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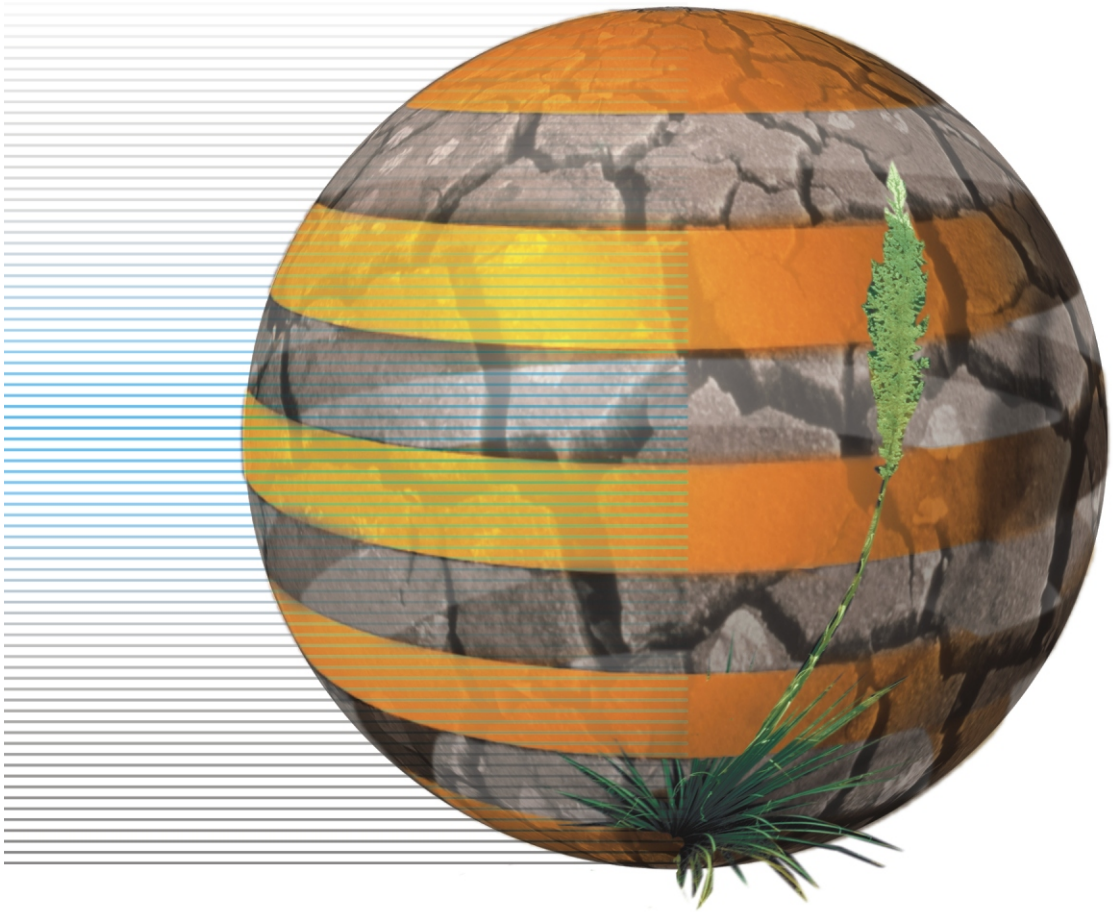

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Perspectives for a Healthy Brain Aging

EONIA

„Ana Aslan“ International Academy of Aging

Editorial office:
office@brainaging.org
Tel. / fax: +40 1 3127721
2 Morarilor Str., Bucharest 2, Romania

Roxana Nat aslan@pcnet.ro
Gunilla Johansson
Gunilla.Johansson@neurotec.ki.se

Publishing manager: Laurenţiu Nat
eonia@pcnet.ro

Cover: Gheorghe Popescu

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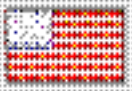

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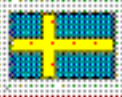

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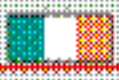

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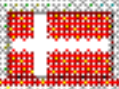

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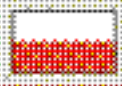

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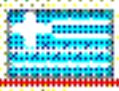

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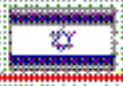

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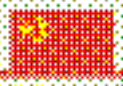

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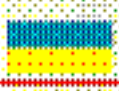

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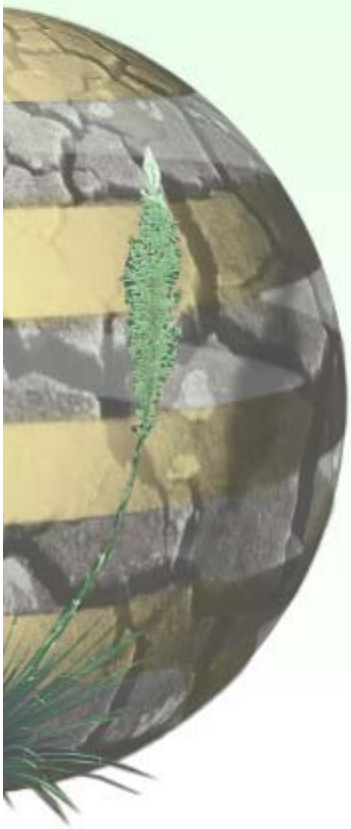


Alzheimer's disease is not only an economic and social problem but also a great scientific challenge. Our journal "Brain Aging" aims at highlighting the latest basic and clinical scientific findings in both normal aging and pathological aging, including Alzheimer's disease. Alzheimer's disease is quantitatively very different from normal aging. In normal aging, we also experience everyday lapses in communication and memory, as well as regular destruction of „tired" brain cells. These everyday lapses, nonetheless, do seem worrisome, because who can tell whether they are the early signs of Alzheimer's or just „normal aging?" Some scientists have theorized that the common, everyday lapses of normal aging are the happy result of a „pruning" process, wherein the mature brains discard the insignificant details of an idea or event, and retain only the important „gist" or „essence" of that idea or event. Alzheimer's, on the other hand, is a specific organic condition that unrelentingly destroys brain cells – indiscriminately. The disease slowly destroys or blocks activity in those brain cells responsible for communication and memory, and, in time, debilitates enough vital brain matter to render victims unable to reason, concentrate, or perform even the simple tasks of daily living (such as dressing, driving, and handling money). This systematic breakdown of brain function is irreversible and eventually results in death.

Just a few years ago, individuals with Alzheimer's disease and their families had little cause for hope. No effective treatments existed, and the prospect of any such therapies seemed a long way off. But today, research is advancing at an accelerating pace. Consequently, families have reason to look forward to a brighter future as more care and treatment options have become available.

Recent discoveries support the notion that we may soon be able to delay the onset of the disease through treatments such as anti-neurofibrillary degeneration, anti-amyloidosis, anti-inflammatories, antioxidants, promotion of neurogenesis or hormone replacement therapy as well as those affecting the neurotransmitter systems. Ultimately, our goal is to be able to diagnose individuals with the disease long before symptoms appear and find treatments that will prevent Alzheimer's. The concept of mild cognitive impairment (MCI) has been introduced and trying to define MCI will certainly lead to earlier and better diagnosis of Alzheimer's disease.

Wishing to put together minds focused on Alzheimer's disease research, Alzheimer's Associations in collaboration with World Association of Alzheimer's Disease Scientists (WADS) is organizing the 8th International Conference on Alzheimer's disease and Related Disorders, which will take place in Stockholm, Sweden, between 20-25 of July, 2002.

The Program Committee has invited 135 speakers to participate in the plenary sessions and symposia. These international leaders will lend their expertise to a program covering the entire spectrum of advances, challenges, and critical issues in dementia research.

Around 2000 researchers will present their works in a tremendous "brain storming".

The main topics will be

- Biology of amyloid, tau, inflammation, and other neurodegenerative mechanisms
- Epidemiology and risk factors
- Genetics and genetic testing
- Diagnosis, biomarkers, neuroimaging, and clinical course of Alzheimer's disease and related disorders
- Therapeutic strategies
- Cellular and animal models
- Molecular and cellular processes and pathologies
- Human and animal prion diseases, especially bovine spongiform encephalopathy
- Evidence-based patient management and social and behavioral research.

Since the last Alzheimer's Association meeting, major advances have been made towards understanding the aetiology and the pathogenesis of Alzheimer's disease. These include the discovery of the new genes whose mutations associate with Alzheimer's disease in the familial cases, the mechanisms of neuronal degeneration and the identification of specific steps in the neurodegenerative process that provide targets for therapeutic intervention. Presented at this conference will also be advances in epidemiology, differential diagnoses, psychosocial care, therapeutics and generation of cellular and animal models of β-amyloid and tau pathology, the two hallmark lesions of Alzheimer's disease.

We present some conference themes, which are the cutting edge of Alzheimer's disease research.

Neurofibrillary Degeneration

- Molecular Mechanism of Neurofibrillary Degeneration
- Tau in Alzheimer's Disease: Role in Cellular Transport and APP Trafficking
- Role of GSK3Beta in Tau Phosphorylation
- FTDP17 Mutations and Tau Aggregation

Amyloid Precursor Protein (APP)

- Amyloid Precursor Protein (APP) Processing
- Cell Biology of Gamma-Secretase
- Cholesterol-Dependent Beta-Amyloid Generation
- Use of Cholesterol-Lowering Drugs to Treat Amyloid Pathology
- Beta-Amyloid Degradation and Clearance
- Role of Neprilysin in Preventing Amyloid Formation

Presenilins

- Presenilin Processing
- Presenilin Mutations
- Structure and Function of Presenilins
- Molecular Genetic Mechanisms of Neurodegeneration Associated with Beta-Amyloid Protein
- Regulation WNT-Catenin Signaling by Presenilins
- Nicastrin

Stress-Induced Pathogenic Mechanisms

- Presenilin-Mediated Endoplasmic Reticulum Stress
- Role of the Presenilin Complex in Familial Alzheimer's Disease but Not in Endoplasmic Reticulum Stress
- Cytokines in Alzheimer's Disease
- Oxidation and Apoptosis
- RAGE and ERAB
- Modelling SOD1-Related Oxidative Stress
- Inflammation and Oxidation in Alzheimer's Disease
- Alpha-Synuclein in Aging and Alzheimer's Disease
- Histopathology of Tauopathies

Mechanisms of Neurodegeneration

- Abnormalities in Neurogenesis in Alzheimer's Disease
- Neuroendocrinology of Memory in Alzheimer's Disease
- APOE in Pathogenesis of Alzheimer's Disease
- Neurodegeneration in Alzheimer's Disease and FTDP-17
- Presenilin Mutations and Alzheimer 's Disease Pathogenesis
- Toxicity of APP Fragments

Epidemiology/Lifestyle and Dementia

- Dietary Recommendations to Lower Alzheimer Risk
- Smoking and Alcohol and the Risk of Dementia
- Occupational Factors and Alzheimer's Disease
- Social Engagement

Genetics

- Genetics of Alzheimer's Disease and Related Dementias
- Genetics and Molecular Epidemiology and Cardiovascular Risk Factors of Alzheimer's Disease
- Genetics of Early-Onset Alzheimer's Disease in a Population-Based Sample
- Search for Late-Onset Familial Alzheimer's Disease Genes
- The Search for Novel Alzheimer's Disease Genes
- Progress Toward the Identification of the Chromosome 10 Locus for Late-Onset Alzheimer's Disease
- Genetics of Chromosome 12

Clinical Challenges in Diagnosis and Management

- Challenge of Current Diagnostic Criteria for Alzheimer's Disease
- Neuropathology and Clinical Relevance
- Noncognitive Symptoms in Dementia
- Mild Cognitive Impairment
- Monitoring Cognition Across the Spectrum of Alzheimer's Disease
- Executive Dysfunction in Dementia
- Occurrence and Progression of Severe Dementia
- Atypical Alzheimer's Disease
- Diagnosis and Management of Frontotemporal Dementia
- Lewy Body Dementia

Neuroimaging Techniques to Monitor Disease Progression and Treatment

- Genetic Risk and Imaging
- Using MRI to Measure Progression in Alzheimer's Disease
- Functional MRI Studies in Early Alzheimer's Disease
- Quantitative EEG and Alzheimer 's Disease

The 8th International Conference
on Alzheimer's Disease and Related Disorders
Alzheimer's Association

July 20–25, 2002
Stockholm, Sweden

8

ALZHEIMER'S DISEASE

- PET and Response to Treatment
- Imaging Amyloid
- Visualizing Amyloid Plaques

Biomarkers

- Proteomics and Genomics in Biomarker Research
- Cerebral Spinal Fluid Protein Markers
- Identification of Alzheimer's Disease Subgroups through Cerebral-Spinal Fluid Markers
- Biochemical Quantification of Tau and APP By-Products in the Human Brain
- CSF-Tau, Phospho-Tau, and Beta-Amyloid Profiles in Alzheimer's Disease
- Genetic Modifiers of Plasma Beta-Amyloid
- Differential Gene Expression in Alzheimer's Disease

Issues in Treating Dementia

- Evaluation of Treatment Effects in Alzheimer 's Disease
- Preventive Strategies in Dementia
- Cholinesterase Inhibitors: When to Start? In Whom? When to Stop?
- Pharmacoeconomics and Dementia
- Ethical and Legal Issues in Alzheimer's Disease
- New Targets for Therapy

Amyloid-Lowering Strategies

- Is Lowering Amyloid a Therapeutic Target for Alzheimer 's Disease?
- Inhibitors of the Presenilin/Gamma-Secretase Complex: Biological Tools and Therapeutic Potential
- Vaccination as Prophylaxis
- Beta-Amyloid–Sheet Breakers
- Anti–Beta-Amyloid Aggregant Therapy
- Regulation of Beta-Secretase Activity

Future Strategies in Treatment

- Interaction between Cholinergic Neurotransmission and Underlying Alzheimer Pathology: Therapeutic Implications
- Treating Amyloid in the Periphery and the Brain
- Clearance of Amyloid by Vaccination
- Antioxidant Treatment for Dementia
- Lipid-Lowering Strategies in Dementia
- Future Combination Therapies in Alzheimer's Disease
- Pharmacologic Treatments of Severe Dementia

Animal Models: Biological Functions

- How Do We Bridge the Gap between Animal and Human Cognitive Testing?Presenilin Modifiers in C. elegans
- Beta-Secretase and the Brain's Susceptibility to Beta-Amyloid Deposition
- Presenilin Function
- Presenilin-1 and Its Interaction with Telencephalin and APP
- Presenilin-1 in Cortical Development, Synaptic Plasticity, and the Pathogenesis of Alzheimer's Disease
- Presenilin and Notch Signaling
- From Mice to Men: The Value of Genetically Engineered Mice for Experimental Therapeutics
- Preclinical Prediction of Alzheimer's Disease
- Neuroimaging in Dementia: Beyond Exclusion
- Animal and Cellular Models for Alzheimer's Disease

Disease Model Systems

- Role of the APP Cytoplasmic Domain
- Presenilin-1 Regulates Gamma-Secretase Cleavage, Adhesion, and Signaling of the Cadherin Family of Proteins
- Blocking Amyloid Production: Are Presenilins the Ultimate Target?
- Cholesterol in Alzheimer's Disease
- Amyloid-Induced Neurofibrillary Tangle Formation
- Cross Talk between APP and Tau in Mice

Subcortical Dementia and Related Conditions

- Vascular Cognitive Impairment
- Cognitive Outcomes after Coronary Bypass Surgery
- Dementia in Parkinson's Disease: An Epidemiologic Perspective
- Dementia in Huntington's Disease
- Neuropsychology of Frontotemporal Dementia
- Cognitive Functioning in Late-Life Depression

Variant Creutzfeldt-Jakob Disease (CJD)

- Epidemiology of Bovine Spongiform Encephalopathy
- Clinical Features of Variant CJD
- The Pathology of Variant CJD
- Molecular Pathology of Variant CJD
- Protein Folding and the Species Barrier
- Tetracyclines as a Possible Therapeutic Strategy for Variant CJD

Quality of Life and Alzheimer Care: Integrating Research and Practice

Social and Cultural Resources for People with Alzheimer's Disease and Their Caregivers

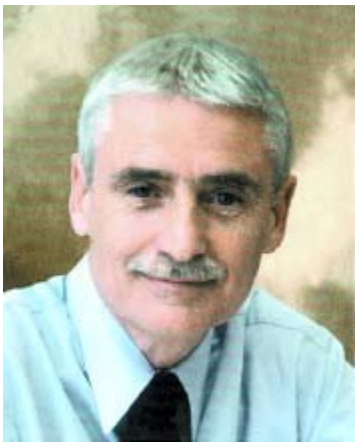
- Psychosocial Intervention in the Management of Dementia

Basic, clinical and social aspects will be analysed in both patients, clinicians and researchers perspectives.

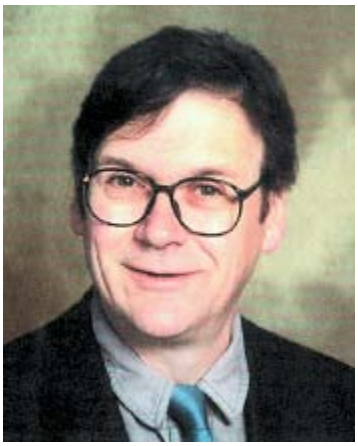
Bengt Winblad, Editor in Chief
Luiza Spiru, Editor
Khalid Iqbal, Editor

The Nobel Prize in Physiology or Medicine 2001

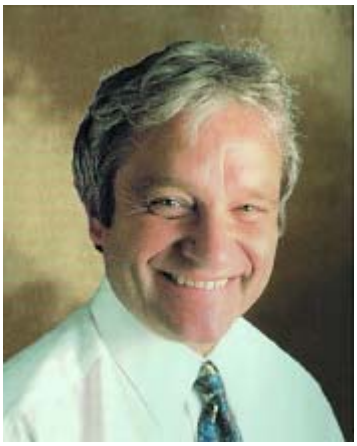
The Nobel Assembly at Karolinska Institutet has awarded the Nobel Prize in Physiology or Medicine jointly to **Leland Hartwell, Tim Hunt** and **Paul Nurse** for their discoveries of „key regulators of the cell cycle“. Using genetic and biochemical methods, they identified the molecules CDK and cyclin that control the cell cycle in eukaryotic organisms. These fundamental discoveries have a profound impact on many aspects of biology and medicine.



Leland Hartwell, born 1939.
Fred Hutchinson Cancer
Research Center,
Seattle, WA, USA.

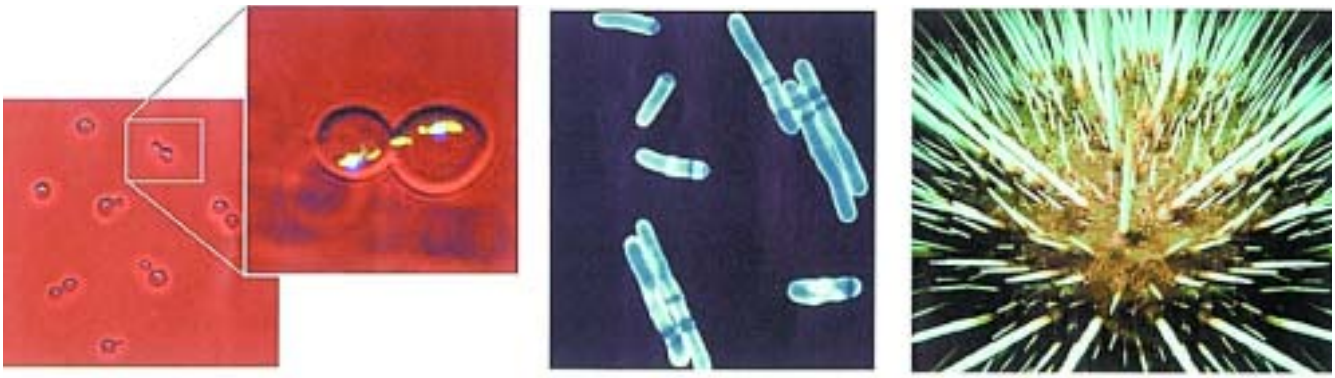


Tim Hunt, born 1943.
Imperial Cancer Research Fund,
Clare Hall Laboratories,
South Mimms, UK.

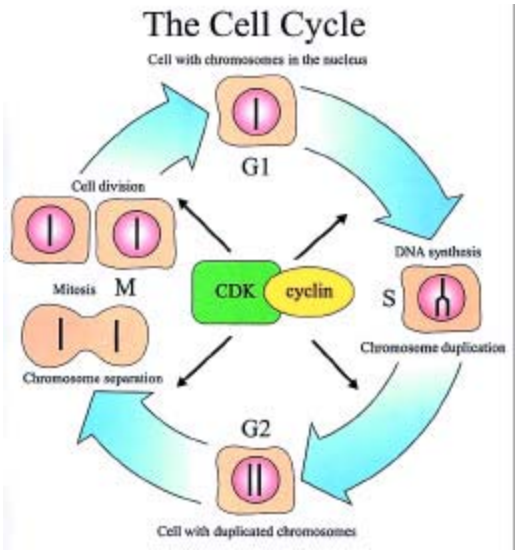


Paul Nurse, born 1949.
Imperial Cancer Research Fund,
Lincoln's Inn Fields,
London, UK.

Organisms consist of cells that multiply through cell division. Before a cell can divide it has to grow in size, duplicate its chromosomes and separate the chromosomes for distribution between the two daughter cells. These different processes are coordinated in the cell cycle.



Important model organisms for this year's Laureates. Leland Hartwell used baker's yeast, *Saccharomyces cerevisiae* (left). Paul Nurse used another type of yeast, *Schizosaccharomyces pombe* (middle). Tim Hunt used sea urchin, *Arbacia* (right).



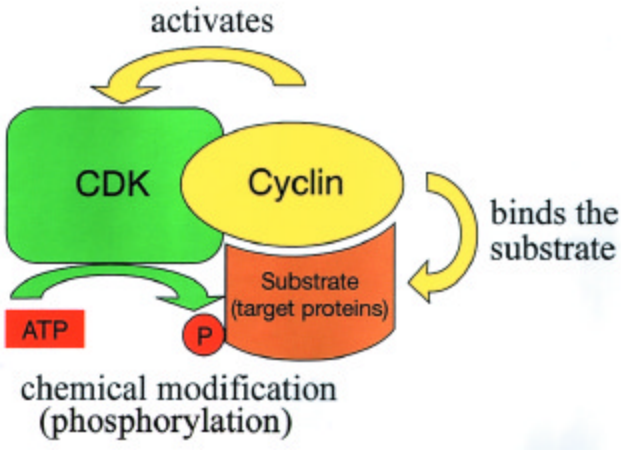
The cell cycle consists of several phases (figure above). In the first phase (G 1) the cell grows. When it has reached its appropriate size it enters the phase of DNA-synthesis (S), where the chromosomes are duplicated. During the next phase (G2) the cell prepares for division. In mitosis (M) the chromosomes separate, and the cell divides into two daughter cells. Through this mechanism the daughter cells receive identical sets of chromosomes. After division, the cells are back in G 1 and the cell cycle is completed. This year's Nobel Laureates have discovered fundamental mechanisms controlling the cell cycle. CDK and cyclin drive the cell from one phase to the next in the cell cycle.

Leland Hartwell used baker's yeast, *Saccharomyces cerevisiae*, as a model system for genetic studies of the cell cycle. In an elegant series of experiments 1970-71, he isolated yeast cells, in which genes controlling the cell cycle were altered (mutated). By this approach, he identified genes specifically involved in cell cycle control, so called CDC-genes (cell division cycle genes). One of these genes, designated CDC28, controls the first step in the progression through the G1-phase of the cell cycle (the function „start"). Hartwell also identified the fundamental role of „checkpoints" in cell cycle control. These checkpoints monitor that all steps in the previous phase have been correctly executed and ensure a correct order between the cell cycle phases.

Paul Nurse identified the key regulator of the cell cycle, the gene *cdc2*, during the years 1976-80. He showed that the product of this gene controls cell division (transition from G2 to M). Nurse discovered the gene *cdc2* in the fission yeast *Schizosaccharomyces pombe*. He later showed that *cdc2* had the same function as the gene *CDC28* in the distantly related baker's yeast.

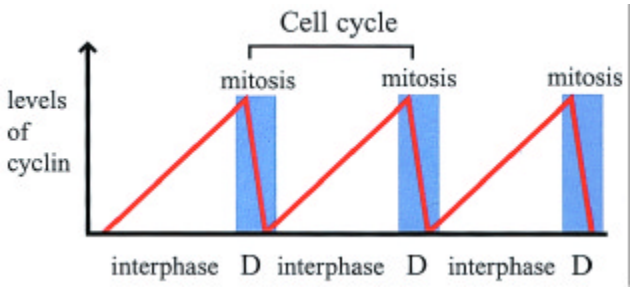
Thus, *cdc2* has more than one function in the cell cycle, controlling both the transition from G 1 to S and G2 to M. In 1987 Paul Nurse isolated the corresponding human gene, later called *CDK1*. These findings showed that the CDK function has been conserved through evolution.

The gene *CDKl* encodes a protein that is a member of a family called cyclin dependent kinases (CDK). These molecules function by linking phosphate groups to other proteins (phosphorylation, figure below). Today half a dozen different CDK-molecules have been found in humans.



CDK and cyclin together form an enzyme that activates other proteins by chemical modification (phosphorylation). The amount of CDK molecules is constant during the cell cycle, but their activities vary because of the regulatory function of the cyclins. CDK can be compared with an engine and cyclin with a gear box controlling whether the engine will run in the idling state or drive the cell forward in the cell cycle.

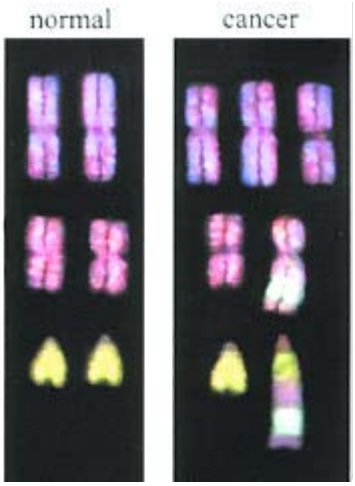
Tim Hunt discovered cyclins, proteins that bind to the CDK molecules. Cyclins regulate the CDK activity and select the target proteins to be phosphorylated. The proteins were named cyclins because of their cyclic variation in amount during the cell cycle (figure below). Hunt's discovery that cyclins were degraded during mitosis turned out to be another fundamental control mechanism in the cell cycle.



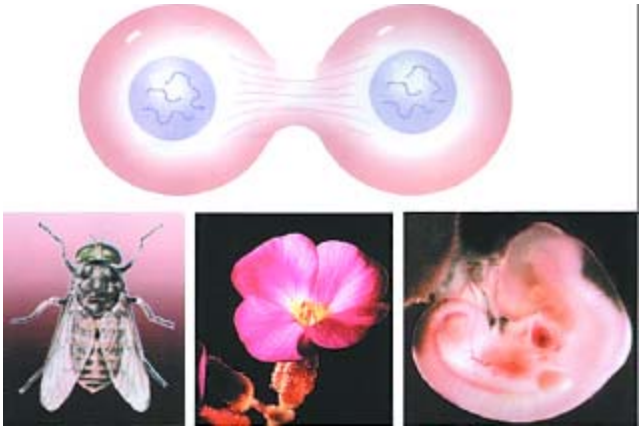
Cyclins are proteins formed and degraded during each cell cycle. Periodic protein degradation is an important control mechanism of the cell cycle. (D = cell division.)

Tim Hunt discovered the first cyclin molecule in 1982, using eggs from sea urchin, *Arbacia*, as a model system. He also found that cyclins, like CDK, were conserved during evolution. Today around ten different cyclins have been found in humans.

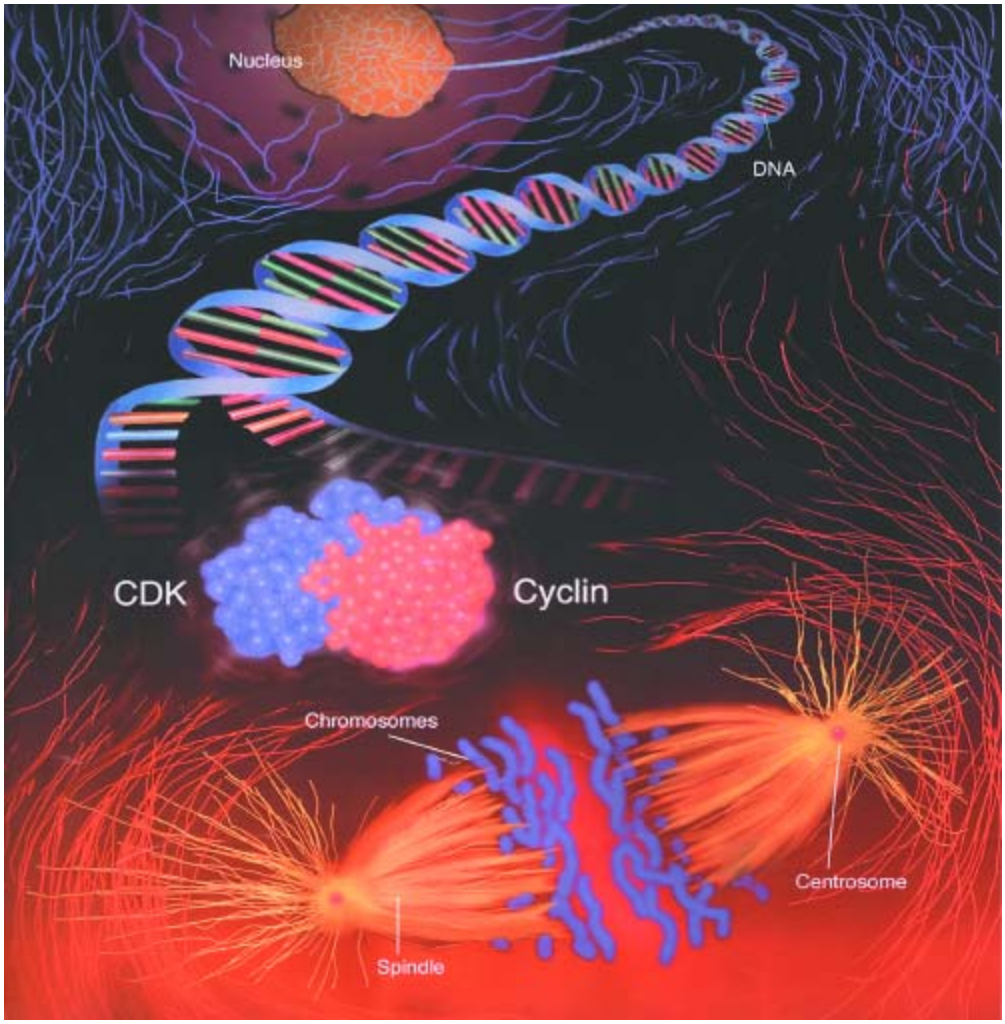
The basic discoveries made by this year's Laureates will have broad applications within many fields of biology and medicine. The discoveries are important in understanding how chromosomal instability develops in cancer cells, i.e. how parts of chromosomes are rearranged, lost or distributed unequally between daughter cells (figure left). The findings in the cell cycle field are about to be applied to tumour diagnostics, and the discoveries may in a long term perspective open new possibilities for cancer therapy.



Chromosomal instability in cancer cells may be the result of defective cell cycle control. The figure shows three pairs of chromosomes (1, 3 and 14) in normal cells (left), compared with the same pairs in cancer cells (right). In cancer cells, the chromosome number may be altered (aneuploidy) and parts of chromosomes may be rearranged (visualized by different colours).



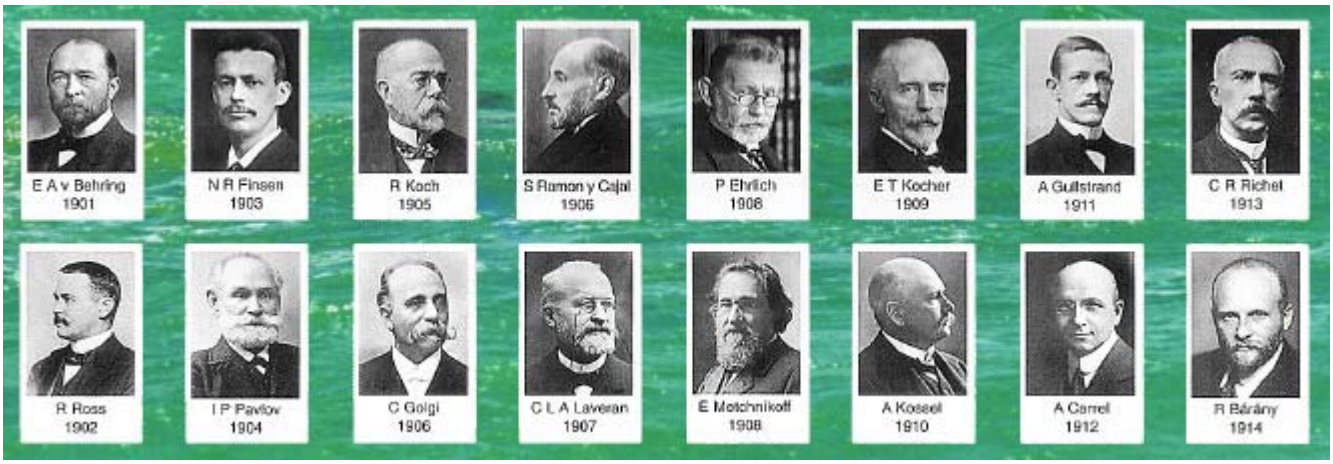
The fundamental molecular mechanisms controlling the cell cycle are highly conserved through evolution and operate in the same manner in yeasts, insects, plants, animals and humans.



CDK and cyclin are key molecules that control and coordinate DNA-synthesis, chromosome separation and cell division. CDK and cyclin together drive the cell from one cell cycle phase to the next.

Nobel Prize Centennial 1901–2001

Awards in Physiology or Medicine
Discoveries of benefit to mankind
A selection of awards in genetics, metabolism and disease



1904 – **The physiology of digestion** – Ivan P Pavlov

1905 – **Discoveries in relation to tuberculosis** – Robert Koch

1911 – **The dioptrics of the eye** – Allvar Gullstrand

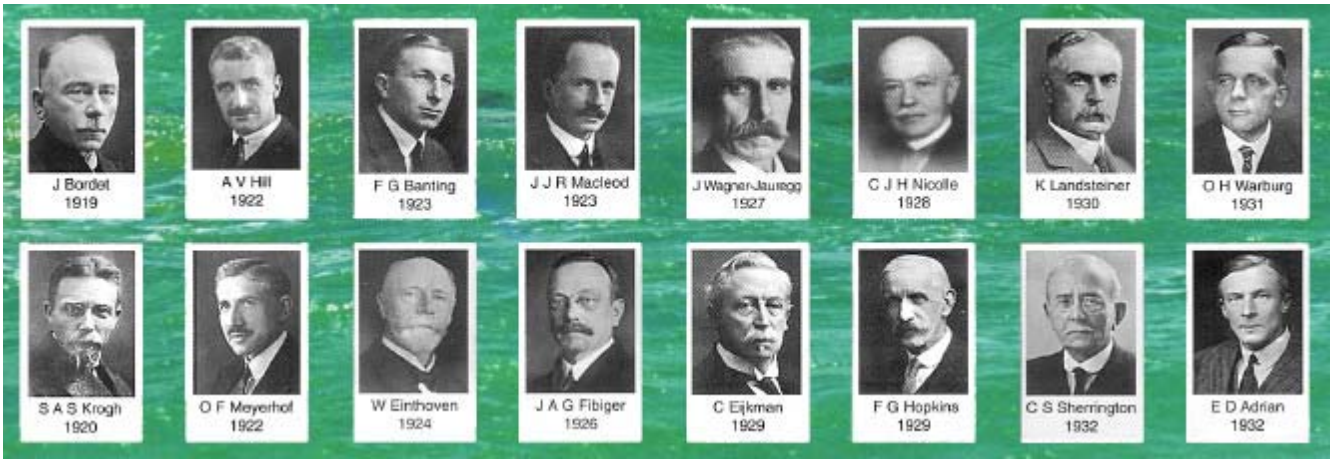
1923 – **The discovery of insulin** – Frederick G Banting and John J R Macleod

1924 – **The electrocardiogram** – Willem Einthoven

1930 – **Human blood groups** – Karl Landsteiner

1933 – **The role played by the chromosome in heredity** – Thomas H Morgan

1945 – **Penicillin and its curative effect in various infectious diseases** – Alexander Fleming, Ernst B Chain and Howard W Florey



1950 – **Hormones of the adrenal cortex, their structure and biological effects** – Edward C Kendall, Tadeus Reichstein and Philip S Hench

1953 – **The citric acid cycle** – Hans A Krebs **Coenzyme A and its importance for intermediary metabolism** – Fritz A Lipmann

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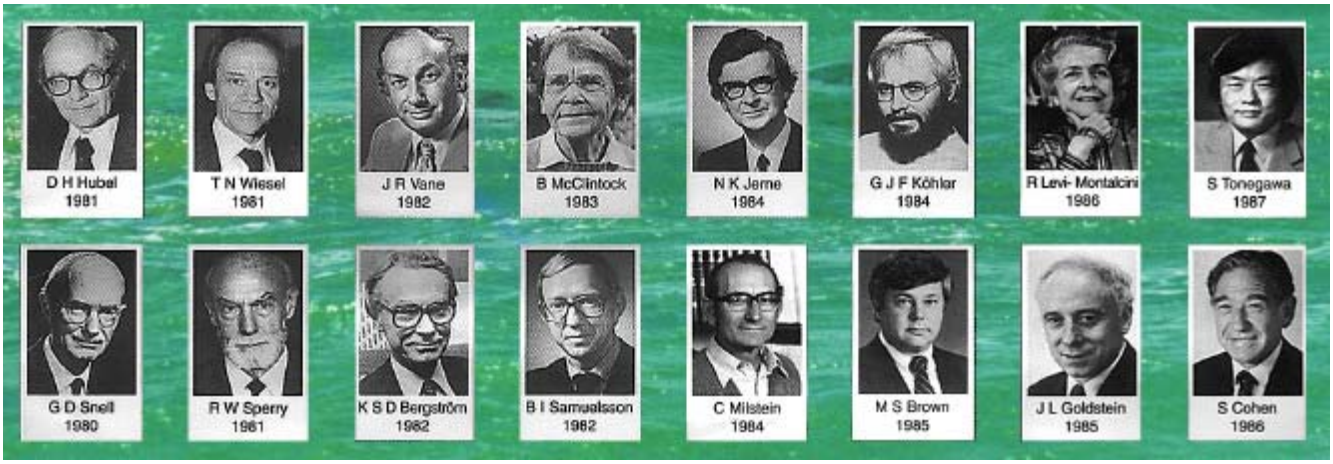

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Beyond Definitions and Stereotypes

Magda Cuturicu[#]
University of Medicine and Pharmacy "Carol Davila", Bucharest

Almost everybody can learn to think or believe, but not a single human being can be taught to feel. Why? Because whenever you think or you believe or you know you're a lot of other people; but the moment you feel, you're nobody – but – yourself.

- e. e. cummings
A Poet's Advice to Students

In our era of narrow specialization, on the one hand (think of *the right eye* oculist), governed by attempts to be as strict and as concise as possible, and a global view of the world on the other hand, we sometimes fail to grasp the vastness and intricacy of phenomena and processes and look at them as only figures in a table or definitions in a dictionary.

In the great museums or cathedrals of the world, tourists no longer look at the paintings or centuries old pulpits or stained glass. They read notes in a guide or watch slides; they buy postcards to remind them not of the real thing – with which they have had no direct experience – but of another postcard in a guide or on a slide. A copy of a copy…

Let's look at the real thing and let's do it with our own eyes!

I. On Definitions

Such terms as Life and Death, or whatever is between the beginning and the end, like development divided into ages, are very difficult to define not only due to the complexity of the terms themselves, but also to the diversity of points of view. And there are obviously as many points of view as there are people in the world, because each of us, besides having all the characteristics of the species Homo Sapiens, also have unique qualities mostly derived from the intricate ways we think and feel, and look at reality.

Life is known to be difficult to define, so much more man's life (Creationism or Evolutionism?), and, when defined, this is usually done in terms of what is known. So, to start with, there is a barrier of time and knowledge. Most definitions of life refer to function and, as function is the object of several sciences – physiology, molecular biology, biochemistry, genetics, pathology, biophysics – there will be several definitions, not one.

Death, on the other hand, is generally defined as the cessation of life, but in so far as one cannot strictly define life, it is so much more daring to attempt to define death. This will be differently defined by a physicist or a histologist, by the attending physician liable to make a mistake or by the priest thinking of the soul leaving the body somewhere in the process.

Apparently not so difficult to define is what happens between the moment life starts (birth) and the moment life ceases (death) that is man's development through different time related stages mainly from the anatomical and physiological point of view, to put it simply.

As Shakespeare so brilliantly wrote, all men and women have their entrances and their exits in this world, as players have on a stage. Between one's entrance and one's exit there is a life span, unique in character and irreversible in time, from infancy to childhood and adolescence, and from there on to adulthood, old age and death.

The concept of old age has only recently (for about a century, as compared to the several thousand years' concern with life and death) become an object of study and researchers still try to define it.

Old age presents patterns that govern the development of all the individuals of the species, but as each individual is unique, to define this uniqueness a great number of sciences are involved: anthropology, sociology, biology, psychology, education and, of course, medicine.

The study of old age is supposed to have started in the 19th century with the publication of a book by Quetelet, a French mathematician – also a psychologist and sociologist – especially concerned with the relationship between age and creativity (1825). Charles Darwin's, cousin, Francis Galton took over the idea underlining the individual differences in relation to age (1883).

 # Correspondence: Magda Cuturicu, Associate Professor of English, Head of the Foreign Languages Department, UMP "Carol Davila", Bucharest, Romania
Phone: 40 1 3159585, e-mail: magdacuturicu@yahoo.com

The psychology of aging also began to be studied; G. Stanley Hall published his book *Senescence: the Last Half of Life* (1922).

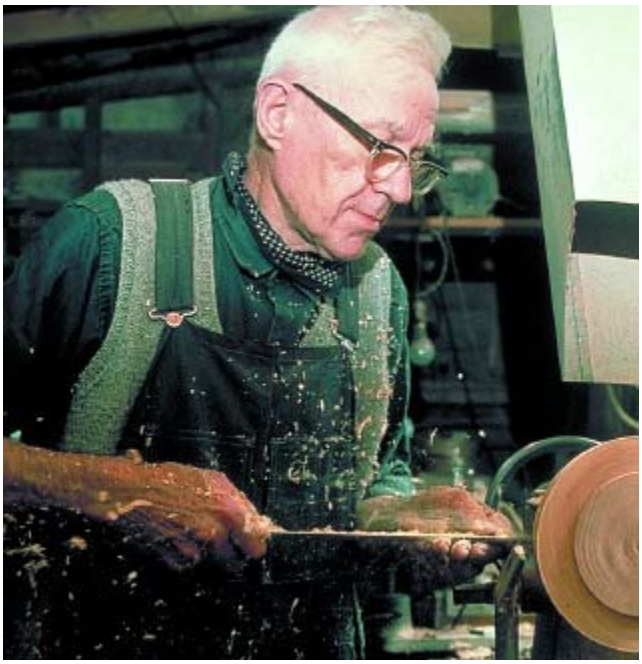
Ample research into the matter was initiated immediately after World War I and research units were devoted to the problem.

Old age has become a major study once the bases of Gerontology and Geriatrics have been laid, and the present medical journal is a proof of the interest of the medical profession in such problems.

Only a very important and complex matter could have given rise to so much research and so many controversies along time, wherefrom the difficulty – almost impossibility – to offer a simple, clear-cut definition of old age. Dorland's Medical Dictionary, a reputed one in the field, does not even mention the term. Neither is *aging* mentioned. Only *age*, as the duration of individual existence measured in units of time, or the measure of some individual attribute in terms of the chronological age of an average normal individual showing the same degree of proficiency, distinction being made between several categories of age, among which anatomical age, physical and physiological age or chronological age.

On the other hand, Webster's defines not only age as *the time that a person or thing has existed since birth or beginning*, but also *old* – stressing that it is a characteristic of aged people, mature in judgement, wise, apparently in contradiction with the definition the same dictionary gives for *old age* – the advanced years of human life when strength and vigor decline.

There appears to be a distinction between judgement and physical strength, while they actually go hand in hand in the process of aging with the individual differences that account for the uniqueness of each human being.



II. On Stereotypes

So many things have been said about old age and in so many ways that they have become negative „stereotypes" with linguistic implications. An old man is now to be called „a senior citizen". An 80 year old man is no longer called an old man but „an elderly person" or „older person". Americans have gone even further to call him „an older American". *Ageism* has thus been created which, besides *sexism*, is just another „-ism" of modern society. Is old age so horrible or indecent to give it another name? Is everything which is said about it actual fact? It may or may not be true. So avoid generalizations!

Old age indeed witnesses a lowering of one's senses, a diminishing of one's strength; it means infections, accidents and tiredness or loss of memory. The stereotype of an old lady looking for her glasses while they are on her forehead may be true. An old man sleeping in a rocking chair with an open newspaper in his hands may also be true.

But also true is the fact that old people have governed the world in perfect health of mind and body, that old men procreated healthy children. Think of Leonardo or Galilei! Or Hippocrates, that old man with a marvelous mind who still astonishes the medical world. Or Chaplin! Or just my grandmother who at 100 still had an intact power of reasoning and judgement!

Are they old, elderly, senior citizens or golden agers? Or are they young? What shall we call them? I will certainly want to be called „an old lady", as I was once called „a young lady".

Or take, on the other hand, Nadia Comãneci who ended her golden career because she was „too old". Or the great Pele who no longer plays football because he is old. And neither does Hagi, for the same reasons. Are they old?

No, on both issues. It is only the stereotype we have created! Let me prove my point.

If you were to choose from the following gifts: a bicycle, a stethoscope, a CD with Pavarotti, a dog, a wheel chair, a book of poems, which would you consider the perfect gift for each of the persons:

- Jane, a medical student, 22
- Tom, a farmer, 40
- Helen, retired, 80

Of course, a stethoscope for Jane and a dog for Tom. But Jane is an opera fan and already has a stethoscope and she would rather have a CD. As for Tom, he has written poetry all his life, so a book of poems would be the perfect choice. No doubt you will choose a wheel chair for Helen to sit by the fireplace. Surprise! Helen still enjoys jogging and riding through the countryside. No need for a wheel chair. Buy her a bicycle instead! She'll enjoy it tremendously!

For all the persons here are not usual. How unusual?, you would ask. Well, they do not fit into your idea about their respective age groups. So, tear away the labels you

have attached to them and get the stereotype out of your system! A stereotype usually limits one's perception of things and makes one unaware of the fact that there will never be two identical persons in any of the categories we have become so accustomed to place people in.

There are as many individual persons as the globe population. If you try to look at things this way, you will be a different person yourself! And a better doctor, perhaps.

So, there is no such simple thing as *one object – one sentence* to define it. Look around you at the diversity of things! Look inside you, at the intricate network of tubes and wires – or shall I call them ducts and axons – which could be labeled and defined, as much as human knowledge allows. But, can you label the way you love, or the way you suffer or die? The way you ride a bike at 80 or the way your son is confined to a wheel chair?

Can you – in one sentence – label and define brain death or clinical death? Or life? Or childhood? *Children will be children*, as the saying goes. Wrong! No child is identical to another. Not even twins, though they may look alike. No old man equals another old man! You can stick labels to each age group, but specific individuals in each will contradict you. After all, we are perfect creations, not carbon copies. Even Cc is different from its parent!

After all, labels belong to goods on a shelf with a price attached to, not to people. People are unique and therefore priceless. *The human body is worth one dollar* is one point of view; considering the raw materials – water, air, coal, iron, etc. – it may be true. But considering the price of, let's say, insulin, hemoglobin, or of bradykinin, or the follicle – stimulating hormone or prolactin worth millions of dollars per gram, where are we? And that is only in terms of biochemistry. Think what man can do for himself or for the others? Plant a tree, build a house, write a poem, cure a disease! Or how much it takes one to put all those chemicals into the perfect match which is life? In this case, a human being is priceless.

So, let us give up stereotypes and one sentence definitions and try to look at such things as Life and Death and Old Age from an unbiased, unlabelled point of view. Try to define these concepts – for they are not just words in a dictionary or a text book – from your own point of view. For you are a unique person who looks at things in a unique way. You may be a doctor or a linguist, a painter or a biochemist, a philosopher or a homeless, a geneticist or a taxi driver, you may be young or old, healthy or ill, and everything else.

Revelation

Do you want the change? you will achieve it!
but when? when? when you will die.
you'll understand then that the only change in life
is the passing out of life. life is a word
life is an idea or may be a tear
or may be something else – a feeling, a prayer, a desire
a drop of bitterness that slides on humanity's channel
always happy with the idea of happiness
fighting good, evil or negligence
put to sleep by belief in believing the existence
believing in the existence of faith
desiring the desire
reading death in the eye of life, but seeing life
life? an eternal hoax!!!

Angela Mãdãlina Lazãr
Student, UMP "Carol Davila" Bucharest

What about you? Can you define Life and Death? But Old Age? Don't forget that beyond definitions and stereotypes lies reality itself. Don't be afraid to grasp it and put it into words!

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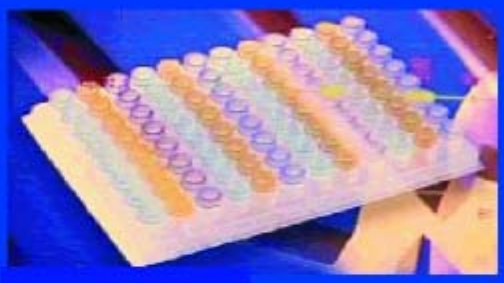

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Pathophysiology of Frontotemporal Dementia - Cytoskeleton Abnormalities and Autoimmunity?

Magnus Sjögren#

Institute of Clinical Neuroscience, Göteborg University, Sahlgrenska University Hospital / Mölndal, Sweden

Abstract

Frontotemporal dementia (FTD) has been estimated to account for between 3 and 10% of all dementia cases. It is characterized by a predominant frontal lobe syndrome including change of personality, disinhibition, mental rigidity and language disturbances. In contrast to patients with Alzheimer's disease, those with FTD initially have intact or only mildly impaired memory and perceptual functions. The differential diagnosis may be difficult. FTD is often first diagnosed as an affective syndrome, psychosis or unspecific dementia. The pathophysiology of FTD is currently unknown, but much speaks in favour of cytoskeleton abnormalities. Tau mutations have been found in hereditary FTD, but in the FTD population in general they are probably infrequent and most cases of FTD seem to be unrelated to them. However, other cytoskeleton abnormalities have been implicated, for instance involvement of the neurofilament proteins. Autoimmunity has been suggested as another possible candidate.

Keywords: dementia; frontotemporal; tau; neurofilament; autoimmunity

Introduction

The concept of frontotemporal dementia

The common denominator of the disorders included in the concept of frontotemporal dementia (FTD) is a predominant frontal lobe syndrome[1]. The most common type of FTD is frontal lobe degeneration of non-Alzheimer type (FLD)[2], followed by Pick's disease (PiD), and amyotrophic lateral sclerosis (ALS) with frontal lobe degeneration of non-Alzheimer type (ALS-FLD)[3]. They are all primary degenerative disorders. Concepts other than FTD have been proposed, for instance frontotemporal lobar degeneration (FTLD)[4] and Pick complex[5], but, so far, these have not been generally accepted. The newer concepts include semantic dementia, corticobasal degeneration, and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17)[6]. The latter is characterized by strong familial inheritance[7]. In most FTDP-17-affected families, mutations in the tau gene in the chromosome 17q21-22 region have been identified[8].

Prevalence

Estimates of the relative prevalence of FTD are based largely on reports with post-mortem data. Some of these reports have estimated the prevalence of PiD among demented patients at approximately 10% (note that the authors regarded all cases of FTD as PiD)[9] and that of FTD at approximately 9%[10]. Others have provided lower figures. One post-mortem study found that an FTD-like disorder (dementia lacking distinctive histological features) had a prevalence of about 3% among demented patients[11]. A community-based clinical study in a diagnostic unit for evaluation of suspected dementia found the prevalence of FTD to be 3.2% among the remitted patients[12]. No genuine epidemiological population-based study has been performed to estimate the prevalence of FTD among the general population. However, in the Netherlands, a nation-wide, telephone-based screening study estimated the prevalence of FTD at approximately 3.4 per million in the age group 40 to 50 years, 10.7 per million in the age group 50 to 60 years, and 28 per million in the age group 60 to 70 years[13]. Several researchers consider FTD as one of the most common types of dementia[2,14,15]. It has long been regarded as a presenile disorder, but a recent epidemiological study found a prevalence of approximately 3% among 85-year olds[16]. Thus, FTD also seems to affect older individuals.

Clinical features

In general, FTD presents with a personality including social misconduct paralleled with a deficit in frontal lobe cognitive functioning[1], i.e., the features of an emerging

Correspondence: Magnus Sjögren, MD, PhD, Institute of Clinical Neuroscience, Psychiatry Section, SE 431 80 Mölndal, Sweden, +46 31 343 2382 (Phone), +46 31 776 9055 (Fax), magnus.sjogren@medfak.gu.se

frontal lobe syndrome. The change in personality does not seem to be an aggravation of the premorbid personality[17] but rather a symptom of a disease. At least initially, other cognitive functions, such as memory and perception, are usually intact or only mildly impaired[18]. However, there is a great variation in the symptomatology of FTD and memory disturbances may sometimes appear early in the course of the disease[19]. Cognitive disturbances that are characteristic of Alzheimer's disease (AD), such as visuospatial difficulties, sensory dysphasia and paraphasia, are usually mild in both the initial and the middle stages of FTD. They may aggravate with the progression of the disease, but frontal features usually predominate even in later stages.

The onset of FTD is insidious and occurs most often in the age span of 45 to 65, that is, in the presenile period[20]. Early features include loss of social awareness, loss of sense of what is proper and often also escalating impulsiveness[10]. Partial or total loss of insight (anosognosia) and loss of mental flexibility are other early features. Another is a successively increasing concrete way of thinking and acting in social contexts i.e. dynamics of social interaction[20]. Patients with FTD are often unable to adapt to the requirements of new situations but manage routine daily activities, such as dressing, finding the way and shopping.

Language disabilities with characteristic economy of speech[21], dysnomia and loss of generative capacity[22] are also common in FTD. These symptoms often gradually evolve into mutism][19]. Some FTD patients are restless and seem to have unimagined powers to drive themselves into repeated misfortunes[10]. Others lack motivation and are most often inactive[1]. Some FTD patients seem to have a mix of these two putative subtypes, with alternating periods of restlessness and inertia.

Emotional bluntness with a characteristic loss of dynamics in affect presentation is frequent in FTD[18]. FTD patients are often described as emotionally cold[18], and an early feature is loss of interest in one's family[10,19]. Emotional unconcern and loss of empathy are also often reported[10,23]. Depressive symptoms may appear and sometimes anxiety too[10], but these features are less common in FTD than in AD[24]. The anxiety in patients with FTD seems often unrelated to the real circumstances. All these changes in affect are more common in the early stages of the disease and are gradually replaced by shallowness of affect[25].

Different types of repetitive and stereotyped behaviour are also seen in FTD patients[26-29]. Some patients present with simple mannerisms or uncomplicated repetitive motor acts, such as hand rubbing or scratching. Others present with more complex acts, such as repeated utterances of parts of sentences or phrases (palilalia), repetitions of another person's verbal expressions (echolalia) or behaviour (echopraxia), repeated singing of the same song, wandering along a fixed path repeatedly, or repeated hand-clapping of a favourite rhythm[1]. Another example is utilization behaviour[30]. This implies that the patient feels forced to elicit the action associated with an object that is presented to him or her, even if the action is inappropriate in the context. For example, when given a pen, the patient starts writing on the table or another object in the near.

Disturbed eating behaviour and changes in appetite and food preference[31] are sometimes found in FTD. Increased craving is sometimes manifested as abuse, for example excessive alcohol drinking or cigarette smoking[31,32]. A sweet food preference has been reported[18] but also intake of non-food substances like dust or clay.

The knowledge of the clinical course of FTD that we have today is based on experience obtained by thorough and repeated clinical observations, often made on post-mortem verified cases of FTD[10,19]. These reports give no comparative information e.g. with AD, but suggest that the initial clinical symptoms of FTD are the features of a predominant frontal lobe syndrome. Other common initial signs are primitive reflexes. Signs of other concurrent regional brain syndromes, such as parietal symptoms, are seldom present early in the course of the disease but may develop gradually. In the intermediate and late stages, neurological signs, such as extrapyramidal signs, may appear[32]. In the late stages, the FTD patient may become mute and severely apathetic[19]. The frontal syndrome usually remains the most salient throughout the course of the disorder.

Diagnosis

Differential diagnosis of such a heterogeneous disease as FTD is difficult, and it is therefore often misdiagnosed.[19] FTD with early onset is initially often taken for an affective disorder, psychosis, or alcohol abuse. Later, and when the onset is late, FTD is commonly mistaken for AD. When neurological signs prevail, FTD is sometimes mistakenly diagnosed as a neurologic disorder, such as Parkinson's disease or motor neuron disease.

The clinical diagnosis of FTD is based on the core and supportive features and exclusion criteria outlined in the Lund/Manchester consensus statement (Figure 1).[3,4] Thus, in clinical practice, clinical observations and results of status examinations of the patient are combined with neuropsychological test results and information gained from laboratory investigations, structural brain imaging (computed tomography [CT] or magnetic resonance imaging [MRI]), functional brain imaging (e.g. regional cerebral blood flow measurements by single photon emission tomography [rCBF-SPET]), and neurophysiological investigations of the brain (electroencephalography [EEG]). According to the consensus criteria, the typical FTD patient has a predominant frontal syndrome, normal results of blood laboratory tests, no or only mild signs of vascular dis-

Core diagnostic features

Behavioural disorder:
- insidious onset and slow progression
- early loss of personal awareness (neglect of personal hygiene and grooming)
- early loss of social awareness (lack of tact, misdemeanours such as shoplifting)
- early signs of disinhibition (such as unrestrained sexuality, violent behaviour, inappropriate jocularity, restless pacing)
- mental rigidity and inflexibility
- hyperorality (oral/dietary changes, overeating, food fads, excessive smoking and alcohol consumption, oral exploration of objects)
- stereotyped and perseverative behaviour (wandering, mannerism such as clapping, singing, dancing, ritualistic preoccupations such as hoarding, toileting and dressing)
- utilization behaviour (unrestrained exploration of objects in the environment)
- distractibility, impulsivity, and impersistence
- early loss of insight into the fact that the altered condition is due to a pathological change of own state

Affective symptoms:
- depression, anxiety, excessive sentimentality, suicidal and fixed ideation, delusion (early and evanescent)
- hypochondriasis, bizarre somatic preoccupation (early and evanescent)
- emotional unconcern (emotional indifference and remoteness, lack of empathy and sympathy, apathy)
- amimia (inertia, aspontaniety)

Speech disorder:
- progressive reduction of speech (aspontaniety and economy of utterance)
- stereotypy of speech (repetition of limited repertoire of words, phrases or themes)
- echolalia and perseveration
- late mutism

Spatial orientation and praxis preserved:
- intact abilities to negotiate the environment

Physical signs:
- early primitive reflexes
- early incontinence
- eate akinesia, rigidity, tremor
- eow and labile blood pressure

Investigation:
- normal EEG despite clinically evident dementia
- brain imaging (structural or functional, or both): predominant frontal or anterior abnormality, or both
- neuropsychology (profound failure on “frontal lobe” tests in the absence of severe amnesia, aphasia, or perceptual spatial disorder)

Supportive diagnostic features:
- onset before 65
- positive family history of similar disorder in a first degree relative
- bulbar palsy, muscular weakness and wasting, fasciculations (motor neuron disease)

Figure 1.
Clinical criteria for frontotemporal dementia according to the Lund/Manchester consensus statement (inclusion criteria only).

orders evident from medical history, status examination or structural brain imaging, a normal EEG pattern and a hypofrontotemporal rCBF pattern. Discriminating different types of FTD is difficult in clinical practice, but language disturbances that progress into severe dysphasia before the onset of behaviour disturbances suggest that the patient is suffering from progressive non-fluent aphasia. Early presence of motor neuron signs indicates FLD-ALS.

One study evaluated the clinical part of the consensus criteria after diagnosing FTD and AD by means of rCBF-SPET alone[33]. This study found that loss of personal awareness, hyperorality, stereotyped and perseverative behaviour, progressive reduction of speech and preserved spatial orientation clearly differentiated FTD and AD patients, whereas clinical variables related to affect and physical findings did not[33]. Other diagnostic measures, such as screening laboratory tests, have not yet been evaluated as diagnostic markers but are commonly used to exclude secondary causes of dementia, for instance hypothyreosis. A hypofrontal rCBF pattern has high sensitivity but low specificity for FTD[34]. Frontal and anterior temporal atrophy may be seen on CT or MRI of the brain in FTD, but there is often generalized atrophy and occasionally even a nearly normal brain image[35]. A normal EEG pattern is usually found in the initial stage of FTD[36,37], but it is also often normal in the initial stage of AD[36,38]. The use of clinical observation, rCBF measurements and EEG in combination has been suggested to increase the precision of the FTD diagnosis[38].

In search of distinctive disease markers of FTD, CSF levels of various structural proteins have been analysed. Analysis of structural proteins that reflect the pathological changes in the brain may enable monitoring of disease progression and the effects of various disease-modifying drugs. The extent of degenerated neurons and their synapses and the number of Pick bodies, neuritic plaques, and neurofibrillary tangles can be compared in patients with FTD and non-demented individuals of similar age. Since the extracellular space in the brain is contiguous with the cerebrospinal canal, the CSF levels of structural proteins may reflect biochemical changes in the brain. For AD, several such markers have been suggested, e.g. CSF-tau, which probably reflects axonal degeneration, and CSF-β-amyloid[1-42], which probably reflects the clearance of this peptide. Studies of patients with FTD have revealed that the CSF-tau levels are usually normal or slightly increased[39,40,41] and the Aβ42 levels are normal or slightly decreased in these patients[41]. An analysis of tau phosphorylated at threonine 181 showed that the CSF levels of this type of tau were normal or slightly decreased in FTD[42]. The light form of neurofilament protein (NFL) has been shown to be increased in FTD[43]. Moreover, a positive correlation has been found between the degree of cognitive impairment and CSF-NFL in FTD, which suggests a pathophysiological relation[43]. These suggested CSF markers may be useful in distinguishing FTD from AD.

Risk factors
Apolipoprotein E (ApoE)

The inheritance of one or two ε4 alleles of ApoE has been found to be associated with a dose-related higher risk of developing AD, especially sporadic or late onset AD, and of having the onset of AD at an earlier age[44,45]. In FTD, only a few similar investigations have been performed, some showing an increase in the ε4 allele frequency in PiD[46,47], others showing a normal frequency in FTD[48-50]. Possibly, both AD and FTD are associated with an increased frequency of the ε4 allele[51]. Some investigators have suggested that homozygosity for ε4 increases the risk for FTD[52] and that inheritance of ε4 lowers the age at onset of FTD in a dose-dependent manner[46]. One investigation found no influence on age at onset for the ApoE genotype in FTDP-17[53].

One study, which investigated the immunoreactivity of ApoE, found that a monoclonal antibody against ApoE binds to neurons and Pick bodies[46]. This suggests that ApoE may be involved in the pathogenesis of FTD through interactions with the neuronal cytoskeleton[46]. As a putative illustration of this, one study found that ApoE3 and ApoE4 (two forms of ApoE) interact with tau and tubulin but not with the intermediate form of neurofilament protein (NFM)[54]. This notion also gains support from a study that found a high ε4 allele frequency together with increased CSF-tau levels[55].

Pathophysiology

For the majority of FTD cases, the pathophysiology of FTD is unknown. Several mechanisms have been proposed, the most widely accepted being a mutation in the tau gene. The first report that eventually led to the description of mutations in the tau gene was published in 1994 by Wilhelmsen and colleagues, who had found a linkage for a FTD-like disorder with a 2-centimorgan region on chromosome 17q21.11[56]. Following this report, several other groups described a similar linkage for disorders that had the clinical features of FTD and a strong heredity but also prominent parkinsonism (FTDP-17)[57-59]. In 1998, Hutton and colleagues described 13 families with FTDP-17, for which they sequenced tau and identified three missense mutations (G272V, P301L and R406W) and three mutations in the 5' splice site of exon 10 of this region[8]. They found that the latter mutations all destabilized a potential stem-loop structure, which is probably involved in the regulation of the alternative splicing of exon 10 in the tau gene. The

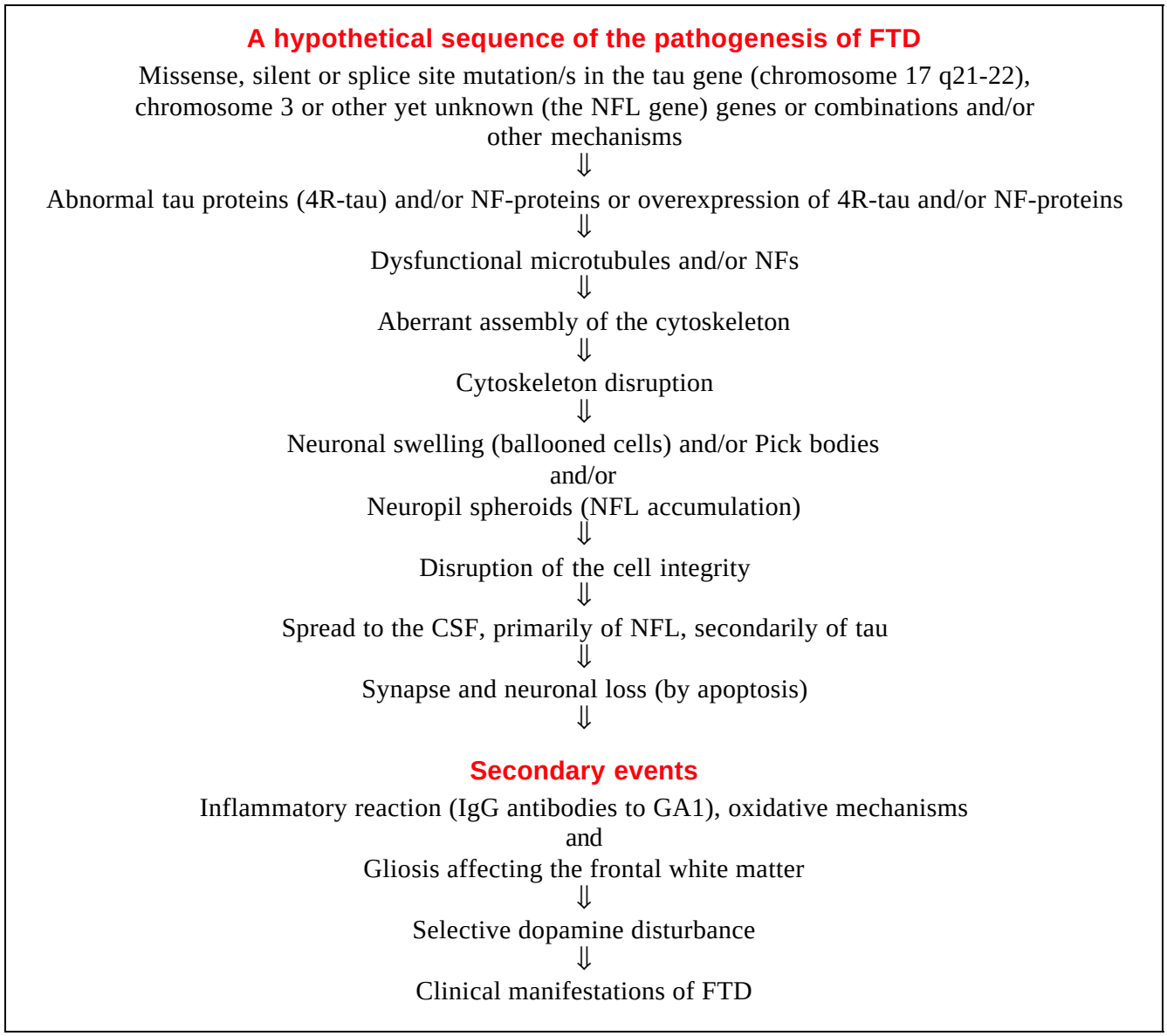

Figure 2.
The cascade of events leading to the clinical manifestations of FTD - a working hypothesis.

result of this mutation is that the 5' splice site is more frequently used and, thus, that an increased proportion of tau that includes exon 10 is transcribed. Increased transcription of exon 10 leads to an increase in the proportion of tau that contains 4 microtubule-binding repeats (4R-tau) and corresponds to the neuropathological changes found in several families with FTDP-17[8]. An increase in 4R-tau may lead to axonal disruption and amyotrophy[60]. Overexpression of exon 10 leads to a reduced ability of tau to promote microtubule assembly[61], which may result in microtubule disruption and loss of cell organisation[62]. Today, over 20 different mutations in the tau gene have been reported in FTDP17 cases and most of these mutations cause an increase in 4R-tau.

However, the prevalence of tau gene mutations is low in the FTD population[55] and, even in the FTDP-17 population, there may be other causes for the development of FTD[61]. There are several reports of FTD without tau pathology[63-65]. Some investigators have even described FTD cases that were devoid of tau in the brain[66]. This does not rule out tau involvement in the pathophysiology of FTD, but it seems probable that other factors have significant roles.

Another hypothesis is that other cytoskeleton proteins are involved in the pathophysiology of FTD[67]. Antibodies to the heavy form of neurofilament protein

(NFH), but also antibodies to tau, have been found to stain neuronal swellings in the neuropil in FTD [68]. Furthermore, ballooned cells (or Pick cells), which are found in PiD[69], have been suggested to be a product of defective axoplasmic transport of neurofilaments[70]. In one study, the ballooned cells contained phosphorylated epitopes that were immunoreactive to antibodies against the neurofilaments but not against neurofibrillary tangles or Pick bodies[70]. Another study found that the ballooned cells also contained alpha B crystallin, a protein which, according to the investigators, was probably involved in the aggregation and remodelling of neurofilaments[69]. However, various cytoskeleton proteins interact and several of these may be involved in the pathophysiology of FTD simultaneously. This notion is supported by findings in studies on FTDP17 transgenic mice that naturally develop tau filaments. When the neurofilament gene (NFL) was knocked out, tau filament formation was inhibited. Thus, the presence of neurofilaments seems to be necessary for tau filaments to develop[71]. The finding that CSF-NFL is correlated to cognitive function in FTD patients may reflect a similar process, even though the patients studied had no tau mutations[43].

A third hypothesis is that autoimmune mechanisms are involved. Several studies[72-74] have found evidence of an ongoing inflammatory process or signs of autoimmunity in AD. Similar processes have only tentatively been suggested to occur in FTD[75]. However, increased serum levels of antibodies against gangliosides have been found in FTD patients[67]. This suggests that autoimmunity may be implicated in the pathophysiology of FTD either as a primary event or secondary to cytoskeleton changes (Figure 2).

Treatment

There are few case reports on FTD patients and only open non-randomized studies including small numbers of FTD patients have hitherto been performed. It is therefore difficult to present general guidelines for the treatment of FTD patients.

Constantinidis and colleagues hypothesized that an excess of zinc, generated by defects in the transport by plasma proteins, accumulates in the brains of patients with FTD. This causes neuronal lesions either by the direct action of zinc itself or by the action of zinc-metalloenzymes, particularly glutamate dehydrogenase[76]. Based on this hypothesis Richards and Constantinidis[77] treated FTD patients with a heavy metal chelator. The patients showed clinical improvement in the following areas: attention, contact, collaboration, initiative, communication, verbal fluency, and comprehension. A decrease in perseveration, echolalia and verbal stereotypes was also observed. In several patients, improvements in EEG pattern was observed.

Cummings and Duchen reported on 5 cases of FTD with pronounced Kluver-Bucy syndromes. In some of the patients, antidepressants, as well as electroconvulsive treatment and L-dopa, were tried, but there was no positive effect[27]. However, Swartz and colleagues tried the antidepressant trazodone in patients with FTD in an open study[78] and found that it effectively reduced behavioural symptoms in some of the patients. Furthermore, Anderson and colleagues reported on 2 cases of FTD with behavioral disturbances and signs of depression. Treatment with lithium and a selective serotonin reuptake inhibitor in combination resulted in improvements regarding behavioural disturbances and depressive signs. The effect lasted at least one year[79].

Coull and colleagues investigated the effect of the α-2-antagonist idazoxan on cognitive function in 3 patients with FTD[80]. They reported that idazoxan (IDZ) produced dose-dependent improvement in tests of planning, sustained attention, verbal fluency and episodic memory. IDZ also seemed to produce deficits in spatial working memory.

Treatment with choline esterase inhibitors (ChEI) has been tried in patients with FTD, but reports and clinical trials are lacking. As the cholinergic system in the brains of FTD patients most likely is normal or only slightly changed[81] and the symptomatology is very different from that in AD patients, ChEI will probably only have a small effect on the cognitive changes seen in FTD patients. However, when there are overlapping symptomatologic features, ChEI may possibly increase cognitive functions in FTD patients too.

Conclusion

FTD is one of the most common dementia disorders, but the prevalence estimates are uncertain. The reason is that diagnosis is difficult because of the clinical heterogeneity of FTD. Diagnostic accuracy can be improved by using thorough clinical status examinations and interviews with the patients and their spouses and by using brain imaging and CSF analysis in concert. Putative pathophysiological mechanisms include tau gene mutations with the development of tau pathology, changes in other cytoskeleton components, and autoimmunity. Curative agents are not available but symptomatic treatment with antidepressant and neuroleptic drugs may reduce behavioural symptoms.

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The Stress-Activated C-jun Amino-Terminal Kinase (JNK) in Alzheimer's Disease Neurofibrillary Degeneration

Jin-Jing Pei#, Wen-Lin An

Karolinska Institutet, Neurotec, Section for Experimental Geriatrics, Huddinge, Sweden

ABSTRACT

The principal protein component of paired helical filaments (PHFs) in Alzheimer's disease is the abnormally hyperphosphorylated tau (PHF-tau). The stress activated C-jun amino-terminal kinase (JNK) has been shown to phosphorylate tau at Ser202/Thr205 and Ser396 seen in PHF-tau. If JNK is involved in the abnormal hyperphosphorylation of tau, they should be activated in neurons undergoing neurofibrillary degeneration. Previously, we found that neurons with tangle-like inclusions positive for active form of JNK appear first in the Pre-* layer of the entorhinal cortex, and then extend into other brain regions co-incident with the progressive sequence of neurofibrillary changes. In the present study, the intraneuronal accumulation of active form of JNK appeared to precede the deposition of PHF-tau in cytoplasm.The level of active JNK was found to be significantly correlated with that of PHF-tau phosphorylated at Ser202/Thr205 (AT8) but not at Ser396 (PHF-1). The data indicate that Ser202/Thr205 is a relatively favourable epitope phosphorylated by JNK in AD brain as compared to Ser396. The increased activation of the stress activated JNK that occurs very early in the disease might be involved in the intraneuronal protein phosphorylation/dephosphorylation imbalance and lead to neurofibrillary degeneration in Alzheimer's disease.

Keywords: Alzheimer's disease; JNK; PHF-tau; Neurofibrillary tangle

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by senile plaques and neurofibrillary tangles (NFTs)[1]. A common intraneuronal feature of AD is the accumulation of the abnormally hyperphosphorylated tau, that aggregates to paired helical filaments (PHF), the principal component of NFTs[2-5]. The accumulation of PHF-tau has been hypothesized to be the result of an altered tau protein kinase / phosphatase balance[3,6] with the equilibrium shifted towards phosphorylation in AD brain.

The stress-activated C-jun amino-terminal kinase (JNK) regulates cellular proliferation, apoptosis, and tissue morphogenesis. JNK is activated upon dual phosphorylation on motif Thr^{183}-Pro^{184}-Tyr^{185} in response to various stressors that prevent cells from relaxing. Consequently, activated JNK phosphorylates and activates several transcription factors, c-Jun, activating transcription factors (ATF2), and E1k[7]. In AD, the JNK pathway is activated by oxidative stress and involved in β-amyloid induced neuronal apoptosis[8-10]. JNK is considered to contribute to PHF-tau formation, because it could phosphorylate tau at Thr181, Ser202/Thr205, Thr231, Ser396, and Ser422 epitopes[11]. However, whether or not β-amyloid induces JNK activation and is involved in tau hyperphosphorylation are not yet well defined. The role of JNK in tau phosphorylation is not likely a sequential event of β-amyloid induced apoptosis, since tau becomes progressively dephosphorylated and proteolytically cleaved during apoptosis execution phase[12-14].

A monoclonal antibody (mAb) AT8 that recognizes a phosphate-dependent epitope of tau at Ser202 /Thr205 has been used for detecting neurons vulnerable to neurofibrillary degeneration (the pretangle neurons) prior to the appearance of NFTs[15]. The neurons with classic tangles could be stained with mAb PHF-1 that recognizes a phosphate-dependent epitope of tau at Ser396/404[16]. Most sites recognized by AT8 and PHF-1, such as Ser202 /Thr205 and

Correspondence: Jin-Jing Pei, MD, PhD, Karolinska Institutet, NEUROTEC, Section for Geriatric Medicine, KFC Plan 4, Novum, S-141 86, Huddinge, Sweden
46-8-58583787 (Phone), 46-8-58583880 (Fax), e-mail: Jin-Jing.Pei@neurotec.ki.se

Ser396 were phosphorylated by JNK *in vitro*[11]. Although active JNK is preferentially localized in NFT-bearing neurons[17], the distribution of active JNK in pretangle neurons and neurons with early or late stage NFTs remains unknown. Thus, using mAb AT8, PHF-1, and polyclonal antibody to active JNK, the intraneuronal relationship between JNK activity and PHF-tau level was analysed. The immunofluorescent data showed that active JNK was accumulated in pretangle neurons distinguished by AT8 and active JNK. The ELISA data showed that the level of active JNK was significantly correlated with AT8-reactive PHF-tau.

Materials and methods

Antibodies

An affinity-purified rabbit antibody to active JNK (phospho JNK) was purchased from New England Biolabs Inc., (Beverly, MA). According to the supplier, phospho-JNK antibody detects the phosphorylated Thr183 and Tyr185 of the active form of p54/p46 JNK, but does not cross-react with either activated ERK1/2 or p38 kinase. The mouse monoclonal antibody (mAb) AT8 to phosphorylated Ser202/Thr205 of PHF-tau was obtained from Innogenetics (anti-human PHF-tau, Zwujndrecht, Belgium). MAb PHF-1 to phosphorylated Ser395/404 was a gift from Dr Peter Davies, Albert Einstein College of Medicine (Bronx, NY) and mAb Tau-1 to non-phosphorylated Ser198/199/202/Thr205 from Dr L Binder, North Western University (Chicago, Illinois).

Double immunofluorescent confocal microscopy

Tissue blocks from 16 cases were obtained at routine autopsy (Table 1). The neurofibrillary degeneration of all cases was staged according to the protocol previously described by Braak and Braak[18]. Blocks of temporal lobe including the entorhinal and temporal cortices, hippocampal formation and / or amygdala were fixed by immersion in a mixture of 4% paraformaldehyde and picric acid, pH 7.0, subsequently kept frozen until use. Tissues were sectioned at 50-100 µm.

Double immunofluorescent staining of floating sections was carried out using CY^{TM}3-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) to stain bound antibody to active JNK (1:100), and CY^{TM}2-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) to stain bound mAb AT8 (1:250). A BioRad Laser Scanning Confocal Imaging System (Radiance Plus) was used to determine co-localization of the CY^{TM}3 (red) labelled active JNK to CY^{TM}2 (green) labelled AT8/PHF-tau. The system is equipped with a Nikon Eclipse inverted microscope (TE300). An argon ion laser that excites at 488 nm with a dichroic beamsplitter 560DCLP and a bandpass filter HQ515/30 was used to detect CY^{TM}2 (green) labelled AT8/PHF-tau. A HeNe laser that excites at 543 nm with E570LP emission filter was used to measure active JNK labelled by CY^{TM}3 (red). Laser light illuminates a Nikon 60× /1.4 NA oil immersion objective. Images scanned on the two channels (red and green) were merged to produce a single profile. Fluorescence images were collected at 2.5x zoom using the BioRad Lasersharp Software Package, and processed using Adobe Photoshop 5.0.

Homogenate preparation and protein measurement

For homogenate preparation, tissue blocks of the medial temporal cortex from 13 control and 22 AD brains were used (Table 2). Grey matter was separated from white matter and homogenized in 10 volumes (ml /g wet tissue) of protease inhibitor cocktail buffer (Sigma, St. Louis, MO) over ice. Protein concentration was measured by Bradford[19], then equalized to 1 mg/ml with protease inhibitor cocktail buffer, and stored at -70°C.

Western blotting

After boiling homogenates for 4 minutes, 20 µg homogenates were loaded and run on 10% SDS-PAGE for about 45 minutes. Proteins were then transferred to nitrocellulose transfer membranes and probed with primary anti-

Table 1. Detailed information of cases used for double immunofluorescent staining.

No.	Sex	Age	Amyloid Deposits	NF Changes
1	F	50	0	0
2	M	55	0	0
3	M	62	0	0
4	M	67	0	0
5	F	53	0	I
6	F	68	0	I-II
7	M	70	0	I-II
8	F	66	0	II
9	F	86	0	II
10	F	86	0	III
11	F	93	0	IV
12	M	85	A	IV
13	F	60	C	V
14	M	75	C	V
15	?	91	C	V
16	?	?	C	V

body to active JNK (1:200). After blocking with 5% (w/v) non-fat milk solution, active JNK was detected using horseradish peroxidase linked anti-rabbit IgG (Amersham Life Science) at 1:2000, and detected by the ECL kit (Amersham Pharmacia Biotech, England).

Indirect enzyme-linked immunosorbent assay (ELISA)

The levels of active JNK and tau were measured by ELISA in homogenates of 22 AD and 13 control cases. Microtiter plates (Nunc-Immuno™, Denmark) were coated in duplicates with 2µg protein/well in 20 mM Tris-HCl, pH7.4, buffered with 2.5 mM EDTA, 2.5 mM EGTA, 0.1% (v/v) protease inhibitor cocktail (Sigma, St. Louis, MO), 0.1% sodium azide, overnight at 4°C. Unspecific protein binding sites were blocked with 3% BSA in 10 mM Tris-HCl buffer with 0.85% NaCl and 0.2% Tween-20 for 1 hour at room temperature. The plates were incubated with rabbit antibody to active JNK (1:200), mAbs Tau-1 (1:40,000), AT8 (1:500) and PHF-1 (1:200) at room temperature for 2 hours. Bound rabbit or mouse antibodies were detected with peroxidase-conjugated anti-rabbit or anti-mouse IgG (1:100-1:2000, Jackson ImmunoResearch laboratories Inc., West Grove, PA), followed by reaction for 10-30 minutes with tetramethylbenzidine (TMB) substrate solution (SMS-gruppen, Denmark). After stopping reactions with 100 µl /well 1M phosphoric acid, absorbances were measured at 450 nm using a Spectra Max 250 ELISA reader (Molecular Devices, Sunnyvale, CA).

Statistical analysis

Levels of active JNK and tau were compared in AD and control homogenates with Student's independent t-test. The Pearson simple correlation analysis was used to estimate the relationship between active JNK and tau level.

Results

Localization of active form of JNK in neurons with different extents of PHF-tau / neurofibrillary changes

We first ran immunoblot with samples from 4 AD and 4 control cases, and found that antibody to active JNK reactes with 54/46 isoforms of JNK, as shown in Figure 1.

In our previous study, we showed that the distribution of active JNK tangle-like neurons coincided with the predictable progressive sequence of neurofibrillary degeneration[17,18]. In order to understand the extent of active JNK involvement in PHF-tau formation, intraneuronal correlation between active JNK and AT8 labelled PHF-tau was analysed in CA1 pyramidal neurons of brains staged at III, IV, and V neurofibrillary degeneration[18] using confocal microscope. Four patterns of neurons could be distinguished.

Table 2. Detailed information of cases used for Western blotting and ELISA analysis.

Code	Sex	Age	Diagnosis	Post-mortem delay (hrs)
1	M	67	Control	2
2	M	75	Control	10
3	F	84	Control	16
4	M	79	Control	17
5	M	82	Control	22
6	M	76	Control	3
7	M	84	Control	7
8	M	83	Control	8
9	F	98	Control	9
10	F	96	Control	10
11	M	79	Control	5
12	F	84	Control	7
13	F	82	Control	4
14	F	90	AD	10
15	M	86	AD	7
16	M	71	AD	7
17	M	88	AD	7
18	F	73	AD	7
19	M	82	AD	6
20	F	100	AD	6
21	F	92	AD	6
22	F	78	AD	6
23	F	97	AD	9
24	F	91	AD	4
25	F	84	AD	4
26	F	87	AD	5
27	F	54	AD	3
28	F	76	AD	6
29	F	82	AD	7
30	F	74	AD	6
31	F	84	AD	7
32	F	84	AD	2
33	F	68	AD	4
34	F	74	AD	4
35	F	86	AD	3

Average age (years): Control, 82.23 ± 8.25, AD, 81.86 ± 10.38 ($p > 0.05$ vs. control). Average post-mortem delay (hours): Control, 9.23 ± 5.9, AD, 5.73 ± 1.96 ($p > 0.05$ vs. control).

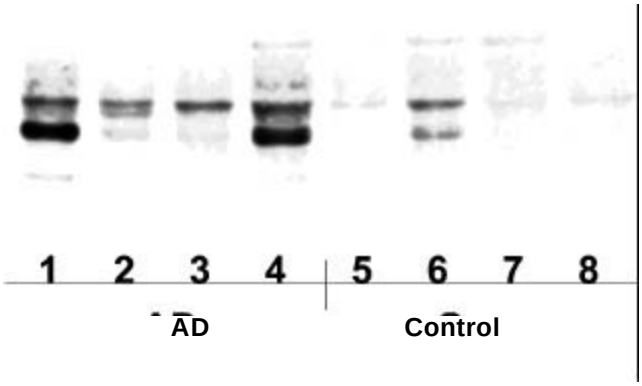

Figure 1.
Immunoblotting of antibody to active JNK in 4 AD and 4 control homogenates. This antibody reacted with two isoforms (54 / 46 kDa) of JNK.

Pattern 1 neurons were without neurofibrillary tangles as determined by AT8 staining, but showed punctuate/granular pattern of labelling with antibody to active JNK (p-JNK) (Figure 2, A1-A3). Around these AT8-immunonegative neurons, neuropil threads were, however, labelled with this antibody. Pattern 2 neurons showed only a small overlap of active JNK with AT8-immunostaining in the non-granular immunostained areas (Figure 2, B1-B3). This is a pretangle-like neuron. Pattern 3 neurons showed most of active JNK (Figure 2, C1) positive structures as filamentous and this staining, and not the granular staining, overlapped with the AT8 staining (Figure 2, C1-C3). This pattern showed an early tangle-like staining. Pattern 4 neurons showed fully mature classical NFT pathology by AT8 immunostaining (Figure 2, D2) and active JNK (Figure 2, D1) immunolabelling that practically overlapped with the AT8 staining (Figure 2, D1-D3).

Levels of active form of JNK and PHF-tau in AD and control brains

Using ELISA, the levels of active JNK, PHF-tau (AT8 and PHF-1), and normal tau (Tau-1) were measured in homogenates from medial temporal cortex of 22 AD and 13 control cases. We found a significant increase of PHF-tau (AT8 and PHF-1, B), a slight increase of active JNK (A), and a slight decrease of normal tau (Tau-1, B) in AD as compared to control (Figure 3).

Correlation of JNK activity with PHF-tau level

The relationship between active JNK and PHF-tau was estimated in AD and control homogenates by Pearson correlation analysis. In AD cases, we found a significant correlation of active JNK with AT8 labelled PHF-tau ($p < 0.01$, Figure 4A). The p value of the correlation between active JNK and PHF-1 labelled PHF-tau is 0.057 (Figure 4B), close to significance. However, active JNK did not show significant correlation with Tau-1 labelled normal tau (Figure 4C). No significant correlation between active JNK and PHF-tau (AT8 and PHF-1), or normal tau (Tau-1) was found in control cases.

Discussion

In our previous study, structures positive for active form of JNK were observed in the earliest to late stages of AD neurofibrillary degeneration in the entorhinal cortex, hippocampus and temporal cortex that corresponds to the different stages of neurofibrillary pathology[17,18]. The present study was concerned whether or not the intraneuronal occurrence of activated JNK correlates to the progressive sequence of AD neurofibrillary pathology proposed by Bancher et al and Braak et al [15,20]. We studied by immunofluorescent confocal microscopy the localization of activated JNK in CA1 pyramidal neurons with different extents of AT8 labelled PHF-tau involvement. By ELISA and simple correlation analysis, the relationship of active JNK level with that of AT8 or PHF-1 labelled PHF-tau was analysed.

We found that four patterns of neurons: normal looking neurons (Pattern 1), pretangle-like neurons (Pattern 2), neurons bearing early stage tangles (Pattern 3) and neurons bearing fully developed tangles (Pattern 4), could be distinguished. The co-labelling of active JNK with NFT is seen in Patterns 3 and 4 neurons. This type of immunostaining which has also been found to be preferentially associated with active but not with inactive GSK-3[21], active MAP kinase[22,23], cdc2[24], cdk5[25,26] and PKA[27] is reminiscent of classical NFTs, as visualized by immunocytochemistry with mAbs Tau-1 or AT8[15,20]. What was most attracting to us is that the neurons prior to developing tangles, in particular the normal looking neurons, accumulate active JNK in various sizes of granular structures that are reminiscent of lysosomes. Studies from Cataldo and colleagues have shown that the lysosomal disturbances develop in AD vulnerable neurons in advance of AD pathologies[28,29]. So far, AT8 is the best antibody to stain early changes of PHF-tau[15]. It is likely that, when the AT8 positive neurofibrillary degeneration is initiated in the distal terminals of neuronal processes[15], the active JNK seems to be sequestered in lysosome-like structures (Pattern 1 neurons). The significance of the con-incidence of these two structures in Pattern 1 neurons is not understood at the current stage. Although JNK is involved in neuronal apoptosis[10], the granules positive for active JNK in Patterns 1 and 2 neurons are not likely the cellular blebbing of the execution phase of apoptosis, due to the fact that tau protein is dephosphorylated and degraded during the phase[12-14]. Taken together, these data suggest that the development of NFT in neurons is progressive, and that the different immunostained structures may represent

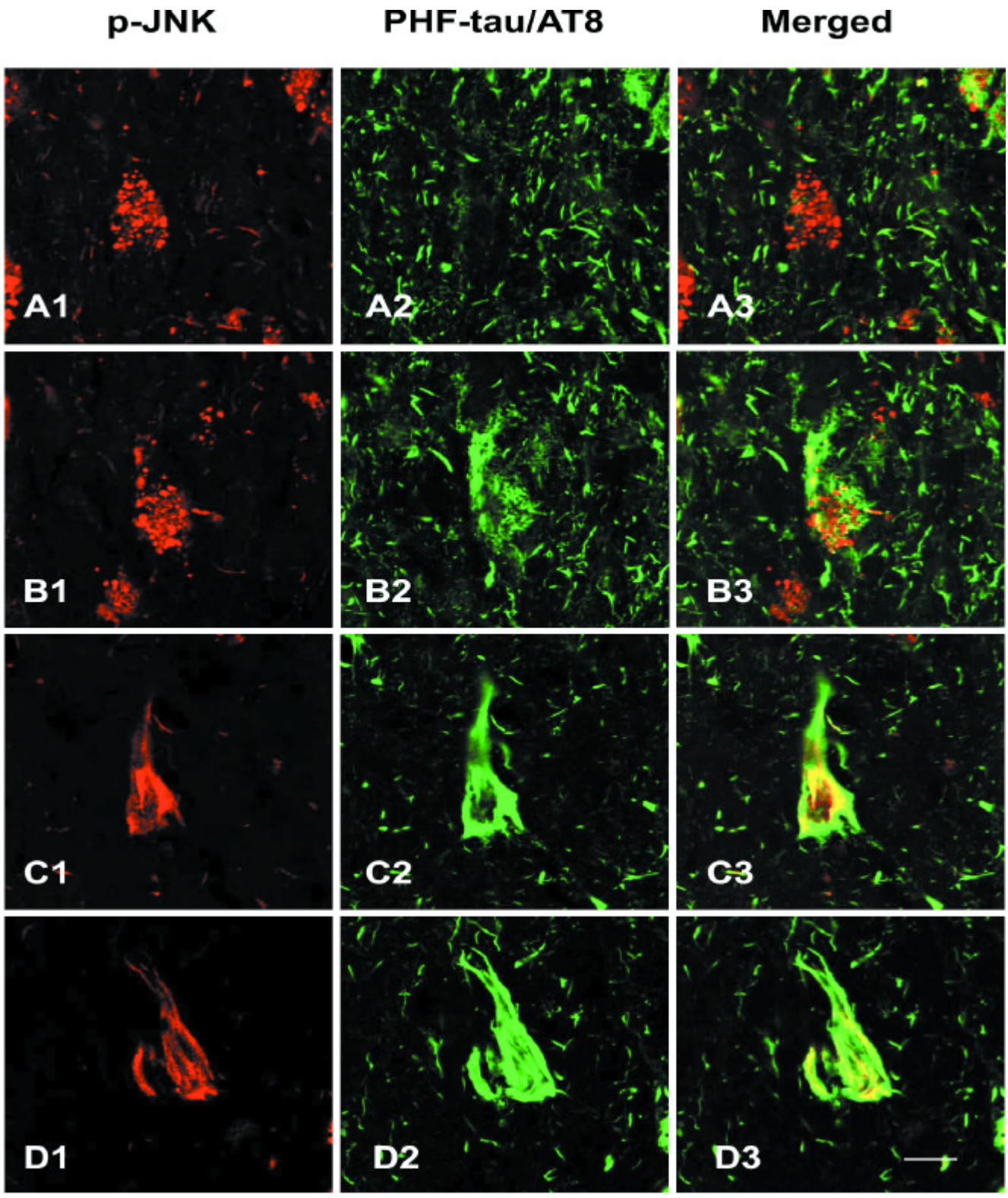

Figure 2.
Demonstration of the intracellular correlation of active JNK (p-JNK) with AT8 labelled PHF-tau in CA1 pyramidal neurons from brains with neurofibrillary stages at III, IV and V with confocal microscope. In the normal looking neurons, only active JNK is positive in granular form (A1, A2 and A3). In the pre-tangle neurons, some of the punctuate stainings of active JNK were partially overlapped with dotted AT8 labelled PHF-tau (B1, B2 and B3). In the neurons bearing early stage tangles, the granular stain of active JNK congregated together and distributed along with the AT8 positive filamentous structures (C1, C2 and C3). At the advanced stage of tangle neurons, active JNK filamentous structures fully overlapped with AT8 positive tangles (D1, D2 and D3). The bar indicates 10μm.

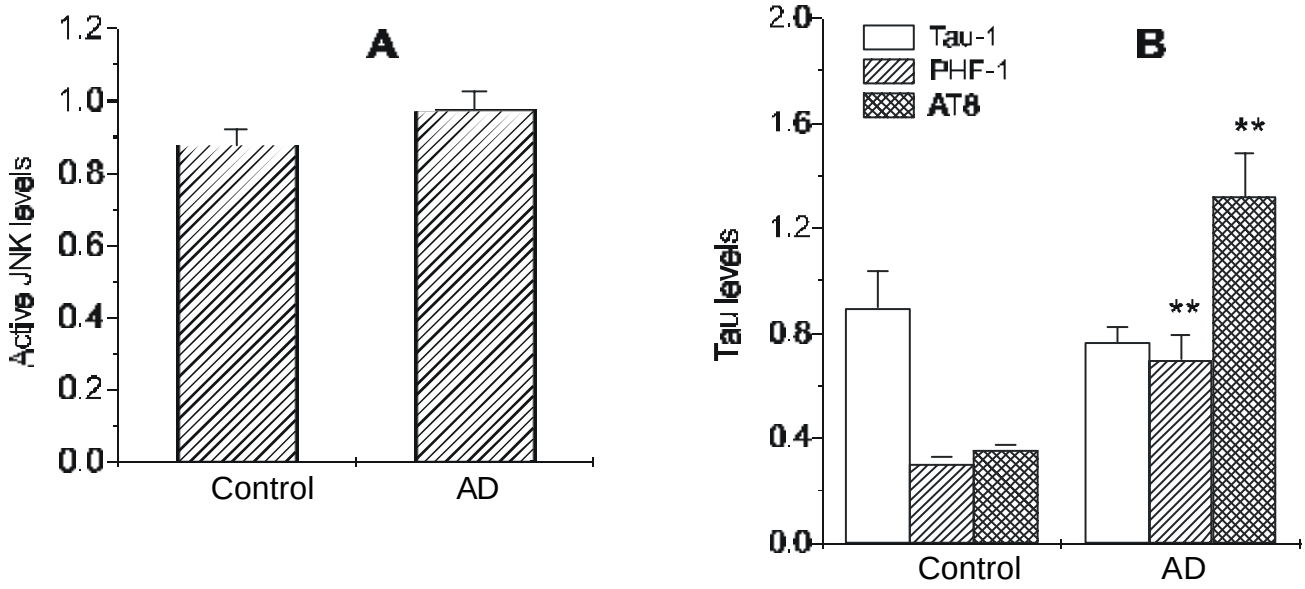

Figure 3.

Levels of active JNK and tau were compared in AD and control (Con) homogenates by Student t-test. A dramatic increase of PHF-tau levels (AT8 and PHF-1) was shown in B, whereas active JNK (A) and normal tau (Tau-1, B) remained unchanged in AD homogenates as compared to control.

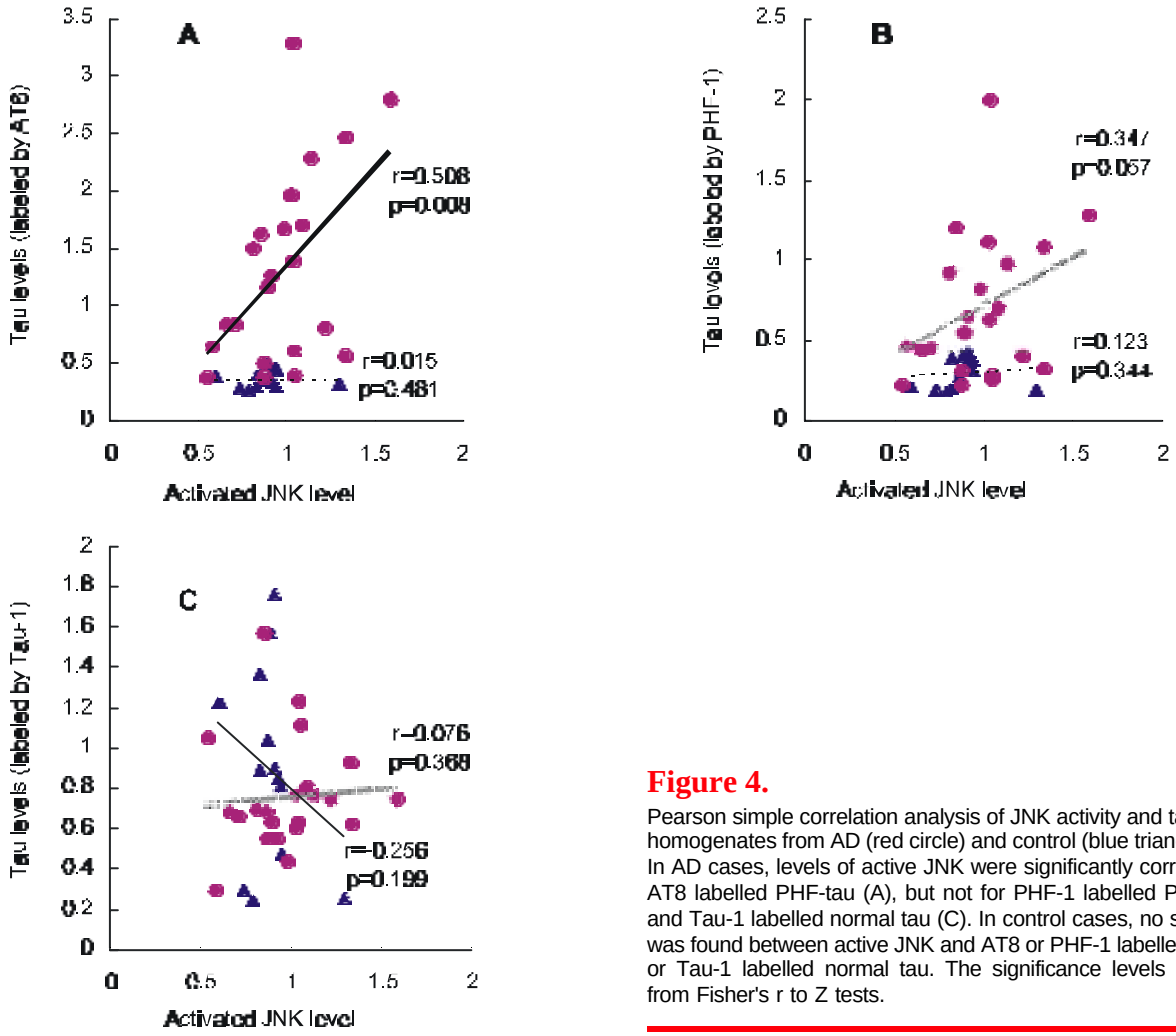

Figure 4.

Pearson simple correlation analysis of JNK activity and tau levels in homogenates from AD (red circle) and control (blue triangle) brains. In AD cases, levels of active JNK were significantly correlated with AT8 labelled PHF-tau (A), but not for PHF-1 labelled PHF-tau (B) and Tau-1 labelled normal tau (C). In control cases, no significance was found between active JNK and AT8 or PHF-1 labelled PHF-tau, or Tau-1 labelled normal tau. The significance levels are shown from Fisher's r to Z tests.

different stages of neurons undergoing neurofibrillary degeneration. Understanding the fact that the Pattern 1 neurons are the most resistant cells left in AD brain to developing tangles is of great interest for further research.

A number of kinases such as glycogen synthase kinase 3 (GSK-3) have been shown to phosphorylate tau at some of the same sites at which PHF tau is phosphorylated[30,31]. The stress-activated JNK also has the ability to phosphorylate tau in vitro at several sites seen in PHF-tau[11]. The significant correlation of active JNK with AT8 but not PHF-1 labelled PHF-tau suggests that Ser202 /Thr205 are more favourable sites than Ser396 in AD brain. It is likely that Ser202 /Thr205 are initially phosphorylated by JNK when cells receive activation of non-relaxing stress factor. However, it appears that only upon sequential phosphorylation with GSK-3 a number of additional sites are phosphorylated and the binding of tau to microtubules and its ability to promote microtubule assembly are inhibited[32,33].

Previously, we have shown that JNK is activated in the brains with neurofibrillary degeneration at stages I, II, and III that are without β-amyloid deposition according to Braak and Braak[17,18]. This suggests that β-amyloid deposition is not necessary for JNK activation and neurofibrillary degeneration. The elevated level of β-amyloid in the brains of individuals with either the sporadic or familial form of AD could act as a stressor to activate the JNK pathway. A major likely causative factor for the activation of JNK and other tau kinases of the AD brain is the reduced activity of both PP-2A/PP1 and tyrosine phosphatases that will keep the tau kinases in phosphorylated/activated state[34,35] and, in the case of PP2A/PP1, also result in hyperphosphorylated tau.

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Alterations of Nitric Oxide Synthase Expression and Activity in Alzheimer's Disease Brains

Andrew Law[1,2], Wen-Hua Zheng[1,3], Serge Gauthier[1,4], Rémi Quirion[1,2,3,4#]

1 Douglas Hospital Research Centre, Verdun, Québec, Canada

2 Department of Neurology and Neurosurgery, McGill University, Montréal, Québec, Canada

3 Department of Pharmacology and Therapeutics, McGill University, Montréal, Québec, Canada

4 McGill Centre for Studies in Aging, Vérdun, Québec, Canada

ABSTRACT

Nitric oxide (NO) is an atypical neurotransmitter that has significant neurophysiological functions, including a role in cognition. NO has also been implicated in neurodegenerative disorder. Alzheimer's disease (AD) is a dementing syndrome with oxidative stress likely being a disease mechanism. This study compared the expression and activity levels of nitric oxide synthase (NOS) in the AD and non-demented post-mortem brains. Our results demonstrated that expression of neuronal NOS (nNOS) was diminished in the AD hippocampus, whereas inducible NOS (iNOS) expressions were higher in the AD frontal cortex and hippocampus. Moreover, in the AD brain, iNOS activity was increased in the hippocampus, and the frontal cortex also exhibited altered NOS activities. Our findings suggest that NO production, especially via iNOS, may be involved in AD pathogenesis.

Keywords: aging, Alzheimer's disease, amyloid, frontal cortex, Griess reaction, hippocampus, inducible nitric oxide synthase, N-iminoethyl-L-lysine, neuronal nitric oxide synthase, nitric oxide, oxidative stress

Introduction

In addition to its vasoactive properties[1], nitric oxide (NO) is also a signal transducing molecule now known to possess neurophysiological roles in the peripheral and central nervous systems, such as being a non-adrenergic non-cholinergic neurotransmitter in the gastrointestinal and urogenital tracts, involved in neurodevelopment, and the apparent retrograde messenger in the formation and maintenance of long term potentiation (LTP) and depression – one of the current electrophysiological models of cognitive functioning[2-5]. However, NO has also been implicated in excitotoxic neuronal death and in a number of neurodegenerative diseases secondary to its free radical properties and concentration-dependent apoptotic effects[6]. NO is produced by three isoforms of nitric oxide synthase (NOS), all of which exist in the brain[7-9]. Previous studies have shown that overproduction of NO by the neuronal (nNOS, type I) or the inducible (iNOS, type II) form of the enzyme is neurotoxic[10-11].

Alzheimer's disease (AD) is a dementing syndrome with cognitive impairment being its primary clinical symptom[12]. AD is histopathologically characterized by extraneuronal amyloid plaque (Aβ) deposition and intraneuronal neurofibrillary tangle (NFT) formation[13,14]. There is increasing evidence to support the relationships between Aβ neurotoxicity, NFT formation, and NO[15,16]. Furthermore, NO appears to be associated with a number of putative mechanisms involved in the pathogenesis of AD, including Ca^{2+} homeostasis disruption, oxidative damage, neuroinflammation, mitochondrial defects, and apoptosis[6].

At present, data regarding the role of NO in the pathogenesis of AD remain controversial[17-20]. The current study describes the possible differences in nNOS and iNOS expressions and enzymatic activities in the hippocampi and frontal cortices of AD and age-matched non-demented control brains.

Correspondence: Dr. Rémi Quirion, Scientific Director, Douglas Hospital Research Centre, 6875 Lasalle Blvd., Verdun, Quebec H4H 1R3, Canada
(514) 762-3048 extension 2934 (Phone), (514) 762-3034 (Fax), e-mail: quirem@douglas.mcgill.ca

Methods

Human brain tissues were obtained from Massachusetts General Hospital, National Neurological Research Specimen Bank of VAMC in Los Angeles, and Kathleen Price Bryan Brain Bank of Duke University (Table 1). All studies performed were in accordance to guidelines on the use of human tissues provided by the Research Ethics Board of Douglas Hospital and McGill University, Montréal, Canada.

Sample preparation

The obtained brain specimens were stored in –80ºC until use. Frontal cortical (Brodmann's areas 8 and 9) and hippocampal tissues were dissected from 7 disease stage-matched (Braak and Braak stage IV-V[21]) Alzheimer's disease and 6 non-demented age-matched control cases. Tissue samples were placed in lysate buffer and homogenized by sonication.

Western blotting

Unless otherwise stated, all materials used were purchased from Sigma, St. Louis, MO. Western blotting was performed as described previously with certain modifications[22]. Briefly, protein concentrations of the homogenized tissue samples were standardized. Samples with equal amounts of protein were placed in buffer (62.5 mM Tris-HCl, pH 6.8, 2% (w/v) sodium dodecyl sulphate, 1% glycerol, 50 mM dithiothreitol, and 0.1% (w/v) bromphenol blue) and were separated by 4-20% polyacrylamide gel electrophoresis, and the resolved proteins were then electrotransferred to Hybond-C nitrocellulose membranes. The membranes were incubated with 5% nonfat milk in TBST (10 mM Tris-HCL, pH 8.0, 150 mM NaCl, and 0.2% Tween 20) for 1 hour at room temperature and then incubated with either nNOS or iNOS primary antibodies (Calbiochem, San Diego, CA) overnight at 4ºC. Membranes were then washed three times with TBST and probed with horseradish peroxidase-conjugated anti-rabbit secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at room temperature for 1 hour. The membranes were subsequently washed six times with TBST to remove unbound secondary antibodies and visualized by the ECL detection kit (Amersham Pharmacia Biotech, Ontario, Canada). The resultant blots were quantified by using the MCID image analyzer system (Image Research Inc., Ontario, Canada).

Pharmacological treatment and NOS assay

N-iminoethyl-L-lysine (L-NIL, an iNOS inhibitor[23]) and all chemicals used for the NOS assay were purchased from Calbiochem (San Diego, CA).

Table 1. Data on Alzheimer's disease (AD) and non-demented elderly control brains used in this study.

Case	Gender	Age	Cause of death*	Length of disease (years)	Post-mortem delay (hours)
AD	female	65	AD	unknown$^{\Psi}$	4
AD	male	68	unknown$^{\Psi}$	5	6
AD	female	69	AD	unknown$^{\Psi}$	10
AD	female	68	AD	1$^{\#}$	8
AD	female	71	pulmonary infection	8	unknown$^{\Psi}$
AD	female	67	unknown$^{\Psi}$	9	11
AD	male	69	AD	7	9
Control	female	67	unknown$^{\Psi}$	n/a	8
Control	male	61	myocardial infarction	n/a	unknown$^{\Psi}$
Control	male	70	automobile accident	n/a	13
Control	male	63	unknown$^{\Psi}$	n/a	7
Control	male	71	colon carcinoma	n/a	6
Control	female	68	cerebrovascular accident	n/a	unknown$^{\Psi}$

***** cause of death refers to diagnosis on death certificate.
# the duration of dementia for this case had not been noted on the patient record, hence the length of disease for this case is based on the time of Alzheimer's disease diagnosis and time of death.
Ψ unknown is assigned when no documentation on relevant information had been recorded.
n/a = non applicable

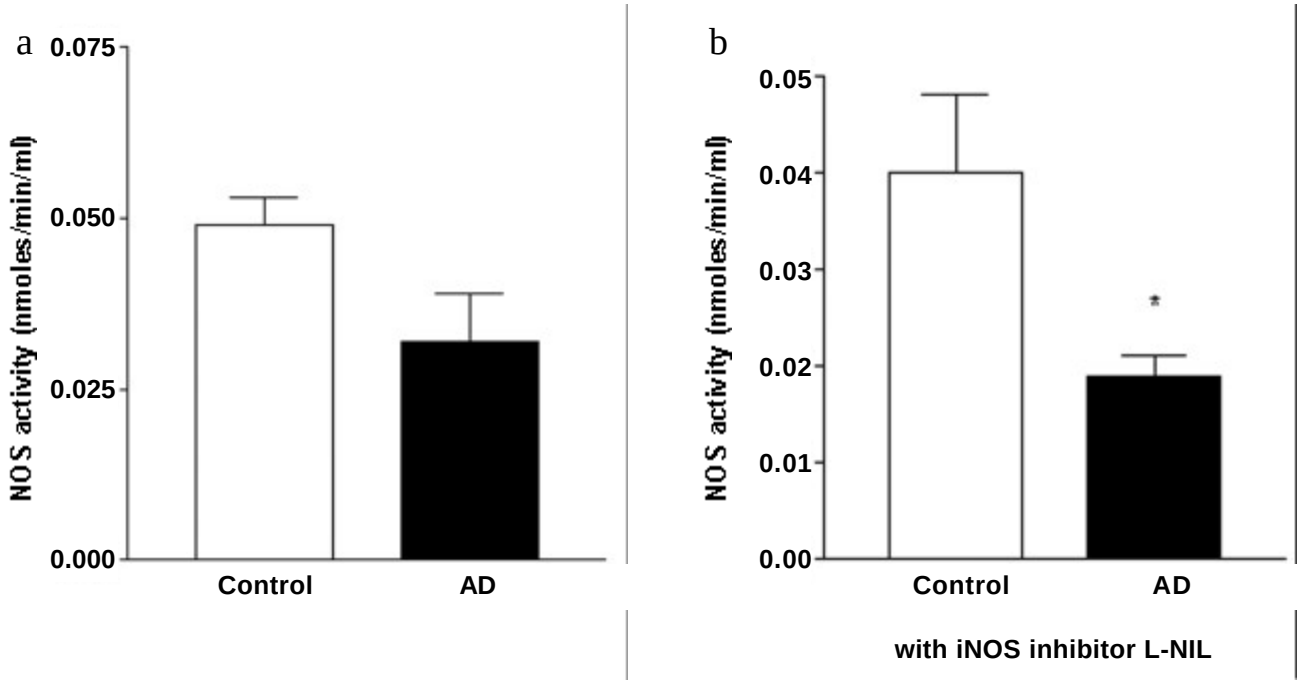

Figure 3.

Hippocampal nitric oxide synthase (NOS) activities in non-demented elderly control and Alzheimer's disease (AD) brains. In samples without N-iminoethyl-L-lysine (L-NIL; iNOS inhibitor) treatment, NOS activities were similar between the control and AD groups **(a)**. In samples treated with L-NIL (50 mM), NOS activity was significantly lower in the AD group **(b)**. All data are presented as mean ± S.E.M. * $p < 0.05$.

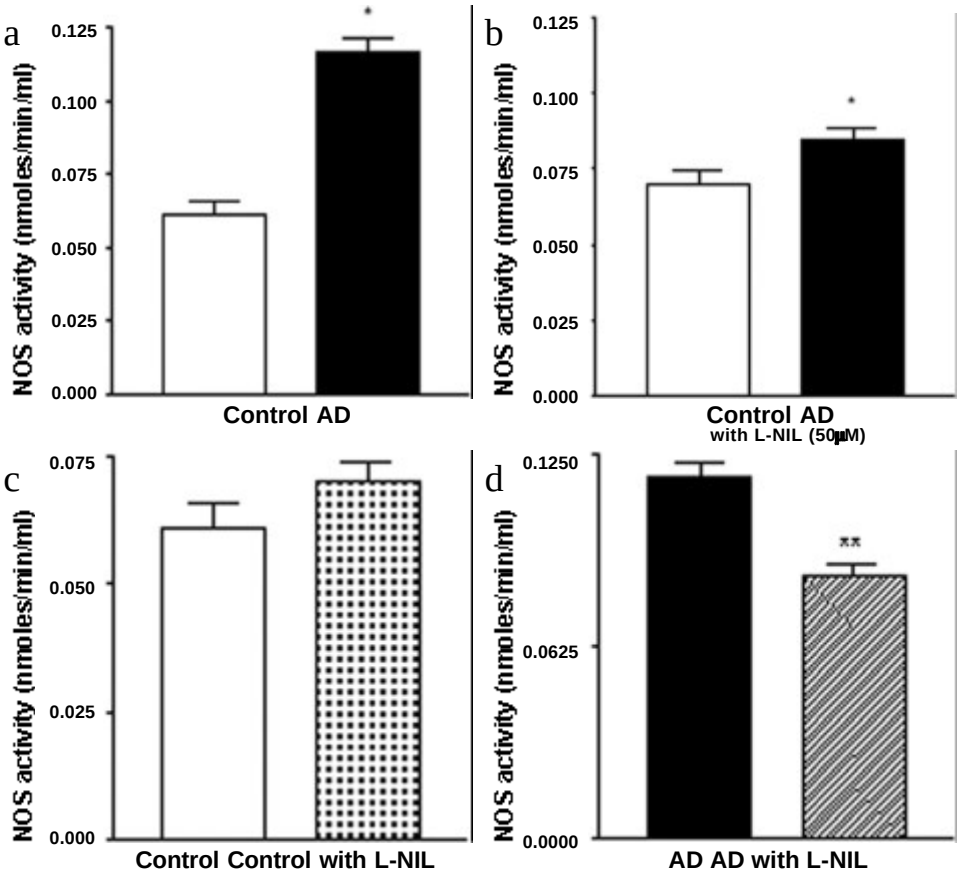

Figure 4.

Cortical nitric oxide synthase (NOS) activity in non-demented control and Alzheimer's disease (AD) brains. In sample without of N-iminoethyl-L-lysine (L-NIL; iNOS inhibitor) treatment, NOS activity was significantly higher in the AD group **(a)**. In samples with L-NIL (50 mM) treatment, NOS activity remained significantly higher in the AD group **(b)**. Comparison between NOS activities for samples with and without L-NIL treatment indicated no significant difference between the control groups **(c)**, whereas AD samples without L-NIL treatment showed significantly higher NOS activity than those with L-NIL treatment **(d)**. All data are presented as mean ± S.E.M. * $p < 0.05$ and ** $p < 0.001$.

Tissue samples were centrifuged at 1400g for 10 minutes and the supernatants were extracted for NOS assaying. NOS activity was inferred by means of NOS colorimetric assay, which measured total nitrite concentration[24]. L-NIL and all chemicals were freshly prepared on the day of the experiment.

L-NIL (50 μM) was added to half of the samples 1 hour prior to the NOS assay. The L-NIL treated samples were kept at 4°C for 45 minutes and at room temperature for the remaining 15 minutes. Triplicates of tissue samples with and without L-NIL treatment, as well as duplicates of nitrate standards were placed in a 96-well microtiter plate with the addition of assay buffer for volume adjustment. Freshly prepared 1 mM NADPH solution and nitrate reductase were then added to each well. These reagents were required for enzyme action and the conversion of nitrate to nitrite, respectively. The samples were then incubated for 1 hour at room temperature, followed by the additions of a cofactor preparation and lactate dehydrogenase solution to all sample wells for excess NADPH removal. Samples were incubated for an additional 20 minutes at room temperature, and Griess reagents were added to all sample wells to convert the nitrite in solution to an azo product with maximum absorbance at a wavelength of 540 nm. The absorbances of samples were quantified at 540 nm by using a micro-plate reader (Bio-Tek Instruments Inc., Ville St-Laurent, Québec). The resultant NOS activity of each sample was then calculated from the nitrate standard curve.

Statistical analysis

Unpaired Student *t* tests and one-way analysis of variance with Bonferroni's multiple comparison tests were used in statistical analysis to compare the obtained quantified data for the Alzheimer's disease and non-demented control groups, with $p < 0.05$ being considered as statistically significant. All data are represented as means ± S.E.M.

Results

The expressions of two NOS isoforms in the hippocampi and frontal cortices of AD and non-demented elderly control brains were examined. NOS activities in the aforementioned brain regions were also measured.

nNOS and iNOS expressions

In the hippocampus, nNOS expressions (Figure 1a) in the AD brains were uniformly minimal, and 4 out of 6 control brains consistently showed high levels of expressions. The basal expressions of iNOS (Figure 1b) in the control brains were insignificant, whereas iNOS expressions in 5 out of 7 AD brains were significantly higher than the control brains. In frontal cortex, similar nNOS expressions (Figure 2a) were observed between the two groups; however, the AD cases uniformly demonstrated significantly higher cortical iNOS expression than the control subjects (Figure 2b).

NOS activities

In the hippocampus (Figure 3a), NOS activities in samples without L-NIL treatments were similar between the AD and control brains (0.032 ± 0.007 nmoles/min/ml for AD cases vs. 0.049 ± 0.004 nmoles/min/ml for controls). In samples treated with L-NIL (Figure 3b), NOS activities in the AD hippocampus were significantly lowered than the control cases (0.019 ± 0.002 nmoles/min/ml for AD cases vs. 0.040 ± 0.008 nmoles/min/ml for controls). In samples without L-NIL treatments (Figure 4a), AD cases showed significantly higher cortical NOS activity (0.12 ± 0.005 nmoles/min/ml) than the controls (0.061 ± 0.005 nmoles/min/ml). In samples with L-NIL treatment (Figure 4b), cortical NOS activity in the AD group remained significantly higher than the control group (0.085 ± 0.007 nmoles/min/ml for AD vs. 0.07 ± 0.004 nmoles/min/ml for controls). The control group showed similar NOS activities regardless of L-NIL treatment (Figure 4c), whereas the AD samples without L-NIL treatment exhibited significantly higher cortical NOS activities than those with the drug treatment (Figure 4d).

Discussion

The present study examined the expressions of hippocampal and cortical NOS isoenzymes between AD and non-demented elderly control brains. Hippocampal and cortical NOS activities were also determined. Our results indicated that, in the hippocampus, nNOS expression was in general higher in the control subjects, whereas iNOS expression was generally higher in the AD brains. In the frontal cortex, no significant difference in nNOS expression between the two groups was observed, but iNOS expressions were significantly higher in the AD brains. NOS activities in the AD and control hippocampi were similar in the absence of L-NIL; however, AD hippocampi showed significantly lower NOS activities in the presence of L-NIL. Cortical NOS activities were significantly higher in the AD cases regardless of the presence or absence of L-NIL treatment.

NO is a signal transducing molecule which is also potentially neurotoxic. For example, nNOS-mediated NO release modulates guanosine 3',5'-cyclic monophosphate signaling pathways through guanylate cyclase activation and participates in LTP formation and maintenance[4,25]. Excessive NO production mediated by nNOS has also been shown to be neurotoxic in cerebral ischemia[11]. Aβ plaque deposition is a hallmark of AD, and this peptide appears to be involved in the pathogenesis of the disease[14]. Previous studies from our laboratory demonstrated that Aβ induced nNOS- and iNOS-mediated NO release in cortical primary cell cultures[26]. These results provided evidence toward putative links between NO production and AD. Histopathological studies have identified the co-localizations of Aβ plaques with reactive astrocytes and microglia,

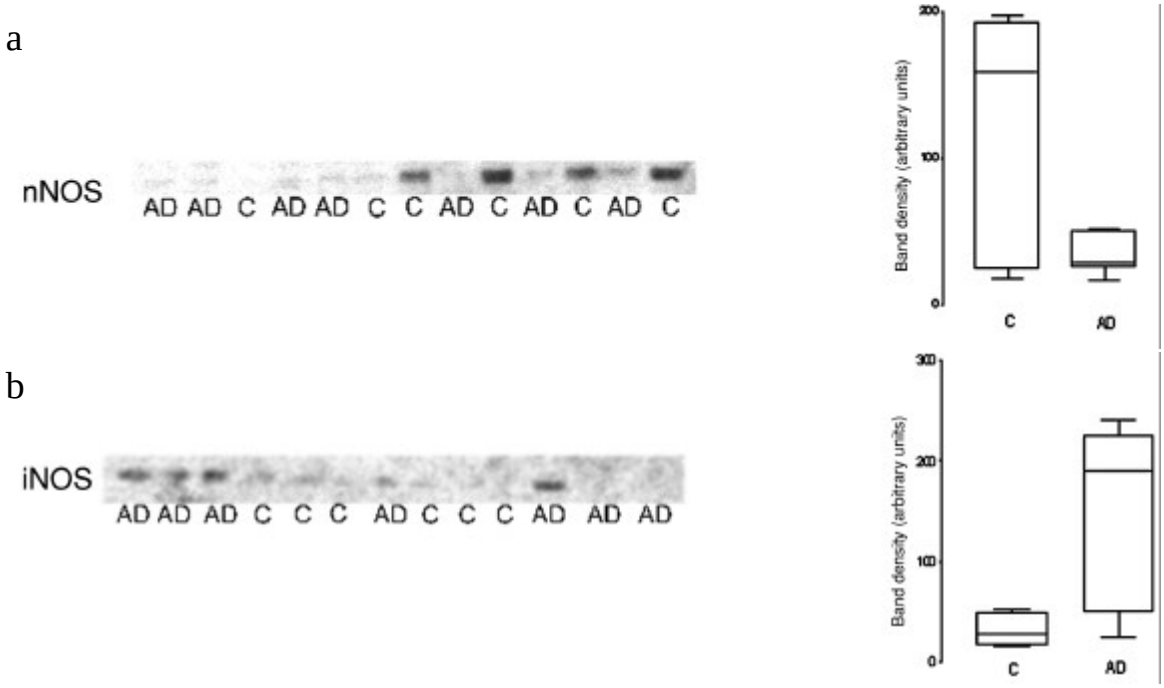

Figure 1.

Hippocampal neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) expressions in non-demented elderly control (C) and Alzheimer's disease (AD) brains. The expressions of nNOS **(a)** were significantly higher in 4 out of 6 control brains, whereas iNOS expressions **(b)** were significantly higher in 4 of the 7 AD brains. Box and whisker plots represent band density distributions for the brains used in the study (n = 6 for C; n = 7 for AD).

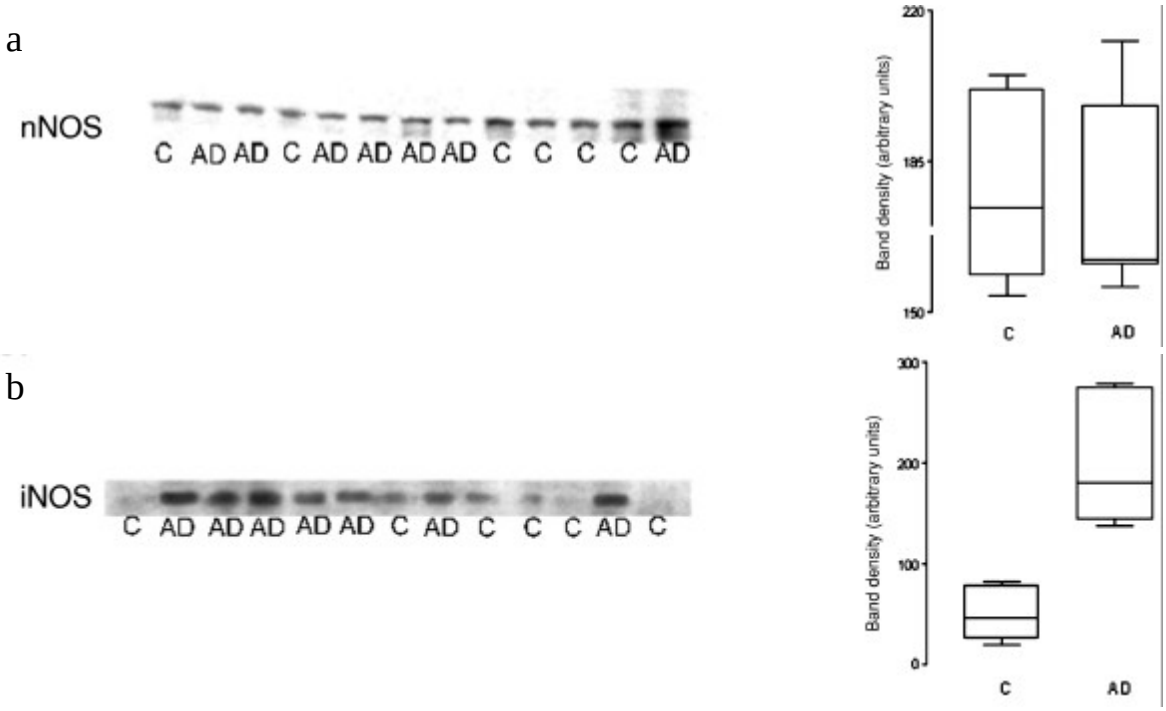

Figure 2.

Cortical neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) expressions in non-demented control (C) and Alzheimer's disease (AD) brains. Cortical nNOS expressions **(a)** were similar between the AD and control cases, whereas AD cases showed significantly higher iNOS expression **(b)**. Box and whisker plots represent band density distributions for the brains used in the study (n = 6 for C; n = 7 for AD).

and these neuroglial cells respond to a variety of brain insults and are known to release NO through an iNOS-dependent mechanism[27,28]. Furthermore, studies have demonstrated synergy between Aβ and cytokines to induce iNOS-mediated NO release[29]. In the present study, the significantly higher cortical and hippocampal iNOS expressions observed in the AD brain therefore further suggest a possible role for iNOS in AD pathogenesis. Interestingly, however, several AD cases showed minimal hippocampal iNOS expression. Previous studies have reported either increased or the absence of iNOS expression in the AD hippocampus[30,31]. This NOS isoform is an inducible enzyme that exists minimally under physiological conditions. The presence of an inducing factor in the hippocampus is required for significant iNOS upregulation, which could differ between AD cases. Moreover, since hippocampal pathologies appears early during the disease process[32], iNOS expression by the remaining viable cells in the AD hippocampus may diminish at a later disease stage, hence showing an apparent minimal expression level.

Our study showed that hippocampal nNOS expressions were higher in several control subjects. The literature has documented that nNOS-dependent NO production appears to be involved in physiological synaptic events such as LTP, and LTP abnormalities have been observed in an AD animal model[33]. Since neuronal loss is a pathological feature in AD, the diminished nNOS expression in the AD hippocampus may therefore be an indication of the loss of nNOS-containing neurons, with concomitant decrease in nNOS transcription[18,34]. Interestingly, nNOS expression levels between AD and control frontal cortices showed no significant difference. Since the AD brains used in this study were classified as either Braak and Braak stage IV or V - representing substantial neocortical involvement, our findings may suggest that nNOS-positive neurons are probably less vulnerable in toxic insults and neurodegenerative disorders - including AD - as described in several studies[35-37]. Furthermore, if these assumptions regarding hippocampal and cortical neuronal survival are correct, out findings would imply differential vulnerability of nNOS-positive neurons. However, it is important to also consider the complex array of neurochemical and neuro-metabolic changes occurring in the AD brain may affect nNOS regulation, despite the survival of nNOS-containing neurons. For example, elevated cortisol levels have been implicated in AD pathogenesis[38], and glucocorticoids has been shown to downregulate nNOS transcription[39]. Increased expression of heme oxygenase-1 (HO-1) - an inducible heme-metabolizing heat shock protein – has been observed in the AD brain[40], and it has been shown to reduce iNOS expression[41]. It is likely that HO-1 also modulates nNOS expression through a similar heme-competing mechanism. Cerebral glucose metabolism is significantly reduced in AD subjects[42,43]. Previous studies have documented iNOS-mediated NO release during glucose deprivation[44,45], which appears to downregulate nNOS expression[46]. Hence, multiple pathological factors occurring in AD can simultaneously affect the expression of nNOS, and further studies to examine nNOS expression in the AD frontal cortex are warranted.

The present study has shown that cortical NOS activity was significantly higher in the AD brain. Although the NOS assaying method employed did not distinguish between NOS isoforms, our findings regarding cortical nNOS and iNOS expressions (see above) suggest that the difference in NOS activity between AD and control subjects was likely to be iNOS-dependent. Furthermore, the presence of L-NIL (an iNOS-specific inhibitor) significantly decreased cortical NOS activity in the AD brain, thereby demonstrating the involvement of iNOS. However, the findings that cortical NOS activity in the AD brain in the presence of L-NIL remained higher than the control cases suggest the possibility that nNOS also contributed to the increased NOS activity observed in the AD brain, despite unaltered nNOS expression level. In other words, the enzymatic activity of existing nNOS could be increased by certain mediators without concomitant change in expression, and one such candidate could be Aβ. Studies have shown that Aβ can induce nNOS-mediated NO release[26,47], but the mechanism by which Aβ - or other putative pathological factors related to AD – alters nNOS-mediated NO release remains to be elucidated.

In the absence of L-NIL, hippocampal NOS activities were similar between AD and control subjects. However, NOS activity in the AD hippocampus was significantly lowered in samples treated with L-NIL. The findings therefore indicate that much of the hippocampal NOS activity in the AD brain could be ascribed to iNOS. This hypothesis is supported by the observation that iNOS expressions were increased in the AD brains.

Taken together, our results suggest that iNOS expression and activity are altered in the AD brain. Although nNOS has been implicated in neurodegeneration, it is not clear whether changes in cortical nNOS activity are associated with AD pathogenesis. As mentioned earlier, Aβ has been shown to induce nNOS-mediated NO release. Moreover, our findings suggest that iNOS-mediated NO release is likely to carry significance in the pathogenesis of AD.

The therapeutic use of NOS inhibitors and antioxidants as treatment adjuncts in AD has been suggested[48,49], and our laboratories have already demonstrated neuroprotective and neurorescuing properties of iNOS inhibitors and antioxidant against Aβ toxicity in primary cortical cultures[26]. Hence, further pharmacological studies on NO modulating agents are likely to yield promising findings toward the development of new therapeutic strategies for AD.

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Predicting Progression to Dementia in a Memory Clinic Population: Clinical Indicators Are of Limited Value

G.K. Wilcock[#], A.O. Hughes, A. Jordan, S. H. MacGowan

Department of Care of The Elderly, University of Bristol, Frenchay Hospital, Bristol, United Kingdom

ABSTRACT

Introduction: **Many potential risk factors, some more robust than others, have been associated with dementia and are reported in the literature from both epidemiological and clinical studies. We have examined whether it is possible to use the information about some of these factors, collected at initial presentation, to predict which patients have a greater risk of progressing to dementia, and also whether it is possible to use this information to discriminate between different disorders.**

Methods: **Patients referred to the Bristol Memory Disorders Clinic were included in the study. All subjects were assessed medically and neuropsychologically, and assigned appropriate diagnoses using internationally agreed standard criteria. 493 patients had a diagnosis of probable Alzheimer's disease, vascular dementia or probable Lewy body dementia. 92 subjects had an initial diagnosis of cognitive impairment insufficient for a diagnosis of dementia (CIND) and were followed up for a minimum of 2 years (average 4.25 years). A number of clinical and genetic variables were examined to determine whether they predicted progression to dementia, or could be used to assist in distinguishing between different conditions.**

Results: **Binary logistic regression confirmed that only two factors, being aged over 75 years and possessing an APOE e*4 allele, significantly increased the risk of developing Alzheimer's disease in patients initially diagnosed as CIND.**

Prior head injury and diabetes were associated with the diagnosis of vascular dementia.

Conclusions: **The clinical and genetic indicators that were available at the time of a subject's presentation to our memory clinic were of limited use in predicting an individual's progression to dementia, or in distinguishing between different dementias. Their importance may lie more in identifying potentially modifiable risks to the integrity of neuronal reserve, irrespective of the severity of the cognitive impairment or type of dementia.**

Keywords: sub-clinical dementia, Alzheimer's disease, Memory Clinic, disease progression, risk factors

Introduction

There have been many attempts to identify factors that may increase the risk of an individual developing Alzheimer's disease (AD), and also others that may confer protection. Potential risk factors include head injury, especially if associated with either loss of consciousness[1] or the presence of the APOE e*4 genotype[2]. Infection with Herpes Simplex virus (HSV-1) has also been linked with an increased risk of AD in individuals who carry at least one APOE e*4 allele[3]. Other possible risk factors include depression[4], although this could also be a prodromal feature[5], elevated cholesterol levels[6,7], hypertension[8,9] and a low blood pressure[10]. A reduced risk of developing AD has been associated with, for instance, certain drugs, such as oestrogen use[11,12] and non steroidal anti-inflammatory drugs (NSAIDS)[13] among others.

Research has also focused on examining potential genetic risk factors. One gene consistently shown to confer susceptibility to sporadic, as opposed to familial, AD is the APOE e*4 allele[14], contributing significantly to the

Correspondence: Professor G. K. Wilcock, Department of Care of The Elderly, University of Bristol, Frenchay Hospital, Bristol, UK BS16 1LE, United Kingdom.

risk of developing AD in those aged over 65 years[15]. APOE e4 status has also been investigated in relation to other conditions, e.g. vascular dementia[16,17] and Age Associated Memory Impairment[18]. The Angiotensin Converting Enzyme (ACE), has similarly been explored in relation to AD risk, with findings suggesting that the presence of the ACE D allele confers increased risk for AD[19]. Several genes in the HLA system have also been investigated, e.g. HLA-DR3 which has been shown to occur less frequently in subjects with AD[20].

Socio-demographic factors have also been examined. AD has a higher prevalence in women[21], although such findings could be influenced by a female survival bias. Increasing age[22] has long been recognised as a major determinant and low levels of education[23,24] have similarly been implicated. Particular lifestyle risk factors have also been investigated with conflicting results, e.g. alcohol consumption[25,26,27] and smoking[26,27,28,29].

The Bristol Memory Disorders Clinic has been collecting comprehensive clinical and neuropsychological data on all individuals seen since the early 1990s, and more recently the results of genetic analysis of blood samples, which are recorded anonymously. In view of the comprehensive nature of our data, and the continued uncertainty as to the value of clinical and genetic factors in predicting disease, we explored the possibility that such factors could assist in determining which subjects in our patient cohort were more likely to progress or develop specific diagnoses.

We specifically examined two issues:

1. Were any of the factors examined able to predict progression from an initial diagnosis of cognitive impairment insufficient for a diagnosis of dementia (CIND) to Alzheimer's disease?
2. Was it possible to use such factors to differentiate between Alzheimer's disease, vascular dementia and Lewy body dementia?

Methods

Subjects

Patients are referred to the Bristol Memory Disorders Clinic with a variety of complaints, ranging from subjective minor memory impairments to established dementia. All undergo a full medical assessment and are also neuropsychologically assessed using a validated battery described in detail elsewhere[30].

The clinic has local ethics committee approval to undertake genotyping for research purposes and all those who had consented to genotyping at a visit to the Memory Disorders Clinic prior to 10th January 2001 were included in the study. This produced a population of 1026 patients. 339 (33%) of these had a final diagnosis of probable AD, 130 (13%) a diagnosis of vascular dementia and a small group of 24 (2%) had a diagnosis of probable Lewy body dementia. The remainder (52%) were diagnosed with a wide variety of other types of dementia and memory problems. Diagnoses of probable AD and Lewy body dementia were made using standard criteria[31,32]. A diagnosis of vascular dementia was based on the Hachinski Ischaemia Scale. Although more recently introduced vascular criteria are also currently used in the clinic; they were not available for the earlier subjects.

There was a group of 92 (9%) patients for whom the diagnosis at first visit was CIND, who had subsequently been followed-up for a period of at least two years (average follow-up period was 4.5 years). All had objective evidence of memory impairment on formal assessment and 11 also had minor impairment in other cognitive domains, but none fulfilled the criteria for dementia. The last known diagnosis on these patients placed 55 of them still with CIND (after an average follow-up of 4.25 years), 16 with probable AD (average follow-up 5 years), 16 with non-AD dementia (average follow-up 4.3 years) and 4 with other specified memory loss problems (average follow-up 6 years).

The database

The database contains a wide diversity of information on all individuals included in this study. The nature of these variables can be broadly categorised as:

1. those associated with genetic information (e.g. APOE e*4, ACE, HLA-DR3);
2. those concerned with lifestyle and medical history (e.g. past medical history, alcohol consumption, age);
3. those associated with features of dementia / memory loss (e.g. diagnosis, symptom course);
4. neuropsychological assessment variables;
5. and those concerned with clinical assessment (e.g. findings on examination and laboratory test results).

Data on most of the neuropsychological variables and a number of the clinical assessment variables were available for every visit made by the patient to the Bristol Memory Disorders Clinic.

For the purpose of the investigations reported in this paper, data analysed comprised all the genetic information and the following clinical indicators: atrial fibrillation, arthritis, cold sores, diabetes, head injury, hypertension, prescription of ace-inhibitor drugs, beta blockers and NSAIDs. The diagnostic information used was as outlined in the subjects section above, and the gender and age of all patients was known.

Statistical analysis

Data was analysed initially using a univariate approach to explore relationships in the data and investigate which factors might be associated with diagnosis. To confirm the independence of any such associations, a logistic (binary or

multinomial) regression was then carried out on the same data. 95% confidence intervals and p values less than 0.05 are reported as significant throughout.

Results

CIND progressing to AD

Our interest was to discover if any of our variables might be associated with progession from CIND to AD. Univariate chi-square analysis (using Fisher's Exact probability test because of small numbers) produced significant relationships for possession of an APOE e*4 allele and age, but for none of the other information (Table 1).

Binary logistic regression confirmed that there were only two significant indicators of progression to AD. These were: being over 75 years of age increased the risk of an AD diagnosis to 5.1 (95% confidence interval 1.4 to 18.6) and having an APOE e*4 allele increased the risk to 4.4 (95% CI 1.2 to 16.0)

There was a group of 16 patients in our data who were admitted with CIND for whom non-AD dementia was a final diagnosis after follow-up. These patients did not differ from those remaining CIND with respect to any of our variables except for age (p=0.004) (data not shown). Patients who were over 75 were more likely to have progressed to a specific dementia.

Differentiation of types of dementia

The clinical data set allowed investigation of these same genetic and clinical factors in an attempt to discover if any of them differentiated between the three common presenting dementias – AD, vascular dementia and Lewy Body dementia. Since follow-up for at least two years was not required for this analysis, a much larger group of patients with clinical and genetic profiles was available for study, ranging from 419 to 493 depending upon the factor being investigated.

The results of the univariate analyses are shown in Table 2. The only significant relationships are for gender

Table 1. Effect of risk factors on conversion to AD.

Risk indicator present at initial diagnosis	CIND after at least 2 years follow-up n/total (%)	AD diagnosed after follow-up n/total (%)	Probability of association (Fishers Exact test)
APOE ε*2	7/55 (13%)	2/16 (13%)	n.s.
APOE ε*3	51/55 (93%)	16/16 (100%)	n.s.
APOE ε*4	18/55 (33%)	10/16 (63%)	0.043
DR3 allele	11/46 (24%)	3/12 (25%)	n.s.
ACE D allele	33/44 (47%)	10/12 (83%)	n.s.
ACE I allele	30/44 (68%)	8/12 (67%)	n.s.
Arthritis	2.55 (4%)	1/16 (6%)	n.s.
Cold sores	7/55 (13%)	0/16 (0%)	n.s.
Diabetes	2/55 (4%)	2/16 (13%)	n.s.
Head injury	14/53 (26%)	2/16 (13%)	n.s.
Hypertension	13/54 (24%)	2/16 (13%)	n.s.
Ace inhibitors	4/55 (7%)	1/16 (6%)	n.s.
Beta blockers	1/55 (2%)	0/16 (0%)	n.s.
NSAIDs	6/55 (11%)	0/16 (0%)	n.s.
Female	21/55 (38%)	6/16 (38%)	n.s.
75 yrs & over	18/55 (33%)	10/16 (63%)	0.043

n.s. = not statistically significant

(females more likely to have a diagnosis of AD and males to have Lewy body disease), diabetes (associated with vascular dementia), hypertension (again associated with vascular dementia) and head injury (related to vascular dementia and Lewy Body Disease).

In a multinomial logistic regression comparing all three diagnostic groups, the features of gender (p=0.012, likelihood ratio test (LRT)), diabetes (p=0.024, LRT) and hypertension (p<0.0005, LRT) are the only significant indicators. However, close examination of the classification table produced by the best fit model shows that only 15% of vascular dementia patients are being correctly identified and none of the Lewy body disease patients. This would suggest that at best it might be possible to differentiate on these variables the AD patients from the vascular patients.

To examine this, a stepwise logistic regression was performed for these two groups. Three variables were found to be significant, hypertension (p < 0.0005), diabetes (p = 0.017), and head injury (p = 0.039). Expressed as the presence of this risk factor suggesting a vascular dementia, the increased risk with diabetes was 3.78 (95% CI 1.27 to 11.26), with head injury was 2.02 (95% CI 1.04 to 3.92) and with hypertension was 2.52 (95% CI 1.52 to 4.19)

Discussion

Considerable confusion surrounds the categorisation and terminology for patients who have cognitive impairment that is not severe enough to justify a diagnosis of dementia. More than ten different diagnostic classifications, using varying but overlapping definitions, have been proposed and the majority of the literature supports the concept that a significant proportion of these people go on to develop a dementia, especially if they have an amnestic component to their impairment[33,34,35]. Several studies, reviewed by Petersen et al.[36,37], present evidence to suggest that performance on specific memory tests may

Table 2. Relationship between risk factor and type of dementia.

Risk indicator present at diagnosis	AD	Vascular dementia	Lewy Body disease	Probability of association
APOE ε*2	23/338 (7%)	10/130 (8%)	2/24 (8%)	n.s.
APOE ε*3	283/338(84%)	109/130(84%)	22/24 (92%)	n.s.
APOE ε*4	217/338(64%)	76/130 (59%)	14/24 (58%)	n.s.
DR3 allele	82/305 (27%)	26/106 (25%)	3/19 (16%)	n.s.
ACE D allele	240/297(81%)	80/105 (76%)	12/17 (71%)	n.s.
ACE I allele	205/297(69%)	73/105 (70%)	12/17 (71%)	n.s.
Atrial Fibrillation	6/339 (2%)	5/130 (4%)	1/24 (4%)	n.s.
Arthritis	12/339 (4%)	6/130 (5%)	1/24 (4%)	n.s.
Cold sores	34/339 (10%)	9/130 (7%)	1/24 (4%)	n.s.
Diabetes	7/339 (2%)	14/130 (11%)	3/24 (13%)	<0.0005
Head injury	35/330 (11%)	30/125 (24%)	4/23 (17%)	0.001
Hypertension	83/224 (25%)	56/128 (44%)	3/24 (13%)	<0.0005
Ace inhibitors	12/339 (4%)	7/130 (5%)	0/24 (0%)	n.s.
Beta blockers	14/339 (4%)	5/130 (4%)	1/24 (4%)	n.s.
NSAIDs	19/339 (6%)	11/130 (9%)	0/24 (0%)	n.s.
Female	213/339(63%)	69/130 (53%)	5/24 (21%)	< 0.005
75 yrs & over	177/339(52%)	81/130 (62%)	10/24 (42%)	n.s.

n.s. = not statistically significant

help predict those who are most likely to progress to dementia, although this is not a universal[38].

Other factors, e.g. those already mentioned in the introduction, have also been proposed as potential risk factors for dementia, and we have therefore investigated their potential as predictors of eventual dementia in our group of subjects who did not fulfil the diagnostic criteria at presentation. Only possession of an APOE e*4 allele and being aged over 75 years increased this risk in our memory clinic population. APOE e*4 allele carriage is known to be associated with memory complaints and to accelerate cognitive decline[39], and it is therefore not surprising that we found it to be a harbinger of conversion to dementia. It is, however, not routinely measured as part of the clinical protocol. The sensitivity and specificity of its reliability as an indicator of progression to AD requires further evaluation before deciding whether it is ethically justifiable for use as an adjunct to clinical and cognitive testing.

Several studies have raised the possibility that high blood pressure may increase the risk of Alzheimer's disease as well as other dementias[6,40] but we did not find this to be the case, as others have[41]. The interrelationship between blood pressure and AD is still being unravelled, and although it may have a part to play in the pathophysiology, our findings do not support a predictive role in determining which patients will progress to AD.

Differentiating between different types of dementia is becoming increasingly important with the advent of treatments. Three factors, hypertension, prior head injury and diabetes, were associated with an increased risk of vascular dementia. As hypertension is an intrinsic part of the Hachinski scale that was used in the diagnostic process, only prior head injury and diabetes are possible indicators, but the increase in level of risk is probably not sufficient to allow adequate sensitivity and specificity for clinical purposes.

It is possible, and perhaps even probable, that dementia in most individuals, especially the elderly, is multifactorial in nature, even though we traditionally try to arrive at a single diagnosis, or usually at most, two underlying diagnoses. The importance of exploring the potential role of the factors investigated in this paper may lie in their contribution to a loss of neuronal function and/or reserve, irrespective of the type of dementia, rather than their value in discriminating between different dementias. Attention to those factors that are modifiable may help retard the rate of future cognitive decline.

Similarly, although it is clear that carriage of an APOE e*4 allele is a risk factor for AD, this has also been reported to occur more frequently in both Lewy body dementia and vascular dementia[42,43,44,45,46]. In addition, it has been reported that APOE e*4 is associated with low levels of cholinergic markers in the brain in non-demented individuals, which has important implications for our understanding of its role in AD[47]. Greater understanding of the contribution of APOE e*4 to neurodegeneration may have a potential therapeutic impact on dementia and cognitive impairment in general, rather than in AD alone.

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Cognitive Compensation Can Overcome Age-associated Physiological Losses in Processing Odors, Pictures, and Flavors

Robert J.F. Elsner[#]
University College Cork, Cork, Ireland

ABSTRACT

It is well known that degradation occurs in the nervous system during senescence, and that olfaction is among the most seriously impacted by age. The purpose of this study was to explore age associated changes in different sensory modalities in order to assess hypothetical compensatory strategies employed to overcome physiological losses. Flavor, vision, and olfaction were assessed separately using a three-choice forced alternative mechanism with stimuli that had been piloted as approaching equivalence. Thirty healthy, community-dwelling individuals (21 female, 9 male) aged 18 to 79 (M = 42, SD = 21.7) tested four series of stimuli that included odors, flavors, and two sets of pictures in booths under fluorescent lighting. No age-associated differences were found in overall abilities, indicating compensatory strategies being employed on the cognitive level. Relationships were found between odor and picture recognition, but not between flavor and odors or pictures. Depressed affect was associated with misidentification of preferred flavors as the correct answer. Conclusions were made that the relationship between the physical deterioration of the sensory systems that accompanies age are moderated by cognitive strategies.

Keywords: cognitive compensation, odor memory, taste, vision, elderly, aging

Introduction

The literature on the physiological manifestations of loss that occur with aging are too numerous to list. Older adults have been theorized to have fewer processing resources available in order to learn and retrieve new information[1]. As much of the information on visual and auditory loss will be familiar to most readers, losses involving olfaction will be highlighted here as a specific pathway to ameliorating lexicality effects associated with cognitive function and potential compensation.

To truly discern the origins of decline, the best approach is the multimodal approach that has been taken by Herz[2], Kemp and Gilbert[3], Larsson and Bäckman[4], and Stevens, Cruz, Marks, and Lakatos[5]. Such examinations allow the detection of relative changes in each sensory system and indicate whether changes are central or peripheral in the nervous system or in central or peripheral processing. Elsner[6] demonstrated the ability for centenarians to retain olfactory function regardless of other sensory system abilities, contrary to expectations, but in support of the comensitory strategies posited by Lindenberger and Baltes[7]. As Elsner[8] points out, environmental exposure and medication use are typically greater factors for olfactory degradation than chronological age due to their direct influence on the neural base for the sensation tested.

Doty[9] warns that a number of nominally distinct olfactory tests, to a large degree, measure the same component of variance in normal subjects. Thus, as his example went, if an odor memory test is given to a person who has experienced cumulative damage to the olfactory neuroepethilium, the low scores may have nothing to do with neural circuits *per se*, but the interpretation would typically be dysfunction on the cognitive level. This idea supports the basic findings of Baltes and Lindenberger[10] that brain structure and function in aging may be a common cause for both sensory and cognitive changes. Baltes and Lindenberger examined 687 individuals aged 25 to 101 years using hearing, vision, and a battery of intelligence tests. The link that they found between sensory and intellectual functioning increased substantially from adulthood to old age. They suggest that much of the age-associated decline in sensory performance typically observed in older people is caused by the same set of factors as those which bring about declines in complex cognition. Thus sensors tasks are more easily understood than complex tasks, both in terms of the cognitive processing of the individual tested and the psychometric tests being employed.

Correspondence: Robert J.F. Elsner MD, PhD, University College Cork, Cork, Ireland
+353-21-490-3126 (Phone), +353-21-427-0244 (Fax), e-mail: relsner@ucc.ie

Performance decreases in a wide variety of tests of speeded performance during the aging process[1,11,12]. Salthouse[1] posited that performance decreases are the consequence of cognitive speed decreases. Performance decrements have been observed in tests of intelligence[13,14], attention[15,16], and memory[17].

Lawless[18] reported that recognition performance for complex figures was uniformly higher across time as compared with memory for common odors and abstract visual stimuli in a sample of young adults. Using unfamiliar symbols, faces, and common odors as test materials, two studies have indicated that aging seems to take a particular toll on odor memory[19,20]. In these studies, age-related deficits in recognition memory were considerably larger for odors. Younger participants remembered the odors much like they remembered the other materials, immediately, two weeks, and 6.5 months after inspection. Odor memory of the older adults was at chance performance levels after the first test.

Parity of research abilities in the modalities achieved through multimodal investigations would open the possibility for true comparison of the sensory systems, as well as understanding of the potential cognitive compensations for physical loss. It would be possible to negate the issues of differing sensory loads or lexicality effects of the various stimuli that limit true comparisons at this point[21,22]. This type of testing would allow for a settling of the debate[10] over the common cause theory versus the aging-induced cognitive load hypothesis, which considers relatively simple sensory tasks to increase in cognitive complexity and demands as participants age. By maintaining comparability of stimuli across the sensory modalities when stimuli were adjusted relative to their absolute thresholds, changes in performance across all modalities would be more indicative of the common cause stance. Specific deficits in sensory systems which the individual's experiences have developed lower automaticity effects, such as olfaction and possibly haptic sensation, would be supportive of the aging-induced cognitive load hypothesis due to the relative changes in demand characteristics for these sensory systems. From the literature, there are two main confounds that must be controlled for in such examinations, environmental and medication risk for decreased olfactory ability[8,23] and the level of depression in the sample[24,25].

As memory can be greatly affected by changes in signal input, significant considerations for tests of olfaction among older individuals are olfactory adaptation and recovery. In an examination of these phenomena, Stevens, Cain, and Oatley[26] found that older individuals adapt more quickly and recover more slowly than younger people do. Unfortunately, few tests of age-associated change in olfaction have considered concomitant variables, such as depression, that often afflict older adults and confound results.

Although there are numerous instruments for assessing levels of depression, few have been validated across age cohorts and cultures. The Center for Epidemiological Studies Depression Scale (CES-D[27]) has been validated for use with a number of diverse age and cultural groups.

Olfaction in aging opens possibilities for research into memory that other modalities of sensory input may not be able to address. Smith and Park[28] eloquently state that "there is evidence to suggest that memory representations can be lexical (i.e., meaning-based abstractions reflecting the linguistic structure of the input) or they can be visuospatial (i.e., perception-based abstractions reflecting the spatial structure of the input), or they can be both" (p. 69). But taken to other sensory systems, this would infer that all olfactorial memory, for example, is mediated by either vision or the lexical representations and descriptives of the odor experience. For the average psychometric task, this fits well into Paivio's (1971) dual code theory, which states that items processed by both the visuospatial sketchpad and the phonoarticulatory loop are richest, therefore most easily remembered and retrieved. This theory leaves us to wonder along what processing lines the olfactorial stimuli are processed, as they are not inherently lexical, and some can be strongly non-lexical. Elsner[29] posits that there is a chemosensory loop off of Baddeley's [30] Central Executive processor that allows for minimal lexical or even non-lexical representations to be processed, and thus allows for compensation at the cognitive level for losses on the physiologic base. A representation of this theoretical model appears in Figure 1. The purpose of this paper is to explore specific issues of cognitive compensation for physiologic loss in sensory systems while controlling for depression.

Methods

Participants

A total of 30 healthy, community-dwelling individuals (21 female, 9 male) aged 18 to 79 (M = 42, SD = 21.7)

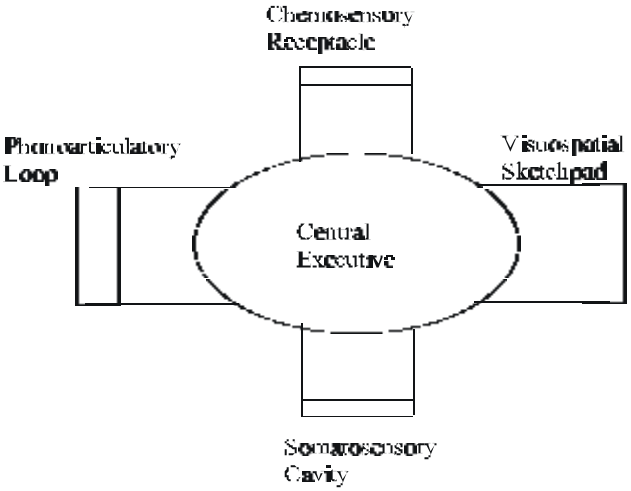

Figure 1.
Revision of Baddeley's model of working memory with inclusion of Chemosensory and Somatosensory modules (from Elsner[29]).

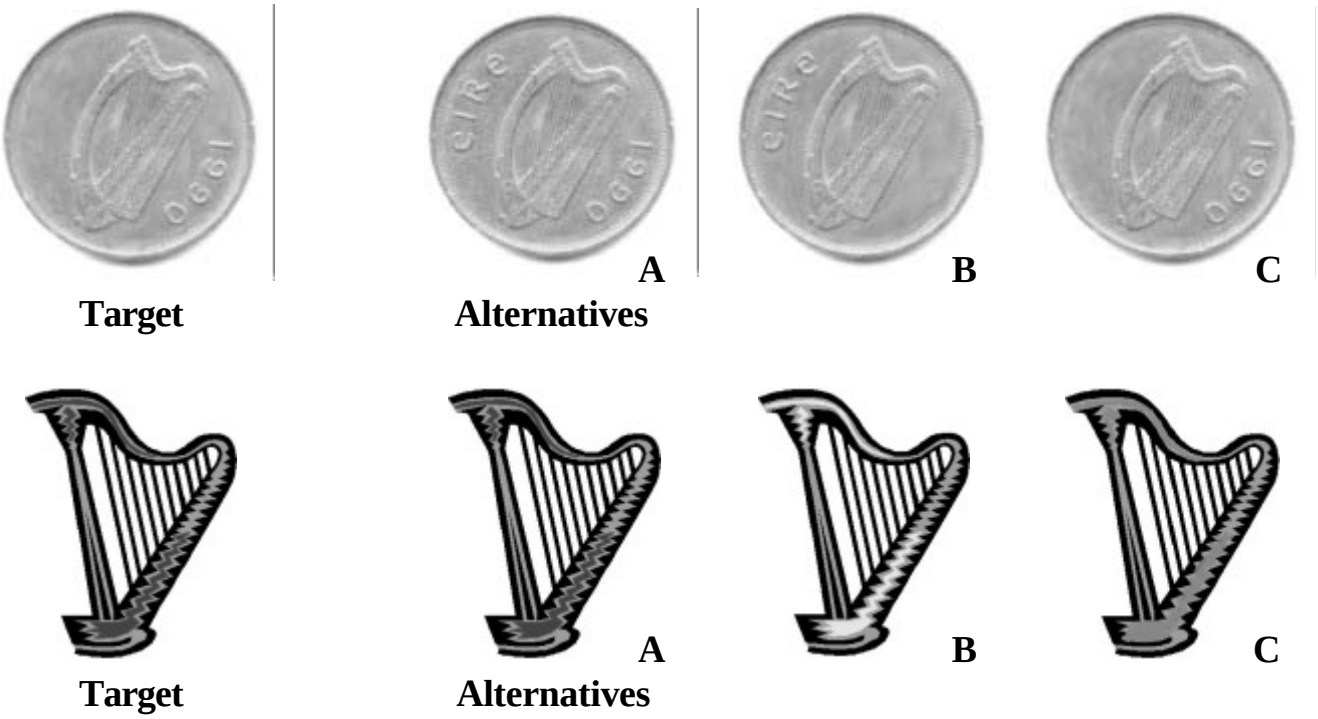

Figure 2.
Example of visual stimuli: Target and three forced-choice alternatives for samples two and three of the eight presented.

from local urban and suburban areas, as well as students at the University, volunteered to participate. Volunteers were recruited through newspaper ads, fliers, and word of mouth, and were required to have no medical conditions, have no history of working in high-risk occupations for olfactory loss[8], taking no medicines, and have been regular consumers of sweetcorn, custard, and orange juice. All participated for the benefit of advancing science and were provided refreshments after testing.

Procedure

Participants were given instructions on the procedures for the experiment and encouraged to ask questions. All participants gave informed consent before participating. Participants entered the individual, positive-pressure sensory booths, maintained at 19°C throughout testing. In order to assess the visual functions best, fluorescent white lighting was used instead of the traditional blanket red lighting typically used in sensory boot testing. All samples were marked with random three-digit code numbers. Participants were given all samples to be tested through pass-thru doors in the booths and marked responses on plain white paper ballots. Participants responded to a depression assessment instrument upon entrance into the sensory booths. All sensory testing maintained an identical procedure of a single stimulus followed by three forced-choice alternatives. Order of presentation for stimuli were picture, odor, picture, flavor, with the series repeated four times. The average time of administration of the test was approximately 30 minutes.

Instruments

Depression. As depression can severely impact memory regardless of modality, a baseline measure of depression was established using the Center for Epidemiological Studies Depression Scale (CES-D[27]). As with the other tasks employed, there was no time limit set on completion of this task.

Visual recognition memory. Visual stimuli presented were eight sets of grayscale images adapted from the literature. Examples of the stimuli are presented in Figure 2. Targets included black circles of varying size (28, 32, & 36 mm), coins with date, country, or neither removed, harps with different shadow details, inverted grids, Muller-Lyer-type images of varying line thickness (1, 2, & 3-points), circle-triangle cutout-illusions of varying grayscale of components (25, 40, & 50% gray), grayscale squares (25, 40, & 50% gray), and squares of various sizes (40, 45, & 50 mm).

Odor recognition memory. Odorants were presented in amber 4ml screw-top glass vials (Supelco, Bellefonte, PA, USA) with PTFE-lined solid caps in order to provide a non-reactive container that limits color biases to which olfaction is sensitive[31,32]. All vials were labeled with random three-digit numbers. The four sets of odorants used

were: 1) raspberry (Metarom Corp., Newport, VT, USA), apple (Metarom), and rum butter toffee (Edlong Corp., Elk Grove Village, IL, USA); 2) essence of arrack (Edlong), dark cocoa (Edlong), and caramel (Edlong); 3) anise (McCormick, Hunt Valley, MD, USA), peppermint (McCormick), and cinnamon (Frontier, Norway, IA, USA); and 4) hazelnut (Bell Flavors and Fragrances, Northbrook, IL, USA), almond (McCormick), and rum butter toffee.

Flavor recognition memory. As flavor is a complex combination of taste and retronasal olfaction, three foods in different phases were chosen to explore a single attribute of flavor, that of sweetness. Although addition of sweetness might initially be seen as only a taste change, not a flavor change, the dynamics between taste and flavor are such that this difference is negligible in a complex system. Unsweetened orange juice (Aldi, Warwickshire, UK); instant custard (Bird's Cheltenham, UK); and sweetcorn without added sugar or salt (Tesco, Dublin, Ireland) were chosen to fit the liquid, semisolid, and solid food categories. The custard was prepared according to directions using spring water (Ballygowan, Co. Cork, Ireland). It was then poured into a large stainless steel vat, separated into three smaller stainless steel pots, and sugar added to the pots at 0,.5, and 1% levels. This process was repeated for the other two products using the same levels of sugar. As none of the products were visually altered by the addition of sugar and all fit within a yellow-orange range, blanket red lighting was not used in order to facilitate use of the visual stimuli.

Results

Scores on the CES-D ranged from 0-31 with a mean score of 9.9 (SD = 7.7). Although this indicates that the average participant was not depressed (over 11), many were within the range, and some were severely depressed. This is quite important from the perspective of avoiding extreme effects in the data. Elevated scores on the CES-D were correlated with the preference for the sweetest custard ($R = 0.044428$, $p < 0.0139$) and for the sweetest orange juice ($R = 0.37561$, $p < 0.0408$), although no explanation can be given as to the reason for this effect. Age was not related to the depression index in this population.

Age was negatively correlated with ability to correctly identify the second odor ($R = -0.4493$, $p < 0.0127$), but not the other odors. Difficulty with this set was not completely unexpected, as that set was the most difficult: the target was anise, and it was matched with peppermint and cinnamon, all of which have a trigeminal component. When the veridicality of responses for the four flavors presented were regressed against age, no significant relationships were found ($F = 1.83$ [4, 29]; $p < 0.1537$), reinforcing the position that the olfactory difficulties presented in the second odor set were due to confusion more than overall ability decrease. Age and CES-D score were not related to the efficiency for identifying pictures ($F = 1.03$ [2, 29]; $p < 0.37$), odors ($F = 0.46$ [2, 29]; $p < 0.6332$), or flavors ($F = 0.04$ [2, 29]; $p < 0.9564$). No interaction effects were evident between age and CES-D in regard to preference or memory. CES-D scores were, however, significantly related ($F = 9.11$ [1, 29]; $p < 0.0054$) to the correct identification of flavors that were also preferred. In such an instance, it appears that preference overrode correct identification.

When the relationships between the efficiency of identifying the pictures, odors, and flavors were examined, specific relationships were found. When treated as a dependant variable, picture efficiency was related ($F = 3.93$ [2, 29]; $p < 0.0317$) to odors and flavors, however very differently. When separated out in the equation, flavor was not found to be significant ($F = 0.01$ [1, 29]; $p < 0.9285$), while odor was ($F = 7.86$ [1, 29]; $p < 0.0092$). When flavor was treated as the dependant, neither of the others was found to be associated ($F = 0.20$ [2, 29]; $p < 0.8179$). When odor was treated as the independent, ($F = 4.10$ [2, 29]; $p < 0.0278$), flavor was not found to be significant ($F = 0.35$ [1, 29]; $p < 0.5612$), while picture efficiency was ($F = 7.86$ [1, 29]; $p < 0.0092$), replicating the previous two sets of results.

To further explore this issue, GLM procedures compared picture and odor components, and found that the efficiency with which pictures were correctly identified was significant ($F = 5.90$ [1, 29]; $p < 0.0219$) for the third picture, the harps appearing in Figure 2. As flavor was considered further, the influence of the preference indicated a bias to respond that the most preferred sample was the same as the target. Flavor memory was significantly associated ($F = 3.71$ [2, 29]; $p < 0.0377$) with the correct identification of items when those items were also the preferred items, although overall preference was not a major contributor to this equation. As correct identification was associated with preference in individuals with higher CES-D scores, this indicates a memory effect of depression.

Discussion

The lack of age-associated change in the overall efficiencies strongly supports the hypothesis that older adults are able to compensate for sensory losses through cognitive means. Perhaps the relationship between the picture and odor efficiencies in this study might best be considered as describing the similarities in highly-lexical tasks better than the relatively static lexical task of flavor differentiation resulting from the flavor task. Although there were few overt lexical differences between the various stimuli in the visual task, the level of automaticity for all visual stimuli is always expected to be much higher than for other sensory systems, this increasing the potential for the more covert lexical components as adding influence[33].

Several studies[34,35] demonstrated that there is a reduction in odor recognition memory and odor identification in older individuals, though not without some method-

ological concerns [35,36]. Murphy, Nordin, and Acosta[35] comment on age-associated differences in encoding and retrieval and warn that some tasks may purport to examine recall but are not controlling for encoding due to individual variations in labeling. As these concerns have been addressed here to some degree, the lack of age-associated changes in the current study may be more indicative of the heterogeneity of older adults and differing levels of cognitive compensation than of the trends within aging adults.

White and Treisman[37] compared the content and order of stimuli on short-term memory for odors, and contended that there is an olfactory short-term memory but without the primacy that arises from differential rehearsal that occurs in verbal memory. They further argued that this odor memory is capable of incorporating verbal labels but is not dependent on such and, thus, is a separate system. This stance is important for issues of cognitive compensation. If there is a separate system, as posited by Elsner[29], then this would allow for compensation through the phonoarticulatory loop in instances where the neural pathways for a specific sensory system may be operating only minimally if contextual variables of the stimuli are able to be capitalized upon. If preference overrides memory in the decision process among sub-clinically depressed individuals, then the limbic nature of the olfactory system and its constituent structures must be examined in such a compensatory scheme.

Lindenberger & Baltes[10] posed the question: "is there a central processor that controls both peripheral and central processes in normal aging?" And as the peripheral and/or central function begin to decline, as often observed in aging, how do these functions interact or summate to influence the observe performance? Koss, Weiffenbach, Haxby, & Friedland (1988) suggested that early chemosensory impairment in Alzheimer's disease is due to central rather than peripheral dysfunction (for a discussion on Alzheimer's diagnosis and olfaction, see Elsner[38]). Using this as a guide, it is likely that cognition itself can compensate for physiologic loss for normative aging, but cannot compensate for pathologies affecting the nervous system, thus introducing drastic change and loss in such populations.

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MRI of Wilson's Disease

George Iana[1#], Adina Ioana Chirita[1], Silviu Manescu[2]

1 Department of Radiology and Imagistics, University Emergency Hospital, Bucharest, Romania

2 Department of Neurology, University Emergency Hospital, Bucharest, Romania

Abstract

Wilson's disease is an inherited metabolic and neurodegenerative disease produced by an abnormal copper metabolism which involves the liver and the central nervous system. It may be familial. It is characterised by: 1) deficiency of a serum protein for copper, the ceruloplasmine, 2) deficient biliary excretion of copper and 3) excessive copper deposition in the liver and brain.A reported MRI feature of the brain is the absence of the high signal on T2wi of the lateral putamina, thalami. Other subcortical central nervous centres can be affected. We reported a 28-year-old woman case with a 7-year history of hepato-leticular degenerescence. MRI showed symmetric lesions on the grey matter ganglia associated with asymmetric white matter subcortical lesions. The MRI particularity of the case is this variety that associates typical symmetric lesions in the central nervous centres, hyper intense on T2wi, and uncommon asymmetric subcortical white matter lesions.

Keywords: Wilson's disease, grey matter neurodegenerative disorder, Magnetic Resonance Imaging

Introduction

A broad spectrum of inherited metabolic and neurodegenerative disorders can involve the grey matter (GM), primarily or exclusively. On Table 1 are primarily presented the neurovegetative diseases that involve nervous ganglia centres (NGC).

GM metabolic disorders can be subdivided into those that affect the cortex and those that involve the deep grey nuclei.

Disorders that affect deep GM nuclei include Wilson's disease, Leigh disease, juvenile Huntington's disease and Hallervorden-Spatz disease.

There are disorders that primarily involve the cortical GM include neuronal ceroid-lipofuscinosis (Batten disease), mucolipidosis, glycogen storage diseases and gangliosidosis. Inherited metabolic and neurovegetative disorders that affect both the grey matter and the white matter (WM) mentioned in the specialised literature are: mitochondrial disorders (MELAS, MERRF), organic acidemias and acidurias, gangliosidosis, globoid cell leukodistrophy (Krabe's disease) and almost any later-or end-stage leukodistrophy. Wilson's disease is not mentioned in this category of affections.

Wilson's disease is known as a hepatolenticular degeneration. It is an autosomal recessive inherited metabolic disease. An affected nuclear-encoded gene has been described on chromosome13. It is characterised by:

- deficiency of ceruloplasmin (serum transport protein for copper)
- deficient biliary excretion of copper
- excessive copper deposition in the liver and brain

Pathological anomalies are cirrhosis of the liver and degenerative alterations in the basal ganglia: spongy degeneration, cavitation, neuronal loss with astrocytic proliferation.

The onset of clinical features in Wilson's disease usually start between 8 and 16 years. Reported clinical features are extrapyramidal and cerebellar signs, dementia and psychosis, Kayser-Fleischer rings (brownish coloration on the outer margin of the cornea), progressive liver disease.

The reported imaging features on CT are bilateral putaminal low-density lesions associated with mild non-specific atrophy.

The MR characteristic signs in Wilson's disease are:

- bilateral, symmetric lateral putamina, thalami hiperintense on T2wi (asymmetric or unilateral involvement may occur)
- caudate, red nuclei, substantia nigra may also be affected
- liver failure may cause high signal in palidi, dorsal midbrain on T1wi.

Correspondence: George Iana, MD, PhD, Department of MRI, University Hospital
Sp. Independentei 169, Bucharest 5, Romania.
Tel.: +40 1 6372190 / 233 / 234, Fax: +40 1 2110538.

Subcortical and periventricular white matter involvement may occur but it is uncommon.

Case report

We shall report the case of a 28–year-old woman with a 7–year history of hepato-leticular degenerescence. No family history.

The clinical onset was at the age of 21, manifested with right hand tremor and involuntary movements. These abnormalities were extended at the lower extremities. She experienced three convulsive generalised crises with postural instability and digestive symptoms. During that period, the patient was repeatedly hospitalised.

The Kayser-Fleisher rings associated with high levels of blood copper were diagnosed one year after the onset. A diagnosis of hepato-leticular degenerescence was established and a treatment with D-Penicilamine was administrated with a positive response starting with that period of time. The patient presented a tentative of suicide 7 years after the onset. The patient discontinued the treatment one year ago.

At the moment, she has come to our hospital for digestive complaints and moderate depression. Clinical neurological examination shows hippomaniac episodes, dysphonia, exaggerate emotional instability and intellectual degradation. Objective examination shows moderate hepatomegaly, complex tremor and dyskinesia of the lower extremities aggravated by emotions, ataxia, extrapyramidal hypertonia, Noica probe positive bilateral, voluntary movements, and present Kayser-Fleisher rings. Biological exams: (copper in) blood copper 50,5 µg%, ceruloplasmine 3,6 mg%, urinary copper 134,4 µg/24 h.

Normal tests of haemoglobin, haematocrit WBC, glycaemia, uric acid, electrophoresis for protein, transaminases, cholesterol and bilirubine, alkaline phosphatase 60UI/l.

Moderate hepatomegaly with diffuse nonhomogenous echostructure on the ultrasound exam. MRI was performed at 1T system. We found moderate high signal intensity in the sequences T2wi and FLAIR on the thalami (Figure 1a,e;

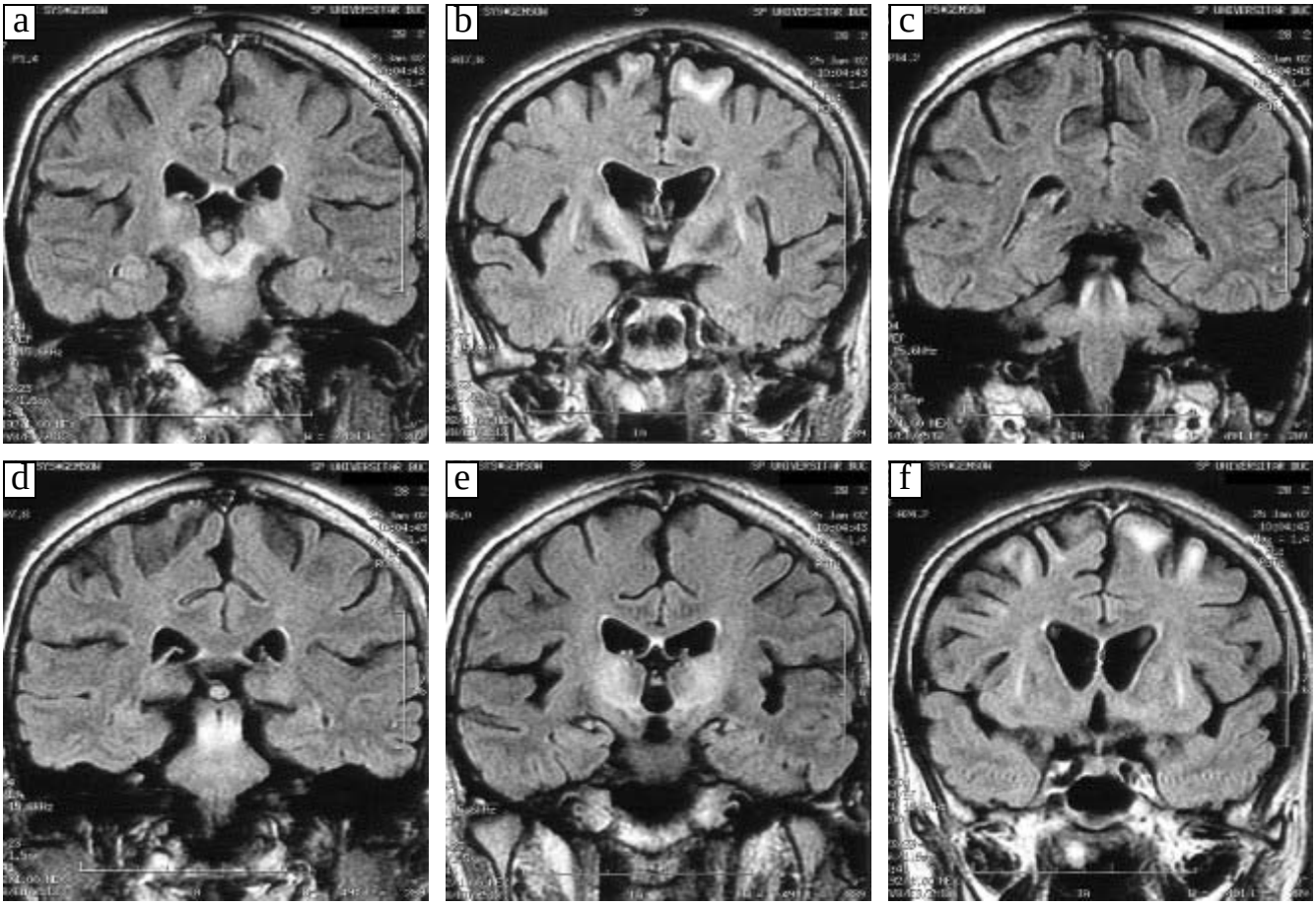

Figure 1.
Coronal FLAIR Sequences on the brain of a patient with Wilson disease.

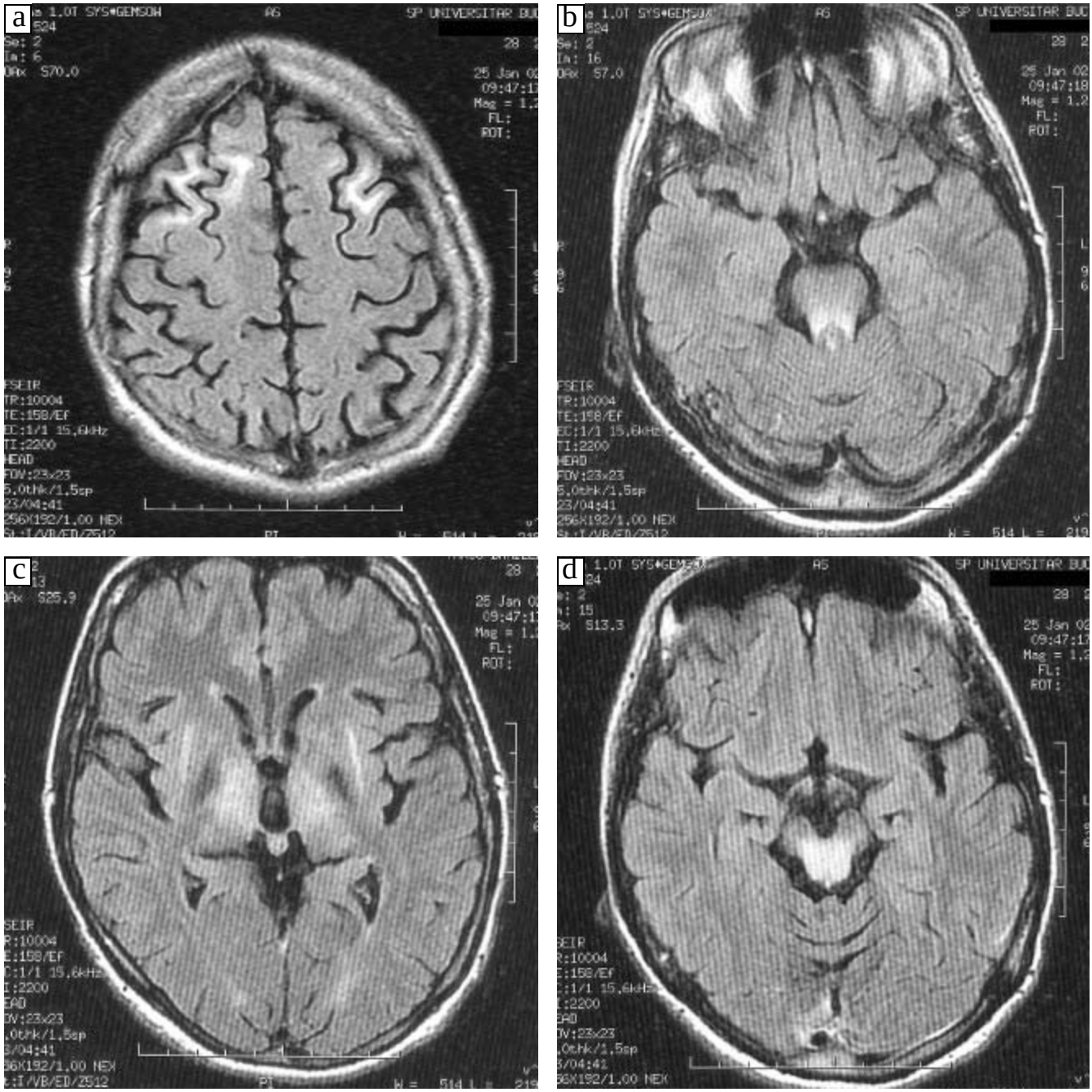

Figure 2.
Coronal T2wi Sequences on the brain of a patient with Wilson disease.

Figure 3b), red nuclei and substantia nigra (Figure 1a) and upper cerebellar peduncles (Figure 1c,d; Figure 2b). High signal intensity on T2W and FLAIR at the external capsule level (Figure 1 buff) Globi pallidi present important hyposignal intensity on T1wi and T2 wi that suggests the "tiger eyes" sign (Figure 1b,e; Figure 2c; Figure b). Lobe caudate with normal morphology does not present significantly modified signal MR intensity. We also found asymmetric high signal intensity on T2wi and FLAIR sequences on the subcortical frontal WM (Figure 1b,f; Figure 2a).

A diagnosis of hepato-lenticular degenerescence was established and a D-Penicilamine treatment was administered with positive evolution (decrease of dyskinesia and intentional tremor).

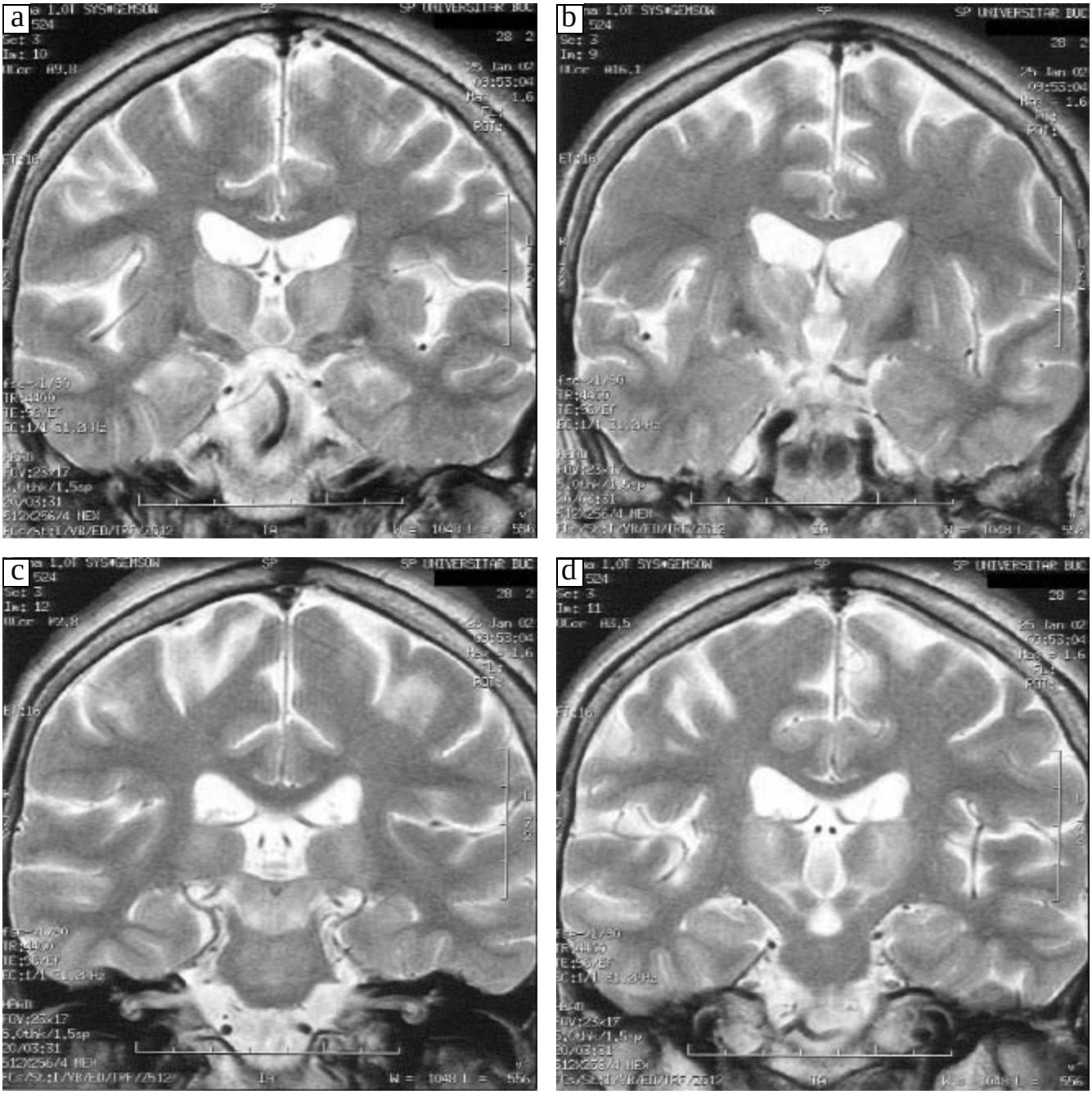

Figure 3.
Transversal FLAIR Sequences on the brain of a patient with Wilson disease.

Discussion

Clinically, our patient presented the symptoms that enter classical table of Wilson's disease: extrapyramidal and cerebellar signs, dementia and psychosis, Kayser-Fleischer rings, progressive liver disease.

Particular for this case is the late onset not associated with a family history. The question is: is this neurovegetative disease inherited or acquired?

The clinical data exclude an acquired toxic disorder and suggest the diagnosis of an inherited disorder. Among the inherited neurovegetative causes, the diseases that could

Table 1.

Neurovegetative diseases that involve NGC
Inherited: - *that involve NGC:* *Metabolic encephalopathy (Wilson's disease)* *Hallervorden – Spatz disease* *Leigh disease (subacute necrotizing encephalomyelopathy)* - *that involve NGC and subcortical WM:* *Mucopolisaccharidosis (Canavan disease)* *Mitochondrial disorders (MELAS, MERRF)* *Gangliosidosis* *Leukodistrophy (Krabe's disease)* - *that involve NGC cortical GM:* *Huntington's disease* *Aminoacidopathies*
Acquired: - *vascular* - *toxic metabolic: CO, H_2S, methanol, etc* - *multisystem atrophy* - *neoplasms(multicentric glioma, lymphoma)*

Table 2.

Wilson's disease *(Hepatolenticular Degeneration)*	***Halervorden-Spatz disease***	***Huntington's disease***	***Leigh disease*** *(Subacute Necrotizing Encephalomyelopathy)*
familial transmission autosomal recessive	familial transmission autosomal recessive	familial transmission autosomal dominant	familial transmission autosomal recessive
- deficiency of ceruloplasmine - deficient biliary excretion of copper - excessive copper deposition (liver, brain)	- vacuolisation, iron deposits in globi pallidi, substantia nigra	mutation on short arm of chromosome 4	mitochondrial encephalopathy (deficiency of multiple enzymes)
- cerebellar atrophy - NGC (caudate, putamena) abnormal signal	- globi pallidi atrophy - pallo-nigral low signal on T2wi - bilateral antero-medial hyperintense foci within low-signal globus pallidus may give "eye-of-the-tiger" sign	- caudate atrophy - caudate: hypo / hyper T2 wi - putamina: bilateral high signal intensity T2 wi - ballooning of the frontal horns of the lateral ventricles, cortical (frontal) atrophy	- NGC (globi pallidi, putamena, caudate) - thalami, - periaqueductal GM - cerebral peduncules symmetric high signal T2 wi WM less severely affected cortical GM sometimes
- onset 8-16 years - extrapyramidal and cerebellar signs - dementia and phychosis - Kayser-Fleischer rings - progressive liver disease	- pyramidal, extrapyramidal signs: rigidity, choreoathetoid movements - dementia in young adults	coreiform disorders mental abnormalities behavioural disturbances typical onset: 40-50 years of age	- hypotonic seizure, - ataxia, - ophtalmoplegia

be discussed are: Wilson's disease, Leigh disease, juvenile Huntington's disease and Hallervorden-Spatz disease (Table 2).

Clinical and biological data suggest Wilson's disease, but cannot exclude Hallervorden-Spartz disease or Leigh disease. The absence of coreiform movements excludes Huntington's disease.

MRI signs that involve both bilateral symmetric basal ganglia and asymmetric subcortical white matter (WM). The association of the symmetric T2wi hypersignals in putamina with important low signal intensity on T1wi and T2wi on globi pallidi is more characteristic for Halervorden-Spartz syndrome. Also, the asymmetrical subcortical WM hypersignal on are uncommon for a Wilson disease but not exclude it.

However, the MR aspect associated with clinical findings rulled out acquired causes like: metabolic toxic, vascular or tumoral causes.

Conclusion

We consider that the present case is interesting because of the differences between the specifically clinical and biological aspects correlated with the good response to a specific treatment and the MRI aspect, suggestive for a neurodegenerative disorder, but not specific for one of them, and uncommon for Wilson's disease.

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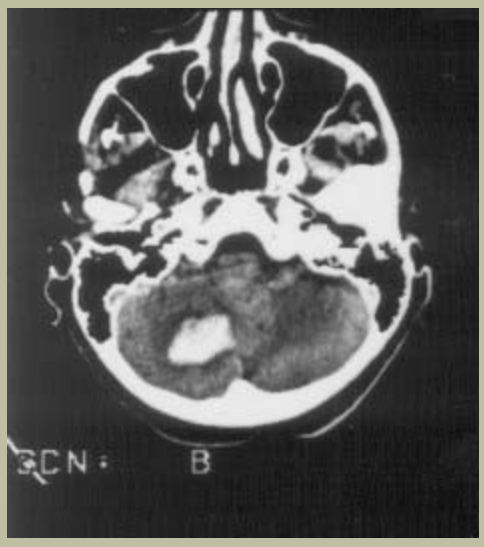
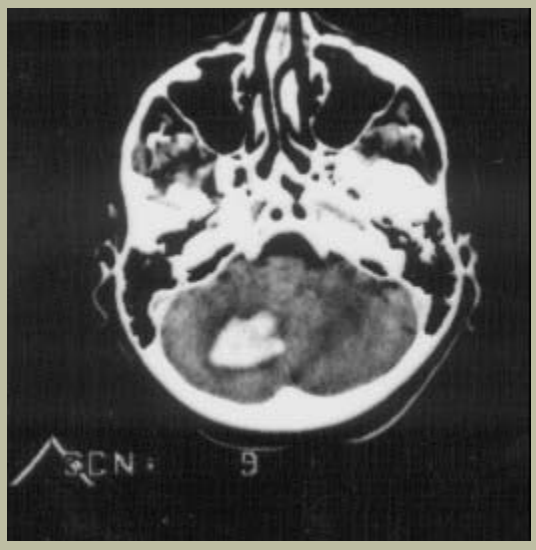
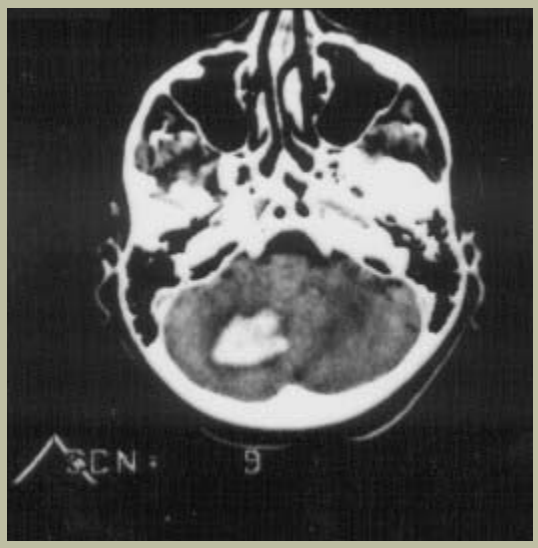
2. Grossman R., Yousem D., *Neuroradiology*, Mosby – New York 1998; 242-245

Right Cerebellar Hemorrhage

Paul Pătraºcu#
Neurosurgical Clinic, University Hospital Bucharest, „Carol Davila" University of Medicine and Pharmacy







Patient: L.E., female, 64 years old

Right cerebellar syndrome: acute onset with headache, ataxia, and dizziness

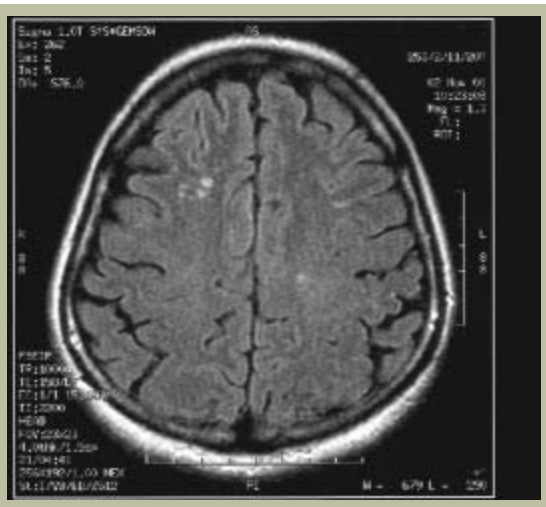
Etiology: untreated systemic hypertension

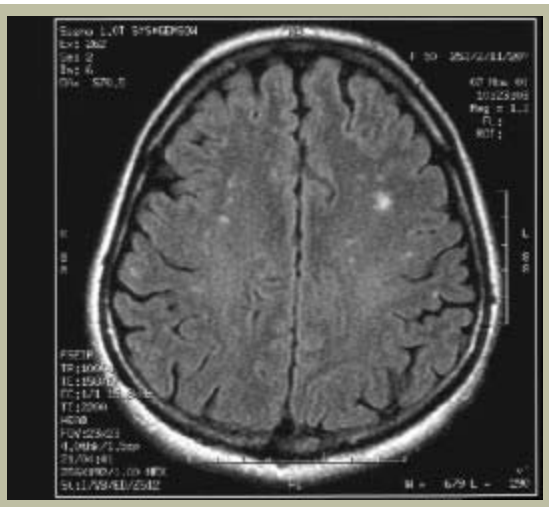
Correspondence: Paul Pătraºcu MD, Neurosurgical Clinic, University Hospital Bucharest, „Carol Davila" University of Medicine and Pharmacy, Splaiul Independentei 159, Bucharest 5, Romania, Tel.: +40 1 6372190

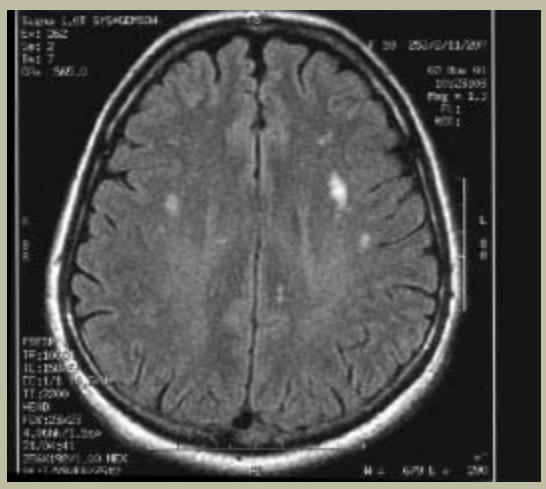
Multiple Small Hemorrhages in the Cerebral Hemispheres

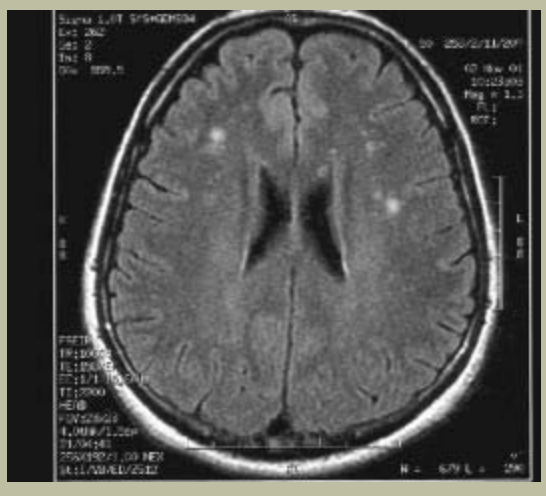
Paul Pãtraºcu#

Neurosurgical Clinic, University Hospital Bucharest, „Carol Davila" University of Medicine and Pharmacy



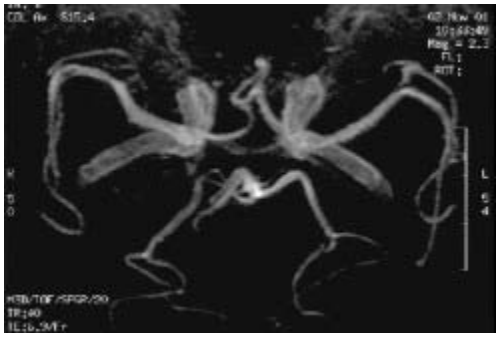






Patient: D.R., male, 50 years old

Acute onset with headache, photophobia, neck stiffnessy



MRI angiography shows no cerebral arteriovenous malformation

 # Correspondence: Paul Pãtraºcu MD, Neurosurgical Clinic, University Hospital Bucharest, „Carol Davila" University of Medicine and Pharmacy, Splaiul Independentei 159, Bucharest 5, Romania, Tel.: +40 1 6372190

News Update

3 December 2001: EP Public Health Committee discusses European Health Forum

On 3 December 2001, the Public Health Committee of the European Parliament held an exchange of views with the Commission on the functioning of the European Health Forum. The Commission explained its desire to create a Health Policy Forum with representatives of the different stakeholders, while at the same time creating a broad open Forum, which would meet once a year. To further involve the public at large and disseminate in an efficient way documents to all parties, a virtual forum will also be created.

12 December 2001: European Parliament adopts Public Health Programme in second reading

At its plenary session of 12 December 2001, the European Parliament adopted the Public Health Programme (2001-2006) at its second reading. Alzheimer Europe regrets that no majority was found for an amendment, which would have allowed European public health organisations to be funded through the programme, but nevertheless welcomes the possibility for NGOs to have specific projects funded by the programme.

16 December 2001: Alzheimer Europe Board adopts positions on genetic testing and research with people with dementia

17 December 2001: Alzheimer Europe organises its fourth Forum on "The genetics of Alzheimer's disease"

1 Ianuary 2002: "Brain Aging", a new journal on Alzheimer's and Parkinson's disease

Ianuary saw the launch of a new international journal dedicated to Alzheimer's and Parkinson's disease. The journal entitled "Brain Aging" is edited by Bengt Winblad and Alzheimer Europe established close links with this journal, which publishes information about the activities of the organisation.

More information on this journal can be found at: http://www.brainaging.org

4 January 2002: In survey, French admit fear of Alzheimer's disease

In a survey carried out by Sofres and published by Figaro (http://www.lefigaro.fr) in France, 1,000 people were asked about diseases they were afraid of. Alzheimer's disease came second with 41% after cancer (78%), followed by other diseases, such as AIDS (35%), multiple sclerosis (21%) or Parkinson's disease (19%).

8 January 2002: German Alzheimer association launches its new helpline

At her opening welcome, Ms. Heike von Lützau-Hohlbein, Chairperson of the German association, stressed that this new service was an important help for carers, especially since "two thirds of carers stay at home and their need for support and information is great. With the opening of this service, a dream of our association has come true thanks to the support of the German authorities". The number 01803-171017 can be reached from Monday to Thursday from 9.00 till 18.00 and on Friday from 9.00 till 15.00.

8 January 2002: German Alzheimer association calls for better training of professional carers of the elderly

At a press conference in Berlin, Heike von Lützau-Hohlbein, chairperson of the German Alzheimer association stressed that currently, most homes did not have enough trained staff although over half of residents had Alzheimer's disease or another form of dementia. On behalf of the German association, she therefore called for better training in dementia care for nursing home staff and a better taking into account of the special needs of elderly people with dementia through the German care insurance system.

12 January 2002: Cyrus Vance dies with Alzheimer's disease

On 12 December 2002, former Secretary of State Cyrus Vance died at the age of 84. He was Secretary of State under President Jimmy Carter and known for his role in the peace process between Israel and Egypt. Cyrus Vance suffered from Alzheimer's disease.

15 January 2002: The Helpline Manual now available on the Alzheimer Europe Internet site

In response to demands by some of the visitors to the Alzheimer Europe website, we decided to re-include the full version of the Helpline Manual which is meant for Alzheimer associations which either want to set up a helpline or to improve an existing helpline service.

The Helpline Manual can be found at the following address:
http://www.alzheimer-europe.org/JMA/English/helpline.html

16 January 2002: Spanish actor Alfonso del Real dies with Alzheimer's disease

On 16 January 2002, Spanish actor Alfonso del Real died in Mallorca at the age of 85. He suffered from Alzheimer's disease.

17 January 2002: Elan pharmaceuticals temporarily suspends clinical trial of AN-1792

Elan Corporation, plc and Wyeth-Ayerst Laboratories, today reported on developments in the Phase 2A clinical trial of AN-1792, an experimental immunotherapeutic for the treatment of mild to moderate Alzheimer's disease. The companies have decided to temporarily suspend dosing in this Phase 2A study after four patients in France were reported to have clinical signs consistent with inflammation in the central nervous system. All four patients are receiving appropriate medical care and the companies are working with clinical investigators to determine the cause of this development.

17-19 January 2002: Greek Alzheimer's association organises successful second panhellenic conference in Thessaloniki

The Greek Alzheimer's Association organised a highly successful conference on Alzheimer's disease in Thessaloniki from 17 to 19 January 2002 and this in preparation of the Alzheimer Europe Conference which the organisation will be hosting in 2003.

22 January 2002: New research bill passes first hurdle in France

A government-sponsored bill that would allow embryonic research to help the sick but maintain a ban on cloning passed its first hurdle in the French Parliament on 22 January 2002. The government's bill would lift a 1994 ban on embryonic research and allow scientists, under certain conditions, to obtain stem cells from frozen embryos created during in-vitro fertilization. The bill would continue to bar any attempt at cloning embryos for procreative reasons, and would forbid so-called therapeutic cloning, where embryos are cloned for their stem cells and then destroyed. Offenders could face up to 20 years in prison.

23 January 2002: European Commission adopts communication on "Life sciences and biotechnology"

'It aims to help Europe to master the frontier technologies that could make a major contribution to the goal of becoming the world's most competitive, knowledge-based and sustainable economy in a decade. The strategy's cooperative and consistent approach to fostering sustainable development is designed to address the complex ethical and societal concerns and support the broad public debate. In line with the principles of governance, the initiative is based on a wide public consultation that included a conference with a broad range of stakeholders.'

The document can be found on the following web site address:
http://europa.eu.int/comm/biotechnology/pdf/policypaper_en.pdf

28 January 2002: Study shows Exelon may be beneficial for patients with subcortical vascular dementia

Novartis announced on 28 January 2002 the results of a study that showed its Alzheimer's drug Exelon to be effective against subcortical vascular dementia. The group said that 12 dementia patients showed significant improvements after being treated with Exelon.

For more information, please refer to:
http://dominoext.novartis.com/NC/NCPRRE01.nsf/3a45c020b9b5c0fdc1256b230057e9f3/238b84ceeade6e66c1256b4f002387b5?OpenDocument

30 January 2002: German Bundestag votes in favour of allowing embryonic stem cell research

After lengthy debates, which highlighted differing opinions across traditional party lines, the German Bundestag voted today to allow limited imports of embryonic stem cells for stem cell research In Germany. The Bundestag had to debate and make a choice between three different positions:

- a ban on all import of embryonic stem cells and a continuation of the German law that does not allow for the creation of such stem cells in Germany,
- the possibility to allow imports of embryonic stem cells under strict conditions (already existing stem cells and not ones created after the adoption of the law) and a continuation on the ban on stem cell production in Germany,
- the lifting of the ban on stem cell imports and of the ban on stem cell production in Germany.

31 January 2002: Spanish health statistics show a 43% increase of deaths related to Alzheimer's disease in 6 years

According to numbers published by the National Statistics Institute in Spain, the number of deaths caused by Alzheimer's disease had increased by 42.9% since 1995. This makes Alzheimer's disease the disease, which has grown most of all causes of death during that period.

3 February 2002: Alzheimer Stichting Nederland installs Dementia Discrimination Alert

The Alzheimer Society of the Netherlands refuses any pre-selection of patients on grounds connected with dementia. The Society also refers to the recent positions of the National Social Economic Council on the organization of Health Care stating that all citizens are entitled to adequate health care. Only medical necessity for any operation or treatment should be used as selection criteria. Adequate health care is a basic right for every citizen in this country, including those who suffer from dementia. Pre-selection of patients on grounds of dementia is rejected strongly by the Alzheimer Society of The Netherlands.

8 February 2002: Estonia ratifies the Biomedicine Convention and its Protocol on the Prohibition of Cloning Human Beings of the Council of Europe

On 8 February 2002, Ambassador Ants FROSCH, Permanent Representative of Estonia to the Council of Europe, handed to Walter Schwimmer the instruments of ratification of the Convention on Human Rights and Biomedicine and its Additional Protocol on the Prohibition of Cloning Human Beings.

11 February 2002: European Health Ministers address the issue of free movement of patients

At their meeting in Malaga on 11 February 2002, European Health Ministers undertook an initial analysis of the situation regarding health care in Europe in relation to the growing mobility of citizens and recent rulings of the European Court of Justice. A Spanish Presidency summary stresses that ministers recognised that, in certain cases, the movement of patients can have an added value without disturbing the organisation or balance of health systems:

The summary further mentions:

- the creation of highly-specialised reference centres (where patients from throughout the EU could go to be cared for specific pathologies),
- the use of unused capacities for patients on waiting lists in other Member States,
- enhanced co-operation in border regions,
- the care for people living for a long period of time in another State to their own.

15 February 2002: Homocysteine link to Alzheimer's disease

The study, conducted at Boston University School of Medicine in Massachusetts, revealed that the risk of contracting Alzheimer's disease nearly doubles in people with high levels of homocysteine. This raises the possibility of staving off Alzheimer's by consuming more folic acid and vitamins B6 and B12 which can reduce homocysteine levels

20 February 2002: "G10 Medicines" group publishes recommendations

A high level group, "G10 Medicines" met to look at producing practical proposals for improving the competitiveness of the European pharmaceutical industry while ensuring high levels of protection of public health. This group presented its recommendations on 20 February 2002 and a number of these recommendations are of particular interest to patients and to carers. Thus, the group recommends:

- to improve the access of patients to new medicines by calling on Member States to reduce the time between marketing authorisation and pricing and reimbursement decisions,
- to create European virtual institutes of health connection all existing competence centres on fundamental and clinical research into a European network of excellence,
- to continue the ban on advertising for prescription medicines to the general public, while at the same time, in co-operation with all the stakeholders, producing a workable distinction between advertising and information that would allow patients actively seeking information to be able to do so and developing standards to ensure the quality of such information,
- to review the Community legislation relating to patient information leaflets taking into account the views of users as well as regulators and industry,
- to call upon the Commission to provide core funding for European patient groups to enable them to participate independently in the debate and decision making on health matters in the European Union.

20 February 2002: Memantine approved for Treatment of Alzheimer's Disease

At its meeting of 20 February 2002, the Committee of Proprietary Medicinal Products (CPMP) recommended to the EU Commission to approve Memantine for the treatment of moderately severe to severe Alzheimer's Disease. Marketing authorization covering the EU is expected mid-2002, and Merz will launch Memantine during the second half of 2002.

26 February 2002: Public Health Committee of the European Parliament has first exchange of views on the review of the EU pharmaceutical legislation

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The first use of an uncommon abbreviation should be preceded by the full name. Generic names of drugs are preferable. If a brand name is used, it should be in parentheses following the generic name, footnoted with the name and address of the supplier. Subheads should be inserted at suitable intervals.

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Authors should provide a one paragraph (100-150 words) abstract summarizing the main points of the article. The abstract must clearly state the objectives, the methods used, the main results and the conclusions of the reported study.

References

References should be cited in the text in numerically consecutive order. References should be cited in the text by Arabic numerals superscript. Each reference should contain names of all authors. Journal titles should be abbreviated according to Index Medicus.

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No., authors' names, title of the article. In: editors' names, *title of the book*. Place where it was published: Publishing House, Year: pages. [for edited books]

No., authors' names, *title of the book*. Place where it was published: Publishing House, Year. [for monographs]

Exemples:

5. Bierer L.M., Hof P.R., Purohit D.P., Carlin L., Schmeidler J., Davis K.L., Perl D.P., Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch Neurol* 1995; 52: 81-8. [for journal articles]
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7. Luria A., *The Working Brain*. New York: Basic Books, 1973.[for monographs]

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