresearch.co.uk/information-for-researchers/. Brain tissue samples and data are showcased on the MRC UK Brain Bank Network website https://brainbanknetwork.cse.bris.ac.uk/.

Genome-wide association data on all deceased BDR participants, using NeuroChip, have recently been made available to researchers through DPUK and some cases have been subjected to whole-exome sequencing [19]. In the near future, methylome data will be available for the same cases. These developments will allow, for the first time, triangulation of clinical, neuropathological, genetic, and epigenetic data for a large cohort. Studies of RNA expression using RNASeq are in the planning stage.

Studying Synapses in Dementia

Deficits in specific synaptic transmission in AD were first recognized in the 1970s when reductions in cholinergic markers in the brains of people with AD were identified [3]. While this provided the basis for initial, partially successful, approaches to symptomatic treatment [20, 21], it was recognized that other neurotransmitter systems were affected, including the glutamatergic and serotonergic systems [22]. Furthermore, in the early 1990s, as more general markers of synapses such as synaptophysin were investigated, reduction in the number of synapses early in the disease process was recognized [23]. Many studies have confirmed this observation and that synaptic loss, rather than neuronal loss, is the strongest correlate of cognitive decline in AD [24]. Imaging with fluorodeoxyglucose (FDG)-PET may be considered to be a close correlate of synaptic function, as synapses consume a considerable proportion of the 20% of body energy used by the brain. Studies of people with AD using FDG-PET have consistently shown pareto-temporal hypometabolism compared to controls, and this is strongly correlated with cognitive decline in longitudinal studies [25].

As a more detailed understanding of synaptic mechanisms and the role of particular proteins emerged, these became the subject of study in the post-mortem human brain. At the same time, efforts were made to examine differences and similarities between various types of dementia. Table 1 shows a summary of the direction of change of various proteins we have studied in AD and Lewy body dementias. The changes shown are from multiple cortical regions in comparison to control cases. It can be seen that all three dementias are predominantly characterized by decreased levels of the multiple proteins shown (Table 1). This is now an accepted feature of neurodegeneration and is part of the process of cell atrophy and death. Indeed, it is likely that a loss of synaptic proteins represents the first failing in synaptic machinery prior to the loss of synapses followed by neurons. However, not all proteins are decreased, increases have been reported for some synaptic receptor (SNARE) proteins—which may reflect an attempt at compensation for synaptic failures and stresses [26]. The increases in proteins related to the unfolded protein response would support this theory [27]. A key question is whether the synaptic changes seen in dementia represent simply a loss of synapses or alterations in components of the synapse. Expressing values for individual proteins as a ratio to that of, for example synaptophysin, may help clarify this issue.

A number of studies go on to demonstrate the links between these changes in synaptic proteins and clinical symptoms in the patients. Such studies emphasize the importance of the availability of clinical information, ideally with serial assessment. The most abundant clinical data were for cognition and behavioral symptoms tested for on the Neuropsychiatric Inventory [36]. We showed reduced levels of Zinc transporter 3 to be associated with cognitive impairment, depression, delusions, and agitation [28, 37]. Reductions in the levels of PSD95, dynamin1, and proteasome activity were also associated with cognitive impairment [29, 37, 38]. Changes in these proteins were also shown to be linked with the duration of dementia (Vamp2) and parkinsonism (monomeric alpha synuclein) [26]. Reductions in SNAP-25 (AD) and Rab3A (Lewy

Table 1 Summary of changes in synaptic and other neuron-associated proteins in human post-mortem studies.

Protein	AD	DLB	PDD
Zinc transporter protein 3—ZnT3	=	↓	↓
Post-synaptic density protein of 95 kDa—PSD95	↓	↓	↓
78 kDa glucose-regulated protein (GRP-78)	=	↑	↑
Mammalian uncoordinated-18—Munc18	↓	↑	=
Syntaxin1	↑	=	=
Vesicle Associated Membrane Protein 2—Vamp2	↓	=	↑
Monomeric α-synuclein	↓	=	↑
Proteasome subunit 19S ATPase (RPT6)	↓	↓	↓
Synaptosome Associated Protein 47—SNAP47	=	↓	↓
Leucine-rich repeat and fibronectin type-III domain-containing protein 2—LRFNF2	↓	↓	↓
Growth Associated Protein 43—GAP43	=	=	↓
Acetylated tubulin	↓	No data	No data
Neural cell adhesion molecul—NCAM	↓	=	=
Synaptopodin	No data	↓	↓
Neurogranin	↓	↓	↓
Ras-related protein Rab-3A—Rab3A	↓	↓	↓
Synaptosome Associated Protein 25—SNAP25	↓	↓	↓
β-III-tubulin	↓	↓	↓
Synaptophysin	↓	↓	↓
Protein of 25 kDa—P25	No Data	↓	=
Glycogen synthase kinase 3β—GSK3β	↓	↓	↓
Dynamin1	↓	↓	=
Phosphorylated form of calcium-calmodulin kinase II—phosphoCaMKII	↓	↓	↓
Vesicular glutamate transporter 1—VGlut1	↓	=	=

↓ Significantly decreased, ↑ significantly increased, = not significantly different. Data summarized from [26–35]. For details of individual studies, please refer to original cited paper. In all cases, the control and neurodegenerative cases were matched for age, post-mortem delay, and brain pH.

body dementia) were related to cognitive decline [30]. However, likely triggers for decreases in the above proteins remain more elusive but probably include a combination of oxidative stress, neuroinflammation, mitochondrial dysfunction, and neurotoxicity from soluble oligomers of the pathological hallmark proteins such as amyloid beta, tau, and alpha-synuclein. Studies are now underway to examine the potential of various synaptic proteins as biomarkers in *in vivo* imaging [39] and in cerebrospinal fluid.

Future Directions

There is an increasing drive to use the very latest techniques previously only used in animal and cellular models, in the human brain. One such approach, array tomography, allows detailed investigation of synapses. A recent study highlights the approach and demonstrates for the first time that small aggregates of phosphorylated synuclein are likely to interfere with synaptic function in Lewy body dementia [40]. This imaging method requires specialized fixation conditions from fresh tissue and a form of super-resolution microscopy known as stimulated emission depletion. Brain banks will need to respond to such developments by collecting and processing suitable tissue to allow the full potential of these imaging techniques to be realized.

The importance of the human brain to epigenetic studies of dementia has only recently been fully appreciated in that the epigenetic marks found from peripheral samples differ considerably from those of the brain [41]. Even within the brain, cell types have different profiles and therefore the ability to separate cell types for study becomes important. Recently, fluorescence-activated nuclei sorting has been applied to frozen post-mortem tissue to isolate neuronal nuclei using an antibody against the NeuN [42]. This will allow epigenetic, transcriptomic, and proteomic profiling of neurons from healthy and diseased tissues.

Overall, brain banks appear to have a bright future, provided that they are able to be agile in the way that tissue

is collected and stored so as to meet the requirements of cutting-edge research. Furthermore, collecting brains from cohorts who have been prospectively studied allows the maximum possible power to investigate clinico-pathological relationships. Finally, it is vital that brain collection from participants in disease-modifying trials is prioritized as this will help understand the full impact of such treatments.

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