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BrainNet Europe II

Network of European Brain and Tissue Banks for Clinical and Basic Neuroscience

Network of Excellence

Life Sciences, Genomics and Biotechnology for Health

Publishable Final Activity Report

Period covered: from 2004-07-01 to 2009-12-31

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Start date of project: 2004-07-01	Duration: 66 months
Project coordinator name:	Prof. Dr. Hans A. Kretzschmar
Project coordinator organisation name:	Ludwig-Maximilians-Universität München

1. Project execution

BrainNet Europe II is a network of 18 established brain banks across Europe. Having established a network of ten brain banks in preparation for BrainNet Europe II eight new members were integrated to spread excellence in collecting human high quality post mortem brain tissue and to foster research in the cellular and molecular basis of neurological and mental disorders and diseases, gender aspects and process of ageing. Diseases of high frequency and outstanding medical and social importance such as Alzheimer's, Parkinson's, motoneuron disease, multiple sclerosis, schizophrenia and affective disorders are the focus of the network. In addition BNEII wants to contribute to research in rare diseases, a research branch, which can only be worked on successfully on an international European level.

The objectives of BrainNet Europe II are:

1. To exchange experiences in all matters of brain banking between all partners of the BNE consortium with focus on brain bank management, tissue sampling, tissue storage, quality control of the tissue, safety aspects, ethical and legal aspects to reach a common high level of excellence
2. To harmonize neuropathological diagnostics
3. To perform staining and diagnostic quality control exercises
4. To determine the influence of pre- and post-mortem factors and tissue storage on tissue quality
5. To determine the limits of the usability of human post-mortem tissue for sophisticated molecular techniques
6. To develop gold standards for
   - safety provisions
   - histological and immunohistochemical stainings in neuropathological diagnostics
   - neuropathological diagnostic criteria
   - working with human brain tissue using modern technologies (such as laser capture microdissection, mass spectrometry, gene expression profiling etc.)
   - the usage of morphometric methods in neuropathological diagnostics
   - tissue storage and handling
   - the quality of human post mortem tissue samples
   - DNA, RNA and protein extraction
7. To contribute to the harmonization of the ethical codes of conduct of the participating countries
8. To identify the legislation concerning import/export of frozen samples, autopsy, storage and use of frozen and fixed human tissue samples, data protection etc. in participating countries, and to give impulses to uniform this legislation
9. To foster the establishment of donor programs in participating countries
10. To collect brain tissue of patients with psychiatric diseases

11. To exchange cases with difficult or atypical neuropathology ("mystery cases") within the consortium to reach a consensus diagnosis and to improve diagnostic security
12. To utilize the Internet for
    - internal exchange of information
    - spreading of excellence within the consortium
    - spreading of excellence outside the consortium
    - raising scientific awareness regarding human post mortem tissue samples
    - announcement of internal and external training sessions and courses
    - dissemination of information to the general public
    - running a common data base with highest possible IT security and protection of data
13. To train researchers of the consortium and external researchers in the use and application of human nervous system tissues for research, by means of short term visits to other centres, training fellowships, and workshops.
14. To spread excellence beyond the network not only via the BNE website but also by
    - publishing in scientific journals,
    - presenting posters and giving lectures at national and international meetings in the field of neuroscience
    - using electronic media
15. To acquire and distribute well-characterized and high-quality tissues for basic research in neuroscience
16. To disseminate information to the general public on the necessity of brain banking in general and on BNEII in particular, on research activities on human post-mortem tissues samples and their results, and on donor programs.
17. To increase the awareness of standardized neuropathological and clinical diagnostics in neurology and psychiatry at a European level
18. To provide a basis and quality control system for RTD projects dealing with clinical or epidemiological aspects of neurological and psychiatric diseases
19. To integrate new members
20. To investigate important neural parameters in schizophrenia and the influence of pre- and post-mortem variables on these parameters

In order to achieve all objectives a rigorous decision making and management system was established resting on the members of the Network Coordination Committee and being assisted by a company for process organization as a partner of the consortium. A Joint Program of Activities was worked out dividing the work into 25 workpackages.

Contractors involved in the Joint Program of Activities

1. The project was coordinated by Hans Kretzschmar. The Joint Program of Activities was managed by Thomas Arzberger, Ludwig Maximilians University Muenchen, Institute of Neuropathology and Prion Research, Germany.

The other BNE partner institutions (status at the end of the project) and their team leaders during the project:

2. Irina Alafuzoff, Department of Neuroscience and Neurobiology, Section of Neuropathology, University of Kuopio, Finland
3. Safa Al-Sarraj, Department of Clinical Neuropathology-King's College Hospital/Institute of Psychiatry, Kings College London, UK
4. Nenad Bogdanovic, Huddinge Brain Bank, Karolinska Institute, Stockholm, Sweden
5. Herbert Budka, Institute of Neurology, Medical University Vienna, Austria
6. Peter Falkai, Department of Psychiatry and Psychotherapy, University of Goettingen, Germany
7. Isidro Ferrer, Institute for Neuropathology, IDIBELL-Bellvitge, University Hospital, University of Barcelona, Spain
8. Giorgio Giaccone, Division of Neuropathology and Neurology, National Institute for Neurology, Milan, Italy
9. Jean-Jacques Hauw, Laboratory of Neuropathology Raymond Escourolle, Pitie-Salpêtrière Hospital, Pierre and Marie Curie University - Paris VI, France
10. Rivka Ravid (project years 1-2), Inge Huitinga (project years 3-5), Netherlands Brain Bank, Royal Netherlands Academy of Arts and Sciences, Amsterdam
11. Jeanne Bell (project years 1-3), James Ironside (project years 4 and 5), Department of Neuropathology, School of Clinical and Molecular Medicine, University of Edinburgh, UK
12. Nicholas Kopp (project year 1), David Meyronet (project years 2-6), Centre for Pathology and Neuropathology, Hospices Civils de Lyon, France
13. Miklós Palkovits, Department of Anatomy, Semmelweis University, Budapest, Hungary
14. Piero Parchi, Department of Neurological Sciences, University of Bologna, Italy
15. Efstratios Patsouris, Department of Pathology, School of Medicine, National and Capodistrian University of Athens, Greece
16. Richard Reynolds, UK Multiple Sclerosis Tissue Bank, Department of Neuroinflammation, Imperial College London, UK
17. Tamas Revesz, Queen Square Brain Bank, Department of Molecular Neurosciences, Institute of Neurology, University College London, UK
18. Peter Riederer, Clinic and Policlinic for Psychiatry and Psychotherapy, Department of Clinical Neurochemistry, University of Wuerzburg, Germany
19. Ameli Schwalber (years 1 to year 5 month 9, Birgit Fuchs (from year 5 month 10 on), GABO:mi Gesellschaft fuer Ablauforganisation:milliarium mbH & Co. KG, Process Management, Muenchen, Germany

Work performed and end results

1. Exchange of experiences

Experiences in all topics relevant to brain banking and to the Joint Program of Activities were exchanged between BNE partners at the Kick-off Meeting in Munich, at the Network Coordination Committee (NCC) Meetings in Salzburg, Athens, Vienna, London and Munich, and at the Network Governing Board (NGB) Meetings in Munich, Budapest, Venice, Stockholm, Barcelona, Malta and Lisbon. All researchers, doctoral students and technicians working for BNE's Joint Program of Activities were invited to attend the Kick-off Meeting and the NGB meetings. The attendees of these meetings used the opportunity for fruitful discussions in particular during special workpackage (WP) meetings and workshops/training sessions for technicians and brain bank managers.

In addition employees of BNE partners took active part in WP meetings and training sessions/workshops beyond regular NGB meetings organized and held by WP leaders. These separate WP meetings took place for the gene microarray study of the RNA preservation WP (WP17) and for the WP on ethical and legal issues (WP02).

Meetings of the participants in trials and studies on Quality Control (WP03) and Neuropathological Diagnostics (WP15) were regularly organized in Munich as Consensus Meetings for the harmonization of neuropathological diagnostics.

The training courses comprised workshops on microdissection, morphometry, neuropathological diagnostics, immunohistochemical staining methods, data base construction, data protection, DNA analysis techniques, RNA analysis techniques, protein analysis techniques, analysis techniques for neurochemical substances, laser capture microdissection, legal and ethical issues in brain banking and brain donor recruitment. Some of these courses were also open for interested people, mainly scientists and technicians, from outside the BNE consortium.

Furthermore individual training visits to BNE partners took place.

2. Harmonization of Neuropathological Diagnostics

Neuropathological diagnostics in Alzheimer's disease, Lewy Body Disease, beta-Aymloid deposits, FTLD-U, tauopathies and vascular pathology were harmonized:

Diagnostics in Alzheimer's disease concerning neurofibrillary tangle and neuropil thread pathology were harmonized creating a new Alzheimer staging based on immunohistochemistry for hyperphosphorylated tau-protein in collaboration with Professor Heiko Braak (Braak et al. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry 2006, Acta Neuropath 2006, 112:389-404). The usefulness of this new Alzheimer staging system was validated by an inter-rater study finalized in June 2006. The results of this study was published in Brain Pathology in 2008. (Alafuzoff et al. Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. Brain Pathol 2008 Oct; 18(4):484-96)

A second inter-rater study on Parkinson's Disease/Dementia with Lewy Bodies cases based on the two staging systems by Braak and by McKeith was also successfully completed. After a first assessment of the cases, the participants of this inter-rater study met for a consensus/training session in Munich in springtime 2007. It was realized that the current recommendations were not well-defined. Thus, these recommendations

were modified at that consensus meeting in order to facilitate a higher reproducibility and were published in Acta Neuropathologica. (Alafuzoff et al. Staging/typing of Lewy body related alpha-synuclein pathology: a study of BrainNet Europe Consortium. Acta Neuropathol 2009, 117: 635-652)

A third inter-rater study dealing with the regional distribution of intraparenchymal and intravascular beta-amyloid deposits was performed in project year 4. Professor Dietmar Thal, on whose staging system this new inter-rater study was based, was a member of the co-ordinating group of this study. Its results including BNE's recommendations on how to stage and type this kind of lesions were published in Acta Neuropahtologica (Alafuzoff et al. Assessment of beta-amyloid deposits in human brain: a study of the BrainNet Europe Consortium. Acta Neuropathol 2009, 117: 309-320).

An inter-rater study on typing of cases with FTLD-U was carried out. At a consensus meeting in Munich all cases were demonstrated and discussed. A consensus diagnosis was given to all cases, and final consensus recommendations were fixed. Types 2 and 3 according to the classification Cairns et al. appeared to be not reliably distinguishable. A manuscript is in preparation.

A further inter-rater study on cases with sporadic and hereditary forms of tauopathy was launched and performed. First results of this study were presented to study participants at a Consensus Meeting in November 2009 in Munich where all cases were also demonstrated and discussed. The final, detailed analysis of the data is in progress and results are expected to be prepared for a manuscript by the end of October 2010.

All surveys regarding the assessment of vascular pathology given by each BNE partner were collected, and all data is available in a data-file. The results are to be summarized in a publication format. A manuscript going to be submitted for publication in 2010 is in preparation.

3. Staining and diagnostic exercises for quality control

Numerous staining and diagnostic exercises were performed on tissue microarray (TMA) sections of cases with Alzheimer's disease using silver staining methods according to Gallyas and Bielschowsky and immunohistochemical stains with different antibodies against beta-amyloid and hyperphosphorylated tau-protein, of cases with Lewy body disease and multiple system atrophy using immunohistochemical stains with different antibodies against alpha-synuclein, of FTLD-U cases using immunohisto-chemical stains with different antibodies against ubiquitin, p62 and TDP-43, of different tauopathies using immunohistochemical stains for hyperphosphorylated tau-protein (clone AT-8) and 3- and 4-repeat tau-proteins and of glial cells using conventional stains for myelin and immunohistochemical stains for myelin basic protein, glial fibrillary acidic protein, CD68 and KiM1P. The last trial on a TMA section stained with AT-8 was done via an installed Virtual Slide System.

Staining and interpretation of the staining results were carried out by each participant independently. All stains were re-assessed by a reference group. Results of the exercises were discussed at NCC and NGB meetings or in separate WP/consensus meetings.

Reports on the results of the inter-laboratory trials on silver staining methods, the immunohistochemical detection of hyperphosphorylated tau and beta-amyloid in Alzheimer's disease, and on the immunohistochemical detection of alpha-synuclein in LBD/MSA were published in scientific journals: (a) Alafuzoff et al. Interlaboratory

comparison of assessments of Alzheimer disease-related lesions: a study of the BrainNet Europe Consortium. J Neuropathol Exp Neurol 2006, 65:740-57; (b) Alafuzoff et al. Inter-laboratory comparison of neuropathological assessments of β-amyloid protein. A study of the BrainNet Europe consortium. Acta Neuropathol 2008 May, 115(5):533-46; (c) Alafuzoff et al. Assessment of immunohistochemically detectable a-synuclein pathology. A study of the BrainNet Europe Consortium. J Neuropathol Exp Neurol 2008, 67:2:125-143.

Manuscripts on the results of the other three inter-laboratory trials/exercises are in preparation and are going to be submitted for publication in 2010.

A manuscript concerning pitfalls in immunohistochemistry with antibodies against beta-amyloid was accepted for publication in The Journal of Alzheimer's Disease (Aho L et al. Immunohistochemical visualization of amyloid precursor protein and β-amyloid in extra- and intracellular compartments in the human brain).

The results of all our trials have emphasized that systematic quality control is of great importance and that a critical approach towards already published data based on immunohistochemical techniques is recommended.

One of the most interesting observations within the framework of these trials was the general need of training sessions regarding assessment and assignment of lesions, i.e. observations that were thought to be clear were quite variously assigned. In conclusion a continuous training in assessment of complex lesions is recommendable.

4. Determination of the influence of pre- and post-mortem factors and tissue storage on tissue quality (preservation of DNA, RNA, proteins and neurochemical substances)

DNA preservation:

Artificial post-mortem delay up to 48 hours at a storage temperature of 4°C, paraffin-embedding and formalin-fixation up to 6 years showed no negative effect on DNA yield and DNA quality in human brain tissue samples. Known point mutations could be re-identified in all investigated frozen cases that were stored for years, but only in 70% of paraffin-embedded cases and even only in one of six formalin-fixed cases. Known CAG expansions in triplet repeat diseases could be re-identified in all investigated frozen cases that were stored for years and in all paraffin-embedded cases but not in formalin-fixed cases. In addition different detection methods were compared. The Qiagen method was found to be far superior to Genomiphi and TaKaRa EXtaq methods in all above mentioned investigations. These results were published in a scientific journal (Ferrer et al. Effects of formalin fixation, paraffin embedding, and time of storage on DNA preservation in brain tissue: A BrainNet Europe study. Brain Pathol 2007, 17: 297-303.)

Further investigations on the effect of formalin-fixation on DNA preservation revealed that DNA polymorphism studies are not recommended on formalin-fixed and formalin-fixed/paraffin embedded brain sections due to the presence of artefacts related with fixation and processing.

In a separate study DNA preservation has been examined in archival and new paraffin blocks and in wet tissue in a variety of diseases (Alzheimer Disease, Motoneuron Disease, Lewy Body Disease) and significant effects of time in formalin have been found on the quality of DNA extracted from the tissue types. Results showed new

paraffin blocks created from the wet tissue produced a lower yield and lower quality of DNA in most cases. There was no significant effect of storage time in formalin.

DNA quality of tissue samples either fixed in formalin or fixed in formalin-free fixative (RCL2) was compared. DNA quality was determined by gel electrophoresis or bioanalyser: (a) DNA was better preserved in formalin free fixative, (b) amplification efficiency for larger fragments (854bp) was better in formalin-free fixative, (c) genotyping by PCR-RFLP revealed no difference for both fixation methods.

Epigenetic studies of tissue samples with artificially prolonged post-mortem delays have shown that histone tail acetylation is modified with post-mortem delay, whereas methylation of CpG islands in gene promotors is stable up to 48h post mortem. This may impair studies of histone tail acetylation in human post-mortem brains.

This study on effects of postmortem delay on histone tail acetylation in relation to synuclein and UCHL-1, and modifications with disease has been reproduced in triplicate with several samples. Data are already available and a manuscript is in preparation.

First results of the methylation study on gene promoters for MAPT, APP, PSEN1 and UCHL1 genes in different stages of Alzheimer's disease, in Parkinson's disease and in tauopathies were published (Barrachina M and Ferrer I. DNA methylation of Alzheimer disease and tauopathy-related genes in postmortem brain. J Neuropathol Exp Neurol 2009, 68: 880-891): DNA methylation sites were very reproducible in every case. No differences in the percentage of CpG methylation were found between control and disease samples, and among the different pathologies in any region analyzed. However, these results should be interpreted with caution, as small changes in methylation of DNA promoters in vulnerable cells might have not been detected in total homogenates. This is particularly important in long-lasting degenerative diseases in which small modifications may be sufficient to modulate disease progression.

RNA preservation:

Investigations on the effect of ante- and post-mortem variables (gender, age at death, PM delay, length of hospitalisation before death, length of coma, presence of respiratory illness, length of artificial ventilation, month of death, duration at room temperature after death, cold storage duration of the body, duration from death to autopsy, duration from autopsy to freezer, tissue transport conditions, dissection duration, CSF pH and brain pH) on RNA quality determined by a Bioanalyser revealed that only an increasing length of hospitalisation before death and a decrease in brain pH below 6.0 was shown to lead to a decrease in RNA viability. Age, post-mortem delay, freezer interval and CSF pH did not show a significant effect on RNA quality. It was possible to isolate adequate concentrations of good quality RNA from human post-mortem brain tissue even with long post-mortem delays (up to 100hrs) and freezer intervals (up to 7 years). However, when the effects of an increasing number of ante-mortem agonal events were analysed, there was a significant decrease in RNA integrity. These studies have demonstrated that single factors do not have a large deleterious effect on RNA integrity but this can in part be explained by the increasing effort of modern brain banks to control for deleterious post-mortem events and to follow a consensus of best practice that BNE has promoted. The measurement of HIF 1 alpha mRNA levels could be used to indicate the degree of the effect that agonal changes had caused to the tissues. A manuscript on all this has now been published: Durrenberger et al. Effects of antemortem and postmortem variables on human brain mRNA quality: a BrainNet Europe study (2010), J Neuropathol Exp Neurol. 69(1):70-81.

Tissue samples from the UK MS Tissue Bank were studied to determine the suitability of human post-mortem brain RNA for quantitative real time PCR (qRT-PCR) gene expression analysis. Results have shown that a wide range of RNA integrity values can be used for such an analysis and thus optimally prepared human post-mortem RNA is very suitable for QRT-PCR analysis.

Protein preservation:

Investigations on the effect of "artificial" post-mortem delay and storage temperature on protein preservation in human brain tissue samples revealed that proteins were variably vulnerable to post-mortem delay when tissue samples were stored at 4°C or room temperature until freezing, but expression levels were not decreased when samples were stored at 1°C up to 50h after autopsy. These results were published in a scientific journal (Ferrer I et al. Brain protein preservation largely depends on the post-mortem storage temperature: implications for study of proteins in human neurologic diseases and management of brain banks: a BrainNet Europe Study. Neuropathol Exp Neurol 2007, 66:35-46).

Further investigations on the effect of "artificial" post-mortem delay and storage temperature on protein preservation using a 2D-DIGE/MALDI-TOF-TOF approach confirmed the variable vulnerability of proteins to post-mortem delay when tissue samples were stored at 4°C, RT or 37°C. Interestingly many proteins appeared to be increased first after 12 hrs but to be decreased finally after 48 hrs. These results were published in Proteomics (Crecelius et al. Assessing quantitative post-mortem changes in the gray matter of the human frontal cortex proteome by 2-D DIGE. Proteomics 2008, 8: 1276-1291.

The results of a study on the effect of post-mortem delay on post-translational modifications of tau-proteins were published as a Neuroscience Letter (Santepere et al. Low molecular weight species of tau in Alzheimer's disease are dependent on tau phosphorylation sites but not on delayed postmortem delay in tissue processing. Neurosci Lett 2006, 399: 106-110).

The following latest results concerning oxidized and nitrated proteins were obtained by studies using mono- and bi-dimensional gel electrophoresis and Western blotting in combination with in gel digestion and mass spectroscopy: (a) Patterns of modified oxidation and nitration adduct with post-mortem delay, which permits to assess the limitations in time of studies dealing with protein oxidative damage in the post-mortem human brain. (b) Oxidized proteins related to energy metabolism were identified in aged control brains. (c) Oxidized proteins were identified in target and distant brain regions at every stage of Alzheimer's disease, in Parkinson's disease and in Progressive Supranuclear Palsy. (d) Early changes and modified metabolic pathways were identified in Argyrophilic Grain Disease. Three main publications came out (Ferrer I et al. Argyrophilic grain disease. Brain 2008, 131: 1416-1432; Navarro et al. Human brain cortex: mitochondrial oxidative damage and adaptative response in Parkinson disease and dementia with Lewy bodies. Free Radic Biol Med 2009, 46: 1574-1580; Gomez A and Ferrer I. Increased oxidation of certain glycolysis and energy metabolism enzymes in the frontal cortex in Lewy body diseases. J Neurosci Res 2009, 87: 1002-1013).

Observations about preservation and localization of selected transcription factors in correlation to postmortem delay and about functional aspects in normal and pathologic brains have been published. Transcription factors of the Jun, Fos, CREB ATF family have been analyzed. The involvement of YY and other transcription factors in the

regulation of the ADORA2 gene has been discovered, and results have been submitted for publication.

Preservation of neurochemical substances:

Post mortem delay and storage duration seemed to significantly influence tryptophan concentrations. Furthermore significant differences in tryptophan concentrations were found between different Brain Bank centres. pH value of brain tissue, GAD activity and phosphofructokinase activity did not correlate with p.m.-delay, storage time, age, gender and did not reveal differences between brain bank centres.

Tryptophan is present in nearly all proteins. In case of protein degradation, an increase in tryptophan can be observed, which may reflect the processes happening after death. At present, the concentration of tryptophan seems to be the only valid neurochemical marker for quality control of autoptic brain tissue giving information about protein degradation status and metabolic disturbances.

The results of these studies were published in the Journal of Neurochemistry (2009 Sep; 110(5):1400-8: Grünblatt E et al. Tryptophan is a marker of human postmortem brain tissue quality.) and in Neuropathology and Applied Neurobiology (2009, 35(3):329-37: Monoranu et al. pH measurement as quality control on human post mortem brain tissue: a study of the BrainNet Europe consortium).

On samples with high/low tryptophan concentration, a protein analysis (Western blot) was performed, and the activity of the enzymes acetyl- and methyltransferase was measured in these samples by fluorescence ELISA. A publication on the results is in preparation.

In order to examine the influence of postmortem delay and storage temperature on tissue quality, a progressive postmortem delay was simulated. Fresh postmortem cerebellar tissue was stored at different temperatures (4°C, room temperature, 37°C) for up to 72 hours. The pH value was measured after 3, 16, 20, 24, 48 and 72 h. No significant modification of the pH value was detected. The tryptophan concentration will be measured in a couple of months.

In a total of 140 samples from control and suicide/depression patients the concentration of dopamine, noradrenaline and serotonin and of their metabolites was determined by HPLC. The concentrations of dopamine and serotonin were significantly lower in the frontal cortex of suicide/depression patients in comparison to controls. The concentration of the metabolite HVA was significantly lower in the cerebellum of this cohort when compared to controls. Concerning tryptophan concentrations, there was no difference between control and suicide/depression patients.

An immunohistochemical study using antibodies against caspase-3 revealed an increased apoptotic activity in (control) cases from newborns and infants especially in the periventricular germ cell matrix and in the granular cell layer of cerebellum. Furthermore isolated immunopositive cells were found in the penumbra of fresh brain infarction but not in global brain ischemia. No apoptotic activity was detectable in a first cohort of adult control cases with ages between 32-90 years. A quantitative analysis of apoptotic activity does not appear to be a useful marker for quality control of autoptic brain tissue in control cases.

5. Determination of the limits for the usability of human postmortem tissue for sophisticated molecular techniques

RNA:

A comparative microarray study of 6 CNS diseases (Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Huntington's, Schizophrenia, Multiple Sclerosis) has produced an enormous amount of data that will in reality take many more years to analyse. The data has revealed more than 60 genes that are deregulated in common in at least 4 diseases with a fold change of greater than 1.7. Important gene changes across different neurodegenerative diseases were validated using RT-qPCR verification of changes in 20 genes found commonly dysregulated in several diseases. These genes belong to the functional groups: apoptotic pathways, arachidonic acid metabolism, TNF superfamily, immune response, blood brain barrier function, inflammation, and stress response. This data highlighted the involvement of inflammatory pathways in all diseases and therefore a manuscript on common neuroinflammatory pathways in neurodegenerative diseases has been prepared and circulated to all co-authors for reviewing.

50 samples of grey matter lesions and normal appearing grey matter from MS tissues with and without meningeal inflammation and grey matter from controls were microdissected. Quality control was carried out on these samples before hybridisation onto Illumina Human Ref8 microarrays. Following hybridisation, bioinformatics analysis was carried out on the microarray data from the MS datasets of grey matter lesions with and without meningeal inflammation. Real Time qPCR was used to verify some of the major changes to the TNF signalling pathways and this was followed by immunohistochemical analysis. This analysis showed a major downregulation of neuronal genes in MS cases with meningeal inflammation, reflecting in particular a loss of pyramidal neurons in layers III and V of the cortical grey matter and a substantial loss of parvalbumin expressing interneurons. These changes were also seen in the normal appearing grey matter and thus are independent of the demyelination.

In order to increase the amount of data available for our microarray analysis of gene expression in schizophrenia a study of the anterior cingulate cortex of 8 disease and 8 control cases was carried out. Following quality control and initial bioinformatics analysis, the dataset will be further analyzed.

A microarray study of gene expression changes in 2 additional regions of Huntington's disease brains, the dorsal caudate nucleus and the putamen, was carried out after the first study on the ventral caudate nucleus. Bioinformatics analysis revealed that a total of 1,085 genes were upregulated and 1,160 downregulated in common between the three brain regions. This dataset will be further analyzed.

A final microarray study was carried out on cerebral cortical samples from 10 CJD and 10 control brains. Analysis revealed 183 upregulated and 151 downregulated genes. This dataset will also be further analyzed.

<u>All these studies showed that human post mortem brain tissue with a RNA integrity number of more than 6.0 is suitable for gene microarray studies.</u>

Protein:

Three publications related to protein aggregates in degenerative diseases of the nervous system came out with focus on pathology of synapses, aberrant tau

phosphorylation in synucleinopathies, abnormal synuclein in tauopathies and pathologies with aberrant tau deposits and synuclein aggregates.

Several publications related to protein modifications in neurodegenerative diseases in comparison to normal controls came out with focus on oxidative and nitrosative damage of selected proteins in AD, PD, DLB, PSP, PiD, FTLD-tau, FTLD-ubi and AGD. A review in Brain Pathology is also in press.

Results on sub-fractionation studies dealing with tau and alpha-synuclein localization and modifications in tauopathies and alpha-synucleinopathies were published.

Synuclein modifications, including phosphorylation and truncation have been analyzed in Parkinson's Disease. In vitro studies have shown autophagy as one of the mechanism leading to synuclein truncation. A manuscript was submitted for publication.

Activities of mitochondrial complexes have been analyzed and published for AD and PD, and have been submitted for publication for Pick's disease. The study on AD has permitted to elaborate the hypothesis of the exhausted neuron in AD as a result of energy metabolism failure.

The study on Pick's disease was continued. Pick bodies were analyzed by laser capture microdissection, and the Pick body proteome was determined by LC-MS/MS. 24 proteins were identified. The major problem with this analysis was the contamination of the samples with keratin, and the abundance of structural proteins, like glial fibrillary protein, actin and tubulin.

Alterations of the proteome in the frontal cortex were determined by two different approaches: by 2D-DIGE (2 dimensional fluorescence difference gel electrophoresis) with subsequent identification by MALDI-TOF Mass Spectrometry, and by High Performance Liquid Chromatography Mass Spectrometry. 70 differently regulated proteins were detected. Proteins are currently validated by Western blots. A manuscript on the results of this study is going to be submitted for publication in 2010.

These studies showed that human post mortem brain issue can be used for sophisticated methods in protein analysis.

Laser Capture microdissection:

a) mRNA

Reproducibility of staining protocols on mRNA preservation (RNA integrity number, concentration) has been tested on a series of brain samples. These samples have been selected from autopsy brains and surgical lobectomy (epilepsy surgery). The search for PCR enzyme inhibitors in solvents and chemicals used in these protocols was being performed in the same samples by a quantitative RT-PCR method with external standards. A manuscript to be submitted for publication is in preparation.

b) Proteins

Protocols were established to assess protein expression (mass spectrometry, whole proteome and immunocapture) with SELDI TOF technique from brain samples.

The effect of commonly used freezing methods for slides with cryostat sections on protein quality was assessed.

Microdissection of cells by LCM technique can be done on both wet and dried sample sections depending on the equipment used. The effect of dehydration in comparison to the use of wet sections has also been investigated.

The effect of artificially prolonged postmortem delays on protein degradation was assessed at +4°C. Whole proteome SELDI TOFF analyses were performed on samples with increasing pre-analytical or freezing delays and compared to analyses on respective control samples in order to find specific patterns of protein degradation. The identified mass spectra of proteins varied differentially. A method was established to compare results obtained in different experiments. A manuscript on the results of this work is in preparation.

The expression of specific proteins in very low amounts of tissue obtained by Laser Capture Microdissection can also be determined by SELDI TOF analysis in combination with immunocapture. Chips are covered with a specific matrix that allow covalent linking of one selected antibody. Protocols on immunocapture/SELDI-TOF analyses of brain tissue sections were established based on the detection of VEGF as an example.

6. Development of Gold Standards

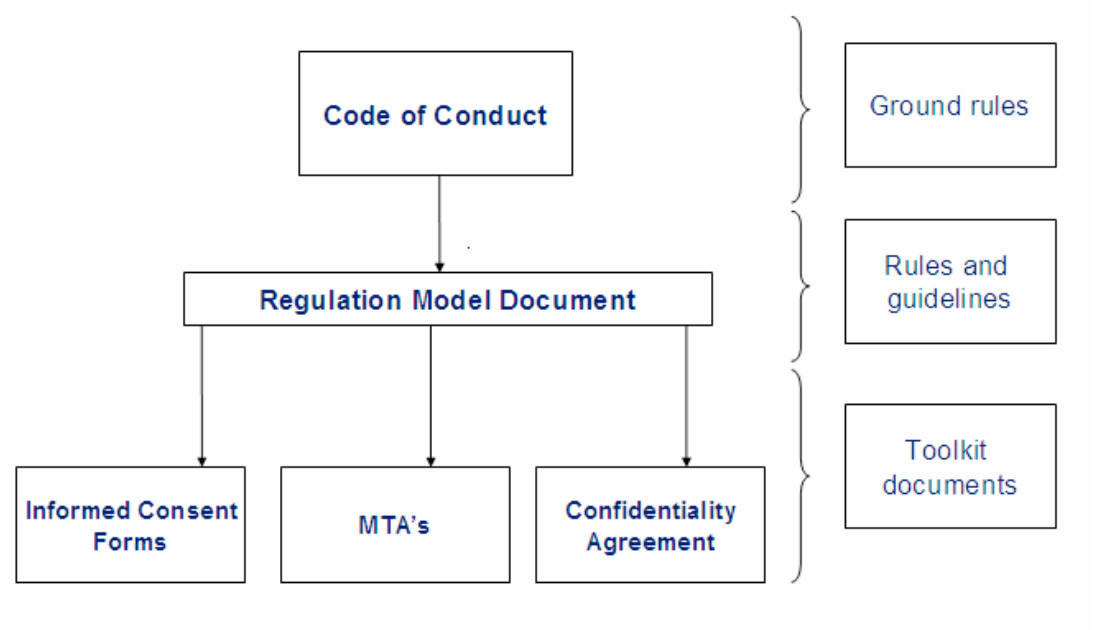
- Gold standards for histological and immunohistochemical staining methods in Alzheimer diagnostics were developed as a result of the staining exercises on Alzheimer cases and published the Journal of Neuropathology and Experimental Neurology (Alafuzoff et al. Interlaboratory comparison of assessments of Alzheimer disease-related lesions: a study of the BrainNet Europe Consortium. J Neuropathol Exp Neurol. 2006 Aug, 65(8):740-57.)
- Gold standards for the immunohistochemical detection of alpha-synuclein in Lewy body disease and Multiple System Atrophy were developed and published (Alafuzoff et al. Assessment of alpha-synuclein pathology: a study of the BrainNet Europe Consortium. J Neuropathol Exp Neurol 2008, 67(2):125-43)
- Gold standards for the immunohistochemical detection of beta-amyloid in the human brain were developed and published (Alafuzoff et al. Inter-laboratory comparison of neuropathological assessments of beta-amyloid protein: a study of the BrainNet Europe consortium. Acta Neuropathol 2008 May, 115(5):533-46)
- Gold standards for the immunohistochemical detection of hyperphosphorylated tau-protein, 3-repeat tau-protein, 4-repeat tau-protein, ubiquitin, p62 and TDP-43 were developed and will be published in 2010.
- Gold standard for the immunohistochemical detection of glial cells (astrocytes, oligodendrocytes, microglia) and myelin were developed and will be published in 2010.
- Gold standards on Alzheimer diagnostics including a new reliable neuropathological staging system as a result of the first BNE inter-rater study were published (Alafuzoff et al. Staging of neurofibrillary tangle pathology in Alzheimer's disease: A study of the BrainNet Europe Consortium. Brain Pathol 2008, 18(4):484-96)
- Gold standards on neuropathological diagnostics in Lewy body disease (modified and simplified staging systems according to Braak and McKeith) were developed as a result of a further inter-rater study and published (Alafuzoff et al. Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium. Acta Neuropathol 2009, 117(6):635-52.
- Gold standards on neuropathological diagnostics concerning beta-amyloid deposits in the human brain were developed and published (Assessment of beta-amyloid

deposits in human brain: a study of the BrainNet Europe Consortium. Acta Neuropathol 2009, 117(3):309-20.

- Gold standards on neuropathological diagnostics in FTLD-U cases, tauopathies and vascular pathology were developed and will be published in 2010.
- Gold standards for minimal and recommended safety provisions concerning the handling of human post mortem tissue were published on the BNE website on the basis of the results of a questionnaire on safety issues and on discussions with the BNE partners at BNE meetings.
- The best protocols for DNA extraction in both frozen and formalin-stored/paraffin embedded human post mortem brain tissue samples were identified, and placed on the BNE website.
- The best protocols for RNA extraction in frozen human post mortem brain tissue samples were determined and placed on the BNE website.
- Best sampling and freezing protocols for Laser Capture Microdissection (LCM) were published within a LCM manual on the BNE website.
- Protocols on the best histological staining methods for tissue sections with respect to highest mRNA yields in LCM were published on the BNE website.
- Investigations were finalized aiming to the development of gold standards for working with human brain tissue using 2D-gel electrophoresis, mass spectrometry and gene expression profiling. Results were published in Brain Pathology (Ferrer et al. Effects of formalin fixation, paraffin embedding, and time of storage on DNA preservation in brain tissue: a BrainNet Europe study. Brain Pathol 2007, 17(3):297-303) and in Proteomics (Crecelius et al. Assessing quantitative post-mortem changes in the gray matter of the human frontal cortex proteome by 2-D DIGE. Proteomics 2008, 8: 1276-1291).
- Gold standards for tissue storage, tissue handling and tissue quality were developed as results of workpackages dealing with the influence of ante- and postmortem parameters for the preservation of DNA, RNA, protein and neurochemical substances and will be summarized in a “Handbook of Brain Banking” in 2010.
- The best method for the usage of morphometric methods in neuropathological diagnostics was identified and shall serve as a gold standard in the future. BNE partners were instructed in this “best method” at two workshops on stereology organized at the University of Aarhus, Denmark in 2005 and 2009.

7. and 8. Ethical and legal issues

In the field of complex legislation and numerous differences in national laws of BNE partners, BNE has been able to set up a structure which focuses on globally accepted bioethical principles and international doctrine. The structure comprised a top-down approach consisting of the following documents (see figure below):



The Code of Conduct for Brain Banking was created in collaboration with subcontracted legal and ethical advisors. It was ratified by all BNE brain bank team leaders in June 2008. This Code of Conduct provides a framework of basic legal and ethical aspects of Brain Banking. It is based on Recommendation Rec(2006)4 on Research on Biological Materials of Human Origin adopted by the Committee of the Ministers of the Council of Europe in March 2006. The Committee of Ministers of the CoE recommends that the governments of the member states adapt their laws and practices to the guidelines contained in the Recommendation and promote the establishment of practice guidelines to ensure compliance with the provisions contained in the Recommendation.

A manuscript containing detailed explanations on the Code of Conduct, the procedure of its establishment, sources and decisions made by the Consortium has been drafted, which is expected to be included in a BNE book on Brain Banking.

In order to promote the ideas contained in the Code of Conduct with a broader audience (scientists, doctors, patient organizations and policymakers), a short, but powerful opinion point has been drafted and will be submitted on short term to a high impact journal. This opinion paper deals with the origin of collections of biomaterial, the differences between collections and biobanks and the pros and contras of transforming a collection into a biobank. Furthermore, issues such as finances, management, responsibilities of the organization where the biobank is located, visibility of output, putative abuse of power by the collector, participation of donors in the biobank management, property rights, integrity of collections vs disseminations of parts of the collection are dealt with. The issues are discussed in relation to the necessity of human tissue for research, European legislation on biobanks, European initiatives on biobanks and the BNE Code of Conduct.

The ground rules laid down in the Code of Conduct were elaborated in a document called Model Brain Bank Regulations containing rules for daily Brain Bank practice such as obtaining, handling and distribution of human biological material. The draft of the Brain Bank Regulations was first reviewed on legal grounds by an external reviewer (Prof. J.K.M. Gevers) and later supplemented by BNE partners. The final documents were posted on the BNE website (www.brainnet-europe.org) under menu item Guidelines for Brain Banking. The pages are openly accessible. Model documents, as

well as detailed instructions on how to use these have been posted on these pages. Model documents are provided in a modifiable format.

A model Material Transfer Agreement including appendices (Material Transfer Statement and Implementing Letter), a model Confidentiality Agreement and SOPs for their use have been developed and tested with non-profit and for-profit users. Web pages, including additional information and flowcharts on material transfer process have been developed, and forms with instructions on how to use them have been posted on the BNE website (www.brainnet-europe.org).

This approach provided the fundaments of a Brain Bank management ensuring optimal transparency and accountability.

A consensus has been reached on a uniform BNE policy with regard to Informed Consent and Authorization. The following statement has been agreed upon by the consortium at the NGB meeting in Stockholm in June 2007 and is part of the Code of Conduct: 'All BNE Brain Banks explicitly underline the importance of Informed Consent or Authorization. Human material and donor data exchanged within the BNE or through the Tissue Request Form must be obtained on the basis of Informed Consent or Authorization, within a period of 12 months after the voting. The individual Brain Banks will be encouraged to bring their internal standard operating procedures in conformity with explicit Informed Consent or Authorization policy.'

During the 2nd international BNE conference in Munich in December 2008 a Workshop on Legal and Ethical Issues and Donor Recruitment in Brain Banking in Europe has been organised where the most prominent ethical and legal problems in Brain Banking were discussed on the basis of recognisable case studies. The workshop was well attended and positively received by the audience. During the plenary session of the BNE conference a lecture on ethics in Brain Banking based on the work of BNE has been given by the leader of the WP on Ethics and Legal Issues, Dr. I. Huitinga.

9. Donor Programs

BNE member countries without donor programs were identified and the possibilities for establishing donor programs with respect to the ethnical and religious background were discussed.

The conclusions obtained from a literature search on published studies pertaining to public attitudes to organ donation for transplantation purposes, autopsy and brain donation served as groundwork for BNE guidelines for establishing and maintaining a donor program. These guidelines will be published on the BNE website.

In addition the results of this literature search were used to design two online surveys: one for BNE partners who run an active Donor Program and the other for BNE partners who do not. The surveys have been developed and administered using open-source software (Lime-Survey). The results obtained by the surveys have been analysed and included in the report "Brain Donation Programs in Europe". The numbers used and conclusions presented in this report have been evaluated by the study participants on their accurateness. This report has been presented during the Workshop on Legal and Ethical Issues and Donor Recruitment in Brain Banking in Europe held at the International BNE Conference in Munich (December 10-12, 2008) and is expected to be included as an article in the BNE book on brain banking.

Model forms for the donors (information leaflet, consent and authorization) have been drafted and submitted for review by the medical ethics committee of the Free University

Medical Center (MEC) in Amsterdam. Concerns expressed by the MEC have been addressed, and the text of the information brochure has been amended in accordance with MEC's advice. Model documents have been submitted for evaluation by a reading group, consisting of doctors, scientists and donors. Comments expressed by the reading group have been implemented. Documents, including instructions on how to use these have been posted on http://www.brainnet-europe.org/index.php?option=com_content&view=article&id=108&Itemid=108.

A donor recruitment flyer has been designed. Once agreed upon the definitive model version, it has been customized by each Brain Bank and translated into the different languages of the BNE partners. The participating Brain Banks are now able to distribute the flyer to their target audiences. The Dutch version of the flyer is currently being used for donor recruitment purposes.

As a donor program requires substantial commitment and infrastructure (secretary, hotline, etc), a checklist containing qualifying criteria has been developed for the use of the flyer. The check list shall help the partners work out what exactly is required before the flyer can be disseminated.

Patient organizations in BNE member states were contacted in order to advertise the work of BNE and in particular to advertise the BNE donor initiative. Representatives of these patient organizations were invited to the Workshop on Legal and Ethical Issues and Donor Recruitment in Brain Banking in Europe that took place during the 2nd International BNE Conference in Munich in December 2008.

A model donor website was developed which should inform potential donors and their families as well as clinicians about the importance of brain donation and the process of the donation. As with the donor flyer, the website should have a flexible concept so that it could be easily adapted to local requirements. All BNE partners received a DVD with all material developed in relation with this donor website for implementation.

A BNE image video about brain banking was created in order to inform a broader public, affected by brain diseases or not, about brain donation and its value and importance for future patients. Another aim was to present BNE as a research project as well as its individual partners to both the scientific and lay public.

10. Collection of brain tissue from patients with psychiatric disorders

A network of Pathology Institutes and Mental Hospitals has been organized to facilitate the collection of brain tissue from patients with psychiatric disorders. A standardized brain preparation protocol for all regions of interest and guidelines for informing the relatives after the patient's death have been developed. 82 cases with psychoses (schizophrenia, major depression, bipolar disorders) have been collected so far.

A prospective donor program has been implemented in cooperation with the Psychiatric Clinic in Liebenburg, Germany. Until now written consent from 7 patients with schizophrenia were obtained for the clinical part of the study and the postmortem investigation after death. Two brains from patients with schizophrenia have been collected.

Guidelines for how to run a neuropsychiatric brain bank were published (Schmitt A et al. How a neuropsychiatric brain bank should be run –A consensus paper of Brainnet Europe II-. Journal of Neural Transmission 2007, 114: 527-537).

11. Mystery Cases

"Mystery cases and Teaching Cases", which are cases with rare, atypical, difficult, combined or so far unknown neuropathological changes, were regularly circulated among BNE partners in order to improve diagnostic security and to reach consensus diagnoses. These cases were discussed at NGB and NCC meetings. In addition three workshops on such cases were given for experienced neuropathologists and young trainees in Vienna in 2006, 2008 and 2009. A summary on each case including a consensus diagnosis, if reached, was placed on the internal part of the BNE website.

20 out of 42 "Mystery and Teaching Cases" presented during the last 5 years were selected to be scanned for BNE's Virtual Slide System. In this way the most important cases can be viewed again and be used for teaching purposes. Future "Mystery Cases" will be discussed also via this tool.

12. Internet Activities

Project Website: www.brainnet-europe.org

The BNE website was re-designed and re-structured two times and its performance continuously optimized. The presentations of the institutions and the profiles of all BNE partners were updated and supplemented with the presentations and profiles of the new partners. A password protected internal website area for BNE members was created and supplied with content concerning meetings, training workshops and courses, project management and WP and documents. A password protected discussion forum was installed.

"Keyword clouds" were implemented on the Website to raise the rating of the Website on search engines such as Google.

The English version of the BNE image-video has been placed on the BNE website. Versions subtitled in French, German, Italian, Swedish and Dutch were finalized and will be available on the BNE website for download soon.

In a plea to reduce running costs, the entire website was migrated to a new hoster and a new content management system technology called Joomla in the last year of the project. There the website can now continue to exist even after the end of the project.

Online "Tissue Finder"data base:

The common BNE database "Tissue Finder" was re-written using a technology (filemaker) that is faster, cheaper and more flexible. The database contains a minimal clinical and neuropathological data set together with information on the availability of certain brain regions and type of storage of tissue samples. It was extended and optimized according to the suggestions of all partners. A data protection concept was written for the Tissue Finder defining usage and methodology of the Tissue Finder and was approved by the data protection officers in BNE partner countries if required. All brain bank partners have now entered all cases relevant for tissue requests into the database.

PDF data base:

A PDF archiving system containing publications with reference to brain banking activities or to the Joint Program of Activities of BNE was implemented on the internal BNE website.

Virtual Slide System:

A Virtual Slide System has been implemented. This system is a Linux based TRIBVN system with 5.1 terabytes of data storage.

Histological slides scanned with high resolution can now be assessed by all BNE partners at their computers via internet. Such a server based Virtual Slide System facilitates and accelerates access, exchange and assessment of Tissue Microarray sections of inter-laboratory quality control trials, sections of "mystery" or rare cases, sections for stereological investigations and sections of inter-rater studies. In addition, these "virtual slides" can be used for internal and external training purposes.

Up to now about 300 slides were scanned and are available on the system. A set of about 100 slides out of the 300 were scanned for the tauopathy section of the server. A further 700 slides of Mystery Cases and cases used in the WP03 trials and WP15 inter-rater studies were selected and are in scanning and processing.

Two major teleslide training sessions have been held for all users in the Consortium at the Consensus Meetings in Munich in March and November 2009.

A first inter-laboratory trial in the scope of the Quality Control WP has been performed via this system (see WP03).

Donor Website

A model donor website was developed which should inform potential donors and their families as well as clinicians about the importance of brain donation and the process of the donation. One BNE partner has already implemented this website, others will follow.

13. Internal and External Training and Exchange

Training sessions and exchange visits were regularly offered to BNE participants.

The following training workshops were organized:

- training workshops on immunohistochemical staining techniques
- three workshops on micro- and macrodissection of human brain tissue
- two workshops on stereology
- three workshops on mystery cases
- two workshops on neuropathological diagnostics (neuropathology of frontotemporal dementias, diagnostics in neurodegenerative diseases)
- workshops on data management, data protection and data base construction
- two trainings for the Virtual Slide System
- workshops on RNA, DNA, protein analysis and analysis of neurochemical substances
- two workshops on laser capture microdissection
- two workshops on ethical and legal issues
- one workshop on donor programs
- five Consensus Meetings on neuropathological diagnostics and quality control of histological stains

- Individual training visits of several BNE scientists took place.

All workshops were well attended and received positive feedbacks.

14. Spreading of excellence beyond the network not only via the BNE website but also by publishing in scientific journals, presenting posters and giving lectures at national and international meetings in the field of neuroscience, using electronic media / 16. Dissemination of information to the general public regarding the necessity of brain banking in general and on BNEII in particular, regarding research activities on human post-mortem tissues samples and their results, and on donor programs

Information on BrainNet Europe, the participating institutions, their research activities, tissue sampling protocols, references for neuropathological and clinical criteria and Model Brain Bank Regulation documents are available for the public on the BNE website.

A BNE poster advertising existence, aims and expertise of BNE II has been prepared and was circulated at meetings of neuropathologists, neurologists and neuroscientists in Europe and in USA. Abstracts from this poster were published in scientific journals.

A BNE flyer has been created. This flyer likewise contains information on the existence, aims and expertise of BNE. It was distributed to neuroscientists at scientific meetings.

Both flyer and poster can be downloaded from the BNE website (http://www.brainnet-europe.org/index.php?option=com_content&view=article&id=122&Itemid=122)

A donor recruitment flyer was designed to attract and inform potential brain donors. It has been customized by each Brain Bank and translated into the different languages of the BNE partners and is ready for distribution. The Dutch and German versions of the flyer are already used for donor recruitment purposes.

A BNE image video has been produced and can be seen on the BNE homepage. It gives information on brain banking in general, on the work of BNE and on brain donation and its value for the future in particular. Furthermore, the video appeals to the public to support BNE's activities.

Numerous lectures on the work of BNEII were given at national and international meetings. For this purpose a project presentation master was created and is available at the dissemination section of the internal BNE website. In this way BNE partners can use a consistent presentation about BNE when they give talks.

BNE II Satellite Symposia were held at the 8th International Conference of Neuropathology in San Francisco, 11-15 September 2007 and at the 9th European Congress of Neuropathology in Athens, 7–10 May 2008.

Two BNE International Conferences on Human Brain Tissue Research were organized in 2006 and 2008. Key topics were Work of BNE, Brain Bank Management, Pathology and Diagnostics of Neurodegenerative Diseases, Research on Human Postmortem Brain Tissue with focus on proteomics, epigenetics, gene expression profiling studies and psychiatric diseases. Special symposia were given on Ethical and Legal Issues and Multiple Sclerosis, and a session was held for patient organizations. Abstracts of these conferences are available in the Journal of Neural Transmission 2006, 113(6): I–XVII and 2008, 115(12):1706-1734.

Furthermore a Workshop on Legal and Ethical Issues and Donor Recruitment in Brain Banking in Europe has been organised at the 2nd International BNE Conference in 2008 where the most prominent ethical and legal problems in Brain Banking were discussed on the basis of recognisable case studies. Donor recruitment materials such as flyers, posters, letters and DVDs were exchanged between participants.

National and international organisations were approached to include information on BNE II in their regular publications.

Results on scientific studies of the Joint Program Activities and on diagnostic trials were published in scientific journals.

BNE's dissimination activities with respect to oral presentations, poster presentations and publications on the work of BNE are listed in the "plan for using and disseminating knowledge" section of the Final Activity Report.

15. Acquisition and distribution of well-characterized high quality tissues

Sampling protocols were developed for different neurodegenerative and psychiatric diseases and placed on the BNE website (http://www.brainnet-europe.org/index.php?option=com_content&view=article&id=99&Itemid=99).

A Tissue Request Form was created and placed on the public BNE website (http://www.brainnet-europe.org/index.php?option=com_content&view=article&id=101&Itemid=101#8309).

A standard operating procedure on how to respond to tissue requests was put on the internal BNE website as well.

Since the Tissue Request Form is available on the BNE website, the BNE office received more than 30 Tissue Requests. These Tissue Requests were either informal and sent via the BNE contact form, or were sent using the Tissue Request Form.

The BNE consortium agreed to plead for the process of providing tissue for reputable pharmaceutical and commercial companies engaged in research and developing of drugs. Such tissue requests should be scrutinised by the local brain bank and a committee of the BNE consortium.

Gold standards for tissue storage, tissue handling and tissue quality were developed as results of WPs dealing with the influence of ante- and postmortem parameters for the preservation of DNA, RNA, protein and neurochemical substances and will be summarized in a "Handbook of Brain Banking" in 2010.

17. Increasing the awareness of standardized neuropathological and clinical diagnostics in neurology and psychiatry at a European level

A list of neuropathological diagnoses with key references was created, regularly updated and supplemented by adding clinical information. This list is published on the BNE website (http://www.brainnet-europe.org/index.php?option=com_content&view=article&id=138&Itemid=248)

A list with key references for clinical diagnoses in neurological and psychiatric diseases created, regularly updated and supplemented by adding clinical diagnostic indicators. This list can be found on the BNE website (http://www.brainnet-europe.org/index.php?option=com_content&view=article&id=139&Itemid=249)

A course on neuropathological diagnostics in neurodegenerative diseases of three days' duration was given to neuropathologists outside BNE in the final project year.

Several publications came out with recommendations created by BNE on how to neuropathologically diagnose Alzheimer's disease, Lewy body disease and intraparenchymal/intravascular beta-amyloid deposits in a standardized way (Alafuzoff et al. Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. Brain Pathol 2008 Oct; 18(4):484-96; Alafuzoff et al. Staging/typing of Lewy body related alpha-synuclein pathology: a study of BrainNet Europe Consortium. Acta Neuropathol 2009, 117: 635-652; Alafuzoff et al. Assessment of beta-amyloid deposits in human brain: a study of the BrainNet Europe Consortium. Acta Neuropathol 2009, 117: 309-320).

Furthermore the neuropathological diagnostic of FTLD-U cases, of cases with sporadic and hereditary forms of tauopathy and of vascular brain pathology was standardized by BNE. Recommendations on how to assess these cases will be submitted for publication in 2010.

Numerous lectures concerning neuropathological diagnostic criteria standardized by BNE were given by BNE partners at international scientific congresses, and at the two International BNE Conferences on Human Brain Tissue Research.

18. Providing a basis and quality control system for RTD projects dealing with clinical or epidemiological aspects of neurobiological and psychiatric diseases

All BNE partners entered their autopsy cases ready for distribution in the common Tissue Finder data base.

A list of recommended histological and immunohistochemical staining methods was created as a result of WP03. The use of these staining methods is mandatory for all BNE partners.

Improved neuropathological diagnostic methods were elaborated and validated for Alzheimer's disease, Lewy Body Disease (Parkinson's disease and Dementia with Lewy Bodies), beta-amyloid deposits, frontotemporal lobar degeneration with ubiquitin-positive inclusions, sporadic and hereditary forms of tauopathy and vascular brain pathology. The use of these harmonized methods is mandatory for all BNE partners.

Studies were performed on the effects of ante- and postmortem parameters and storage conditions on the preservation of DNA, RNA, proteins, neurochemical substances and epigenetic factors. Many results were already published in scientific journals. Further publications will follow in 2010.

All results of the BNE project are going to be summarized in a Handbook of Brain Banking that will be completed by the end of 2010.

19. Integration of new partners

Since the beginning of BNE II three further brain banks were affiliated as associated members without EC funding: the Aarhus Brain Bank in project year two, the Queen Square Brain Bank London in project year 2 and the Neurological Tissue Bank, University of Barcelona in project year 5. The Queen Square Brain Bank London gained full membership after one year of associated membership.

Further applications for associated membership from Brain Banks in Germany, UK and the Netherlands are in the process of consideration.

20. Investigation of neural parameters in schizophrenia

In a genetic study the neuregulin 1 (NRG1) polymorphism SNP8NRG221533 was genotyped. There were no statistically significant differences in NRG1 isoform expression between individuals carrying at least one C allele compared to individuals homozygous for the T allele. Individuals carrying at least one C allele showed decreased expression of the NMDA receptor NR2C subunit. Results have been published (Schmitt A at al. Impact of neuregulin-1 on the pathophysiology of schizophrenia in human post-mortem studies. European Archives of Psychiatry and Clinical Neuroscience 2008, 258, Suppl 5: 35-39).

In formalin-fixed brains the cell numbers of pyramidal neurons, interneurons, astroglia and oligodendroglia were stereologically determined in hippocampal subfields and entorhinal cortex in cases with schizophrenia in comparison to controls. In hippocampal CA4 region oligendrocyte numbers were decreased in cases with schizophrenia. Results have been published in Acta Neuropathological (Schmitt et al. Stereological investigation of posterior hippocampal subregions in schizophrenia. Acta Neuropathologica, 2009, 117(4): 395-407).

Results of the gene microarray study on postmortem brain tissue (temporal cortex) of schizophrenic patients and healthy controls have been confirmed for some genes by RT-PCR in the cDNA of the same tissue. The focus was on immunomodulatory genes, genes for olfactory receptors, myelination-related genes and cytoskeletal genes. Two manuscripts to be submitted for publication are in preparation.

One additional brain region (anterior cingulate cortex) was investigated using Illumina-Chips. qRT-PCR investigations for confirmation of differentially regulated genes have been carried out. A manuscript to be submitted for publication will be prepared.

Results concerning candidate genes in schizophrenia obtained by the gene microarray study have been submitted for publication.

Staining techniques for pyramidal neurons providing good RNA quality and yield were established in postmortem tissue for LCM. cDNA microarrays (Affymetrix) and a subsequent gene set enrichment analysis has been performed on pyramidal cells dissected by LCM from the left hippocampus of schizophrenia patients and controls. qRT-PCR investigations will be carried out by February 2010.

Coordinator contact details:

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2. Dissemination and use

For dissemination and use see Final plan for using and disseminating the knowledge.