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Brain Aging International Journal®
Ana Aslan International Academy of Aging®

The accepted abbreviation for **Brain Aging International Journal** for bibliographic citation is **Brain Aging.**

Scientific board

Why the First International Conference of Brain Aging - An International Journal?

Our Brain Aging International Journal 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 the field of Brain Aging, "Ana Aslan" International Academy of Aging is organizing the First International Conference of Brain Aging - An International Journal which will take place in Bucharest, Romania, between 5-7 of October, 2003 and the Post-Conference Training Course, on 8 of October, 2003, sustained by professors: Laura Fratiglioni (Sweden), Bengt Winblad (Sweden), Bruno Vellas (France), Kurt Jellinger (Switzerland), Khalid Iqbal (US).

The Program Committee has invited 50 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 the field of brain aging.

Around 350 researchers will present their works in a tremendous "brain storming".

The main topics will be:

EPIDEMIOLOGY
GENETICS, BIOMARKERS, NEUROIMAGING and HISTOPATHOLOGY
BASIC MECHANISMS INVOLVED IN BRAIN AGING and DEMENTIAS

THERAPEUTIC STRATEGIES
DIAGNOSIS / IMAGING
ALZHEIMER DISEASE AND NON-ALZHEIMER'S DISEASE DEMENTIAS

Why the Brain Aging International Journal?

Today there are 116 scientific journals and thousands of web sites dedicated to aging, a picture of increasing challenge to professionals dedicated for older people. The multiple pathology commonly seen in elderly people attracts us to a multi dimensional approach of this problem. Both, basic research and clinical trials are the main directions that we want to promote in the field of aging.

Why Brain Aging?

Brain Aging can due to the most devastating brain disorders of elderly people named Alzheimer disease. More than 50% of Alzheimer patients develop behavioral symptoms.

The other related dementia's also transform the elderly patient into a dependent person and this is followed up by family deep involvement and the drama of being clinically apparently healthy. The Journal describes a possible new approach to fighting Alzheimer disease on basic research: especially on stem cells, mechanisms of age-induced death, programmed cell death (apoptosis), programmed death of the body (phenoptosis).

A bridge

The Journal links scientists in Health Care and Basic researchers in the field of Brain Aging (Molecular Medicine, Genetics, Geriatrics and Gerontology, Neuropsychology, Pharmacology and Drug Trials, Epidemiology, Public Health). It promotes new information in the study of new molecules for the therapy of neuro-degenerative diseases in the high field of molecular medicine and in elaborating a new strategy in Community Care and Home Care which should reduce the extremely high costs of long time care of these patients.

Mission

Our goal is to provide relevant, high quality information from diverse sources, to publish with absolute integrity and to be a meeting point for those involved in the field of Brain Aging, serving their needs. If you have original articles, papers, editorials, information or manuscripts we would like to hear from you.

The new millennium offers the potential of greater discoveries in elucidating mechanisms of brain function particularly regarding neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases as well as regarding vascular disorders. The major etiological factor to be considered in these diseases is that their incidence increases dramatically as a function of age.

Within the context of increasing quality of life, a critical question needs to be posed: Is it possible to reduce or forestall the deleterious effects of brain aging and thus slow or even prevent the course of age-related neurodegenerative and vascular diseases?

Arising with the new millennium achievements, this Brain Aging International Journal wants to put together minds focused on mechanisms of brain aging and on interventions which possible alter the course of brain aging and its ultimate expression in cognitive, motor and behavioral changes.

Conference Organizers:

Bengt Winblad, MD, PhD - BAIJ Editor in Chief,
President of "Ana Aslan" International Academy of Aging

Luiza Spiru, MD, PhD - BAIJ Associated Editor,
Executive Vice-President of "Ana Aslan" International Academy of Aging

Khalid Iqbal, PhD - BAIJ Associated Editor,
Scientific Vice-President of "Ana Aslan" International Academy of Aging

"Ana Aslan"
International Academy of Aging

Welcome to the Academy

My colleagues and I are delighted to welcome you to membership in „Ana Aslan“ International Academy of Aging. You will join people from more than 50 countries all over the world, who are members in this organization, committed to medical science, education, culture and art.

The Academy proves itself to be committed to medicine, especially through creating excellent educational and clinical centers for elderly, in accordance with the agreements of World Health Organization, European Union, International and European Consortium in the field of Aging.

You will join a society of scientists, which was founded in 2000. We will invite you to all the conferences, symposia, carried out yearly, which cover all medical, social and educational disciplines.

„Brain Aging“ International Journal issues are available free of charge for years 2001-2002. You may also visit our web site or our on-line journal web site.

We are looking forward to your joining our Academy!

Bengt Winblad MD, PhD
Khalid Iqbal PhD
Luiza Spiru MD, PhD

BRAIN AGING
INTERNATIONAL JOURNAL

„Ana Aslan“ Academy of Aging has the honour to introduce to you probably the most interesting new millennium International Journal, „Brain Aging“.

Ana Aslan was the first one, all over the world, who defined, in 1953, Geriatrics and Gerontology as the most futuristic former millennium clinical specialization.

This great lady, who had the vision in the early 50-ties to define „gerontoprophilaxy“ as the advanced weapon to fight against aging, said that „human life is artificially shorted, but anyone can discover the secrets of longevity“!!

We are pleased to publish under the auspices of „Ana Aslan“ Academy of Aging, the latest news from all over the world, in this so incandescent scientific field, like „brain aging“ is.

This is a journal, which attempts to explain, in a clear and scientific language, how this knowledge may one-day possibly be used to control and overcome brain aging!

We have the mission to encourage also young researchers in publishing, in this field of activity, to grant the most talented of them, every year!

Hopefully, BRAIN AGING will be the host of publishing revolutionary papers, warm scientific information, and Brain Aging scientific timetable.

Read a new Journal, HAVE A NEW FRIEND!!!

Luiza Spiru MD, PhD

Who we are

The objects of the Foundation „Ana Aslan International“ are:

a) initiation and financing of programs concerning the development of the Romanian Geriatrics experience and tradition in the therapy of prevention of aging, in accordance with the present medical norms, in Romania as well as abroad;

b) initiation and financing of programs / scientific research projects concerning the reactions of the ASLAN therapy and of the ASLAN geriatric principles, in the frame of the present medicine and their integration in the international geronto-prophylaxis therapy;

c) supporting and supplying additional financial resources for operating the nurseries for elderly and the social institutions by offering sponsorships and donations in cash or in kind;

d) establishing, organizing and financing, according to the law, of prophylaxis geriatric centers, day-care geriatric assistance, medical offices, individual, grouped or associated, within the medical centers in the city of Bucharest and in the territory, where will be employed: specialist geriatric medicine doctors, psychologists, social assistants, physio-kinetotherapists and other personnel;

e) initiation, support and financing of programs for rehabilitation of elderly, by common action of geriatric assistance services and of the balneo-physiotherapy and rehabilitation;

f) initiation, support and financing of programs aiming at reinvigorating of the Romanian geriatrics, of the traditions of the ASLAN therapy and of ASLAN products, in relation with present science;

g) organizing and operating, according to the law, of medical assistance activities and medical assistance and care for elderly people with total or partial autonomy loss, in medical, socio-medical and psycho-emotional fields.

h) initiation, support and financing of projects and programs for elderly persons, concerning:

- social reintegration, juridical and administrative counselling of elderly persons in need;
- their participation in economic, social and cultural activities;
- support for the payment of current services and obligations, home care, household assistance for elderly persons;
- medical visits and care at home, treatment with the prescribed medicine, granting of sanitary materials, medicines and medical devices;
- hiring of personnel for sickroom services for elderly persons.

i) organization and operation, according to the law, of medical assistance services for persons with no income or with income under the limit of the minimum salary in the country;

j) support and financing of projects and programs for people with no income or having an income under the limit of minimum salary in the country, concerning:

- prevention of social marginalization and assistance the social reintegration of these categories of people;
- their participation in economic, social and cultural activities;
- educational programs concerning qualification, re-qualification and professional reintegration;

k) editing, support and promotion of the publication International Journal of Ageing, under the aegis of the Foundation, with an editorial board established by common agreement of the founders members and the international geriatrics community;

l) acquisition of equipment, books, publications and outfit for medical universities and for scientific research in Geriatrics;

m) establishing a club for the meetings of the founders members, honorary members and their guests;

n) support and development, according to the law, of educational structures able to contribute to the facilitation and optimization of social and professional integration of people

with no income, of disadvantaged, marginalized young persons, who leave the placement centers or having special needs;

o) granting of sponsorships and donations in cash or in kind for the families with reduced income, sole parents or disorganized families, aiming at facilitating their access to lodging, to help covering the expenses for preparation of children at the starting of the school year and for their access to education and formation services.

p) preparation and promotion of programs for equal opportunity employment for women, and for facilitating the access to hiring, professional formation, scientific, social and cultural recognition;

q) providing the material resources for organizing scientific, cultural and sports activities to the benefit of students, professors, medical doctors and other persons with activities in the fields of interest for the Foundation;

r) solving of some problems of the university community in institutions for high level medical education;

s) granting of scholarships for studies and research for teaching and research personnel or to students in high level medical education institutions, scholarships which will support high performance students and teaching personnel, their progress, specialization, participation to scientific research, information and study fellowships in Romania and abroad;

t) editing and/or supporting the material expenses for editing publications, scientific or artistic works in the fields of interest of the Foundation as well as establishing and financing, according to the law, of a publishing house / printing house for achieving this objective.

The objects of „Ana Aslan“ International Academy of Aging are:

- Publication, support and promotion of scientific works and printed materials, including „Brain Aging“ International Journal
- initiation, organization and support of projects and programmes for people of age in the scientific, cultural, educational and communication fields;
- promoting the implementation in the system of services provided for people of age of the the recommendations of the World Assembly of People of Age, of the European Community and of the recommendation expressed on scholarly level by the medicine Universities of Romania, Sweden, Greece, Israel, Thailand, Germany as well as of other countries where the Academy has established branches.
- organization and working of an excellence medical centre containing medical services and services of scientific investigations in various fields to provide for a high level of medical assistance and attendance of medical, socio-medical or psycho-affective nature for people of age who suffer from total or partial loss of their autonomy.
- initiation, organization and support for educational, research and scientific projects and programmes for the medical staff (doctors, nurses, social assistants)and promotion of innovations and their implementation in the system of the services provided for people of age;
- initiation, organization and support for courses, workshops, discussions, conferences in the interest fields of the Academy and of „Ana Aslan International“ Foundation and the promotion of co-operation between the speciality university departments of the Faculties of Medicine in Romania, Sweden, Greece, Israel, Thailand, Germany, as well as in other countries were the academy has branches.
- granting scholarships and research fellowships for students, candidates for doctor’s degree, participants in the university and postgraduate programmes, doctors, in the interest fields of the Academy and of „Ana Aslan International“ Foundation.

"Ana Aslan"
International Academy of Aging

Our task and values

The purpose of the „Ana Aslan“ International Academy of Aging, a scientific and cultural academy organized by the „Ana Aslan“ International Foundation, is to set up, organize and support projects and programs in the medical, cultural, educational and communication fields of aging.

The „Ana Aslan“ International Academy of Aging focuses on health care, training, research and service system innovations that will ensure healthy aging. This includes to:

- **Enhance and expand the training of doctors, nurses, social workers and other health professionals who care for the elderly.**
- **Promote innovations in the integration and delivery of services for all elderly.**
- **Encourage and assist the development of future leaders in the field of aging, both in clinical and basic research.**
- **Expand medical research on aging through focusing on the biology of aging, diseases and disabilities of old age and clinical management issues.**

AREAS OF ACTIVITY

- Clinical Aging Research
- Epidemiological Aging Research
- Gerontological Nursing
- Physiotherapy and Occupational Therapy
- Dementia and Cognitive Disorders
- Neuroscience with focus on Molecular
- Pharmacology
- Social Science
- Health economy
- Drugs trials
- Gerontoprophilaxy

The „Ana Aslan“ International Academy of Aging will provide the hub of a network of research dealing with aging. As an active research center in itself it will also provide a rich environment where doctoral and masters students can learn from senior researchers with a wide variety of academic perspectives. Researchers from many institutions and departments will come to the „Ana Aslan“ Academy of Aging and to the Research and Education Centers so as to meet, attend courses, workshops and seminars and to give support and stimulation.

ACTIONS

Publication, support and promotion of scientific works and printed materials, including „Brain Aging“ an International Journal published by the „Ana Aslan“ International Academy of Aging.

Organization and working of medical research centers and centers of excellence containing medical services and services of scientific investigations in various fields, with the aim of providing a high level of medical, socio-medical nature for elderly people.

Initiation, organization and support for educational, research and scientific projects and programs for the medical staff (doctors, nurses, social assistants).

The First International Conference of Brain Aging – An International Journal

BUCHAREST, ROMANIA
OCTOBER 5th - 8th
THE "MARRIOTT" HOTEL - BUCHAREST, ROMANIA

www.brainaging.ro
or **Contact Organising Committee:**

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BRAIN AGING
INTERNATIONAL JOURNAL

Why a Conference of Brain Aging - An International Journal ?

The Scientific Board of the Brain Aging International Journal has been decided to organize every year an International Meeting on Brain Aging.

Who Should Attend ?

Physicians and health care workers with a special interest in the mental health and illnesses of the elderly.

These include:

Physicians in Family, General and Geriatric Medicine; Psychiatry; Neurology as well as

Nurses; Researches; Psychologists; Social workers; Program Directors; Administrators/Facilitators; Pharmacists;

and others.

The First International Conference of Brain Aging & Aging and Dementia

THE "MARRIOTT" HOTEL

Bucharest, Romania
October 6 - 8, 2003

TOPICS:

- EPIDEMIOLOGY
- GENETICS, BIOMARKERS, NEUROIMAGING and HISTOPATHOLOGY
- BASIC MECHANISMS INVOLVED IN BRAIN AGING and DEMENTIAS
- THERAPEUTIC STRATEGIES
- DIAGNOSIS / IMAGING
- ALZHEIMER DISEASE AND NON-ALZHEIMER'S DISEASE DEMENTIAS

TIME TABLE, REGISTRATION FEES AND DEADLINES

The Conference will be preceded by a one-day Training Course for Romanian Physicians (sponsored by Novartis, Romania)

REGISTRATION FEES:

Course Attendees, special rate (includes Conference) $ 345
Conference Attendees:

Academia	$ 345
Industry	$ 485
Students	$ 200
Accompanying person	$ 120

SATURDAY, OCTOBER 4, 2003 - CONFERENCE REGISTRATION

Sunday, October 5, 2003 - Conference

	Constanta Plenary Session	Salon A	Salon B Oral Presentation	Salon C Oral Presentation	Foyer - Poster Presentation
9:00- 9:20	**Conference Opening Ceremony**				
9:20- 9:40	**Remi Quirion**				
9:40-10:00	**Hilkaa Soininen**				
10:00-10:20	**Kurt Jellinger**				
10:20-10:40	**Ezio Giacobini**				
10:40-11:00	**Khalid Iqbal**				
11:00-11:30	Coffee Break				

	Constanta Plenary Session	Salon A	Salon B Oral Presentation	Salon C Oral Presentation	Foyer - Poster Presentation
11:30-11:50	**Inge Grugke Iqbal**				
11:50-12:10	**Emil Toescu**				
12:10-12:30	**Luiza Spiru**				
12:30-12:50	**Richard Cowburn**				
13:00-14:00	Lunch				
14:00-16:00	Novartis Switzerland Dedicated Symposia - speaker: Prof. Bruno Vellas				
14:00-14:20			**Patrizia Mecocci**	**Caroline Chang**	
14:20-14:40			**Lars-Olaf Wahlund**	**Nicholas Sergeant**	
14:40-15:00			**Lutz Frolich**	**Giulio Maria Pasinetti**	
15:00-15:20			**Robert Elsner**	**Lars Backman**	
15:20-15:40			**Reinhold Schmidt**	**Paolo Prolo**	
15:40-16:00			**Delia Mut Popescu**	**Antonio Metastasio**	
16:00-16:30	Premium Coffee Break				
16:30-18:00	Sicomed Romania Dedicated Symposia				
16:30-16:50			**Claudio Cuello**	**Ng Tze Pin**	
16:50-17:10			**Akihiko Takashima**	**Manfred Windisch**	
17:10-17:30			**Gianluigi Forloni**	**Lin Li**	
17:30-17:50			**Mikal Novak**	**Shi Du Yan**	
19:00	Galla Dinner Ceremony Press Conference sponsored by Lundbeck Romania - New Product Launch				

Monday, October 6, 2003 - Conference

9:00- 9:20	**Gordon Wilcock**				
9:20- 9:40	**Lon Schneider**				
9:40-10:00	**Gunhild Waldemar**				
10:00-10:20	**Raj Kalaria**				
10:20-10:40	**Juergen Sautter**				
10:40-11:00	**Bruno Vellas**				
11:00-11:30	Coffee Break				
11:30-13:00	EBIXA - Lundbeck Denmark Dedicated Symposia and Press Conference speakers: Prof. Bengt Winblad, Prof. Luiza Spiru, Prof. Gruhild Waldeman				
13:00-14:00	Lunch				
14:00-14:20			**Maria Ankarcrona**	**Chengxin Gong**	
14:20-14:40			**Jianzhi Wang**	**Vince S. Thomas**	
14:40-15:00			**Ovidiu Bajenaru**	**Elka Stefanova**	
15:00-15:20			**Catalina Tudose**	**Gary Wenk**	
15:20-15:40			**Eric Salmon**	**Eugeen Vanmechelen**	
15:40-16:00			**Alex. Serbanescu**	**Ioan Romosan**	
16:00-16:30	Premium Coffee Break				

	Constanta Plenary Session	Salon A	Salon B Oral Presentation	Salon C Oral Presentation	Foyer - Poster Presentation
16:30-16:50			**Sandrine Andrieu**	**Sergiu Blumen**	
16:50-17:10			**Magda Tsolaki**	**Simona Sacuiu**	
17:10-17:30			**Mario Impallomeni**	**Tanya Pekmezovic**	
17:30-17:50			**Adrian Wilson**	**Michael Borrie**	
19:00		Dinner - Brain Aging International Journal Scientific Editorial Board			

Tuesday, October 7, 2003 - Conference

	Constanta Plenary Session	Foyer Braila-Ploiesti, Foyer A & B & C, Braila and Ploiesti Poster Presentation
9:00- 9:20	**Masatoshi Takeda**	
9:20- 9:40	**Eva Mandelkow**	
9:40-1 0:00	**Eckhard Mandelkow**	
10:00-10:20	**Danny Michelson**	
10:20-10:40	**Agneta Nordberg**	
10:40-11:00	**Laura Fratiglioni**	
11:00-11:30	Coffee Break	
11:30-13:00	OFFICIAL ROMANIAN B.A.I.J. LAUNCH	
13:00-14:00	Festivity Lunch	

Wednesday, October 8, 2003 - Post Conference Training Course

	Salon A & Salon B
8:45- 9:30	Laura Fratiglioni Epidemiology and Risk Factors
9:30-10:15	Bengt Winblad Treatment strategies of Cognitive Impairments
10:15-11:00	Bruno Vellas Nutritional Aspects on AD Patients
11:00-11:30	Coffee Break
11:30-12:15	Kurt Jellinger Cerebrovascular Pathology and Alzheimer's Disease
12:15-13:00	Khalid Iqbal Neurobiology of Brain Aging and Dementias
13:00-14:00	Lunch
14:00-15:00	Round table 1: Risk Factors and Diagnosis
15:00-16:00	Round table 2: Therapeutics
16:00-16:30	Premium Coffee Break
16:30-17:30	Round table 3: Neuropathology and Molecular Pathology
17:30-18:00	Conclusions, Discussions, Awards

Prevalence of Dementia in Greek Orthodox Monasteries The Role of Diet Poor in Lipids and Style of Life

Tsolaki M[1], Pantazi Ch.[1], Stiliou F., Aminta M.[1], Diudi, P. Karasoulas S., Kazis A.[1], Pollen D.[2] ,

[1] 3rd Department of Neurology, Aristotle University of Thessaloniki

[2] University of Massachusetts, Boston

Abstract

The aim of this study was the prevalence of dementia in Greek Orthodox monasteries. The monks and nuns who live in these monasteries live far from the community, most of them are not married and their diet is poor in lipids. All do not eat meat at all and milk products for more than half a year. WHO questionnaire was translated and used at the first phase. One hundred sixty three subjects above 70 years were asked to complete the questionnaire. Finally, one hundred forty nine monks and nuns were examined (103 nuns and 46 monks) 60-100 years old in 56 monasteries in Greece and Cyprus. Fourteen monks denied to complete the questionnaire. Two general practitioners, two neurologists and a neuropsychologist examined the monks and nuns after the appropriate training. The results showed that only 14 from 149 above 70 years old showed dementia, 9 nuns and 5 monks with mean age 84.25±7.56. The age specific prevalence of AD was 0% between 70-74, 9.68% between 75-79, 8.34% between 80-84, 15.38% between 85-89 and 50% over 90 years old. Conclusively, the prevalence of dementia increased steeply with advancing age in all groups. Dementia is not present in ages between 60 to 75 years in monasteries. This is a great difference between our study results and all other prevalence studies. In Greece, either in community or in monasteries the prevalence rate is lower than in other countries in South Europe. The consumption of meat and the different style of life may be related with low prevalence rates in ages 60-75.

Keywords: Prevalence, dementia, AD, orthodox monasteries

Introduction

Dementia is going to be one of the major challenges of this new century for our societies due to the enormous burden on the health care systems. It is the major cause of death and the most important risk factor for disability and entry into a nursing home in elderly people.

Results from 36 prevalence studies showed that prevalence is equal to 0.3 to 1% in individuals aged 60-64 years and increases to 42.3 to 68.3% in individuals 95 years and older. The only study about prevalence of dementia in Greece reveals lower rates than surveys carried out elsewhere. The age specific prevalence of dementia in Greece was 4,24 between 70-74, 9.82 between 75-79, 10.64% between 80-84, 14.71% between 85-89 and 57.14% over 90 years old.

It is well known that the only generally accepted risk factors are genetics, only for 0.5% of all the cases, age and family history. Although a small number of early onset, dominantly inherited cases of familial AD and FTD are caused by genetic mutations, the cause of most sporadic cases of dementia is presently unknown.

Until recently, the abnormally entwined pairs of tau protein filaments were thought to be innocuous secondary events. However Robert Terry and Robert Katzman a decade ago found that the density of neurofibrillary tangles in AD is related to the severity of dementia while the density of plaques is only weakly correlated with the severity of dementia. There is an hypothesis that AD pathogenesis results from disruption of cholesterol uptake and metabolism and that this in turn results in abnormal trafficking of membrane proteins critical to normal neuronal function and synaptic plasticity.

Prevalence of Dementia in Greek Orthodox Monasteries The Role of Diet Poor in Lipids and Style of Life

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It is well known that the only generally accepted risk factors are genetics, only for 0.5% of all the cases, age and family history. Although a small number of early onset, dominantly inherited cases of familial AD and FTD are caused by genetic mutations, the cause of most sporadic cases of dementia is presently unknown.

Until recently, the abnormally entwined pairs of tau protein filaments were thought to be innocuous secondary events. However Robert Terry and Robert Katzman a decade ago found that the density of neurofibrillary tangles in AD is related to the severity of dementia while the density of plaques is only weakly correlated with the severity of dementia. There is an hypothesis that AD pathogenesis results from disruption of cholesterol uptake and metabolism and that this in turn results in abnormal trafficking of membrane proteins critical to normal neuronal function and synaptic plasticity.

It is well known that the amount of fatty acids is elevated in the brains of AD patients. The results of a recent study indicated that fatty acid oxidation can facilitate the polymerization of tau. However, "overoxidation" of the fatty acids can inhibit the process. The authors of this study postulate that specific fatty acids oxidative products could provide a direct link between oxidative stress mechanisms and the formation of neurofibrillary tangles in AD. The epidemiological reduction of risk of AD by statins, could in part result from changes in APP and β-secretase localization and decreased Aß production in brain.

A high intake of saturated fat and cholesterol and a low intake of polyunsaturated fatty acids have been related to an increased risk of cardiovascular disease. On the other hand, cardiovascular disease has been associated with dementia. Rotterdam study suggests that a high saturated fat and cholesterol intake increases the risk of dementia, whereas fish consumption may decrease the risk.

There is evidence that high serum total cholesterol may be an independent risk factor for AD and some of the effect of the APOE epsilon 4 allele on risk of AD might be mediated through high serum cholesterol vascular risk factors are correlated with dementia.

There is also some evidence that the term vascular dementia is not correct but there are some clinical determinants of post stroke dementia and also some pathological features are the same between AD and VaD.

We decided to study the possibility that diet without meat for many years or diet with meat only the half days of a year in two different communities in Greece and Cyprus could lower the risk of developing dementia and if there was a difference in prevalence rates between these two different communities.

Methods

Study population

Study population consisting of two different groups was identified.

Group A: One hundred sixty three monks or nuns who live in 56 different monasteries in Greece and Cyprus and most of them don't eat meat for many years and eat milk products such as milk, butter, cheese less than half days of a year. Their style of life is also different. They don't smoke and they drink small amounts of wine. Most of them are not married and live far from the community in the monasteries, which are built far from the cities for many years. These people work some hours during the day and pray and read many hours of a day so that to succeed a quality of spiritual life like God. The distinguished nature of the location where the monasteries are built (e.g. Athos Mountain, other mountains with woods and lakes and sea) help monks and nuns to come near God. They sleep about 6 hours a day. Fourteen denied to give any information. We had completed questionnaires from 149, 103 female and 46 male. Their demographics are showed on table 1.

Table 1. Demographics of demented and controls.

	Age Mean (SD)	Education (years) Mean (SD)
Male(n=46)	79.30±7.13	7.77±3.82
Female (n=103)	75.82±7.67	6.41±4.55
	p>0.05	

	Age Mean (SD)	Education (years) Mean (SD)
Dementia	84.25±7.56	5.70±3.92
Control	76.18±7.42	6.77±4.39
	p<0.05	

	MMSE Mean (SD)	FAQ Mean (SD)
Dementia	12.67±3.98	18.38±12.02
Control	24.05±4.29	1.21±2.22
	p<0.001	p<0.05

Group B: Women who were not married but they lived and worked in the community as nurses in Hospitals. They live all together and the aim of their life is to help people and try to live as God wishes. They don't eat meat 40 days before Christmas, 50 days before Easter, 15 days in June and 15 days in August. During the whole year they don't eat meat and milk products every Monday, Wednesday and Friday.

The differences between the two groups are 1. the diet, 2. the location of their houses and 3. the less stress in the A group.

We decided to correlate these results with a third group (C), which is a community door-to-door study with the same stress and the same location of their houses with the B group. The only difference between B and C group was the diet and marital status.

Three trained physicians – one woman, MA and two men, S.K. and F.S. – and one trained neurophychologist, P.N. visited the nuns and monks respectively and completed the WHO questionnaire about dementia. One young trained nurse, E. who lives in the same house with the nurses completed the same questionnaire in Athens. This questionnaire includes subject demographics and characteristics (e.g. smoking, alcohol drinking), questions about cognition which include all the questions of MMSE, Functional Activities Questionnaire (FAQ), symptoms, medical diagnoses (e.g. Diabetes mellitus, Hypertension, Strokes, Depression, Head injuries), hospital admissions, and drug prescriptions (including root of administration, dose, and number of tablets for each prescription). All the information received by investigators is computerized.

We used Mann-Whitney test for the statistical analysis of age, education and tests MMSE and FAQ.

Results

The number of demented persons was 14. From the 14 demented 9 are nuns and 5 monks, with mean age 84.25±7.56 years. The prevalence of dementia above 60 years old is 9.3 with 0% between 60 to 74 years old (table 2).

The prevalence of dementia in unmarried nurses is showed on table 3.

The prevalence of dementia is higher in the community than in nurses and monasteries and the prevalence rate in 70-74 is 4.24% and in 75-79 years 9.82% (table 4).

We can see a table 5 of prevalence rate in the same ages in Pamplona, Spain where olive oil is also used by people and the prevalence rate is twice higher than Greek prevalence.

The number of the examined persons was small so we could not see any differences in the existence in other very important diseases, which could be risk factors for dementia (table 6).

Discussion

This study is a preliminary study. Unfortunately we cannot find many elderly orthodox monks and nuns who follow this kind of diet. Although we visited 56 monasteries only 149 accepted to respond.

Table 2. Age-specific prevalence of dementia in monasteries

Age	D	T	prevalence(%)
60 - 64		8	
65 - 69		14	
70 - 74		39	
75 - 79	2	31	6.4
80 - 84	5	36	13.8
85 - 89	4	13	37.5
90+	3	8	37.5
Total	14	149	9.3

Table 3. Age specific prevalence of dementia in unmarried nurses

Age	D n	T n	prevalence(%)
70 - 74		27	
75 - 79	1	15	6.67
80 - 84	1	9	22.22
85 - 89	1	3	33.33
90+	1	1	100
Total	5	55	9.09

The only very interesting results are that 1. these people live more than other people in the community – we could examine 8 from 149 above 90 years old in the monasteries while we examined only 7 from 365 above 90

Table 4. Age specific prevalence of dementia in Pilea, a city in North Greece

Age	D n	Total n	Prev (%)
70 - 74	5	118	4.24
75 - 79	11	112	9.82
80 - 84	10	94	10.64
85 - 89	5	34	14.71
90+	4	7	57.14
Total	35	365	9.59

Table 5. Age specific prevalence of dementia in Pamplona, Spain

Age	D n	Total n	Prev (%)
70 - 74	9		6.3
75 - 79	35		11.8
80 - 84	50		17.3
85 - 89	70		25.6
90+	30		34.7
Total	194	1019	19

years old in Pilea – and 2. there is no demented patient until 75 years old.

Our results are about the same as the prevalence rates in Africa. The most recent studies reveal rather higher rates but still lower than surveys carried out elsewhere. The possible reasons for these findings in Africa are considered: differential survival rates, the hiding of cases by relatives because of stigma, reluctance to seek medical assistance as inappropriate, poor access to medical care, the feeling that the old person has come to the end of his useful life and defective case-finding techniques. None of these factors can explain the low prevalence rates in our study

Both groups – all of the B group and at the most of the A group – are not married. They were not married because they didn't want to be married in order to give themselves to the God.

The association between marital status and mortality is well known; marital status has also been related to morbidity. There is one study that showed that the age-standardized prevalence of dementia and the proportions of elderly Canadians living in institutions with and without dementia are highest among single people and are also high for those who were previously married.

These associations hold true for both women and men, but the relation between marital status and institutionalization is much stronger for men. If marital status alone was a risk factor for dementia then our groups had to have higher rates of dementia than other groups. Perhaps the cause or the reason why one person is not married is significant.

Table 6. Percentage of other diseases in demented and non-demented persons.

	DIABETES N (%)	HYPERTENSION N (%)	HEAD INJURY N (%)	HEART ATTACK N (%)	STROKE N (%)	PARKINSON N (%)	DEPRESSION N (%)
Dementia	0	1 (14.29%)	1 (16.67%)	2 (28.57%)	1 (25.00%)	0	0
Control	14 (12.73%)	38 (34.55%)	18 (16.67%)	6 (5.66%)	10 (11.49%)	6 (5.77%)	7 (6.73%)

Conclusions

1. The prevalence of dementia increased steeply with advancing age. This is a conclusion as in all other studies all over the world.

2. Dementia is not present in ages between 60 to 75 years in monasteries. This is a great difference between our study results and all other prevalence studies.

3. In Greece, either in community or in monasteries the prevalence rate is lower than in other countries in Southern Europe, such as Pamplona, Spain. This suggest that only the use of olive oil is not useful for lower rates of dementia.

4. The prevalence rate in 80-84 ages in monasteries is half of the rate in the community. This is very important because this age is the mean survival age of people in Greece.

5. Finally, meat may be a risk factor of dementia. People in Pylea were poor for many years and they couldn't eat meat for many years. Nurses eat meat half days of a year and monks and nuns eat no meat at all. The consumption of meat may be related with prevalence rates.

References

1. Fratiglioni L., De-Ronchi D., Aquero-Torres H. Worldwide prevalence and incidence of dementia. *Drugs-Aging* 1999; 15: 365-75.
2. Tsolaki M., Fountoulakis C., Pavlopoulos I., Chatzi E., Kazis A. Prevalence and incidenece of Alzheimer's Disease and other dementingdisorders in Pylea, Greece. *Am J Alzheimer's Disease* 1999; 15: 138-148.
3. Lynch C., Mobley W. Comprehensive theory of Alzheimer's Disease. The effects of cholesterol on membrane receptor trafficking. *Ann N Y Acad Sci* 2000; 924: 104-11.
4. Gamblin TC, King ME, Kuret J., Berry RW, Binder LI. Oxidative regulation of fatty acid-induced tau polymerization *Biochemistry* 2000; 39: 14203-10.
5. Jick H., Zornberg G.L., Jick S.S., Seshadri S., Drachman DA. Statins and the risk of dementia. *Lancet* 2000; 356: 1627-31.
6. Sidera C., Frimpong Manso J., Liu C., Austen B.M. The role of Cholesterol in the Processing of ß-secretase ASP-2, 2nd International Congress on Vascular Dementia, Salzburg January 24-27, 2002; 147-153.
7. Kalmijn S., Launer ., Ott A., Witteman , Hofman A., Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam study. *Ann Neurol* 1997; 42: 776-82.
8. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, Apolipoprotein E and prevalence of dementia and Alzheimer's Disease in the Rotterdam study. *Lancet* 1997; 349: 151-4.
9. Pfeffer RI, Kurosaki TT, Harrah CH Jr Measurement of functional activities of older adults in the community. *J Gerontol* 1982; 37: 323-329.
10. Manubens JM, Martinez-Lage, Lacruz F, Muruzabal J, Larumbe: Prevalence of clinically diagnosed Alzheimer's Disease and other demented disorders in Pamplona. *Neuroepidemiology* 1995; 14: 155-164.
11. Ineichen B. The epidemiology of dementia in Africa: a review. *Soc Sci Med.* 2000; 50: 1673-7.
12. Kristjansson B, Helliwell B, Forbes WF, Hill GB. Marital status, dementia, and institutional residence among elderly Canadians: The Canadian study of Health and Aging. *Chronic Dis Can* 1999; 20: 154-7.

Presenilin Proteins and the γ-Secretase Complex Generating β-Amyloid and APP Intracellular Domain

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Abstract

One characteristic neuropathological finding in brains from Alzheimer's disease patients is the occurrence of amyloid plaques consisting of the amyloid β-peptide (Aβ). The Aβ-peptide is generated from the amyloid precursor protein by the consecutive cleavages of β- and γ-secretase. γ-Secretase is a multisubunit protease consisting of the proteins presenilin, nicastrin, Aph-1 and Pen-2. Intramembrane proteolysis of a number of type I integral membrane proteins critically require γ-secretase activity. For instance, γ-secretase mediates nuclear signalling from the Notch family of cell surface receptors. γ-Secretase hydrolysis is undoubtedly a complex event that will be challenging to mechanistically elucidate. Moreover, the task of developing drugs that specifically target γ-secretase processing of the amyloid precursor protein is likely to be daunting.

Keywords: Alzheimer's disease, RIP, amyloid β-peptide, γ-secretase, presenilin

Alzheimer's disease and the amyloid precursor protein

Alzheimer's disease is neuropathologically characterized by the deposition of the 40-42 residue amyloid β-peptide (Aβ) into plaques in the brain parenchyma and cerebral blood vessels. Neurofibrillary tangles, composed of the protein tau, is another pathological change observed in brain tissue from patients diagnosed with Alzheimer's disease[1].

The amyloid precursor protein (APP), from which Aβ is derived, is a type I membranespanning glycoprotein with its N terminus in the intralumenal space and the C terminus in the cytosol. The function of APP is not fully understood. Its predicted structure suggests that it is a receptor, however no ligand has yet been identified. The APP knockout mouse lack a distinct phenotype[2], precluding the elucidation of the function of APP. Most likely, the APPlike proteins (APLP) 1 and 2, which are highly homologous to APP, can compensate for at least some of the functions of APP in these mice. APP and APLP2, and APLP1 and APLP2, double knockout mice die early after birth, while the APP and APLP1-deficient mice survive and appear normal[3]. APP has, among other things, been suggested to function in cell adhesion[4] and in kinesin-mediated axonal transport of vesicles[5].

APP is cleaved by α- or β-secretase generating the C-terminal fragments C83 or C99, respectively. Additional processing of the C-terminal fragments by γ-secretase produces the p3 or Aγ peptides (fig. 1). The γ-secretase cleavage is unusual in that it occurs within the hydrophobic milieu of the membrane bilayer. This type of cleavage will hereafter be referred to as intramembrane proteolysis.

The α-cleavage is performed by TNF-α converting enzyme (TACE)[6] or ADAM-10[7]. Cleavage at the β-site is executed by β-site APP cleaving enzyme (BACE)[8-11]. Mutations in APP at, or close to, the secretase cleavage sites have been found in patients suffering from familial Alzheimer's disease. The majority of these mutations affect Aβ production by specifically increasing Aβ42 both *in vitro* and *in vivo*[12]. Aβ42 is more prone to aggregate than Aβ40, and is the major Aβ species found in the core of amyloid plaques[13]. Recently, another cleavage event, designated ε-cleavage, has been shown to occur after position 49 in Aβ[14-16].

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The C-terminal protein product resulting from this cleavage is called APP intracellular domain (AICD) (fig. 1). This ε-cleavage event is strikingly similar to the S3 cleavage occurring in the Notch receptor. Further similarities between the processing of Notch and APP is provided by studies suggesting that AICD regulate gene transcription[17]. AICD binds to the nuclear adaptor protein Fe65 and the histone acetyltransferase Tip60[17]. No target gene or genes have so far been reported for this transcriptional regulatory complex. Recently, a physiological signaling role for AICD was reported suggesting that AICD regulates phosphoinositide-mediated calcium signaling[18].

undergoes endoproteolysis, generating a stable N- and C-terminal fragment heterodimer (fig.2).

The PS proteins are critically required for γ- and ε-secretase activity, generating Aβ and AICD, respectively. PS1 and PS2 double knockout mice die before birth and their phenotype resembles the phenotype for Notch 1 knockout mice[23]. Blastocyst-derived stemcells from these PS null mice produce no Aβ[24,25]. Mutagenesis of two conserved aspartates in transmembrane domains (TMD) 6 and 7 (fig. 2) results in PS molecules deficient in supporting Aβ generation[26]. Overexpression of PS in cell culture systems does not lead to increased Aβ formation. Overexpressed PS accumulates as the full length protein, suggesting that endoproteolysis of the full-length PS into the presumably active heterodimer form is highly regulated[27].

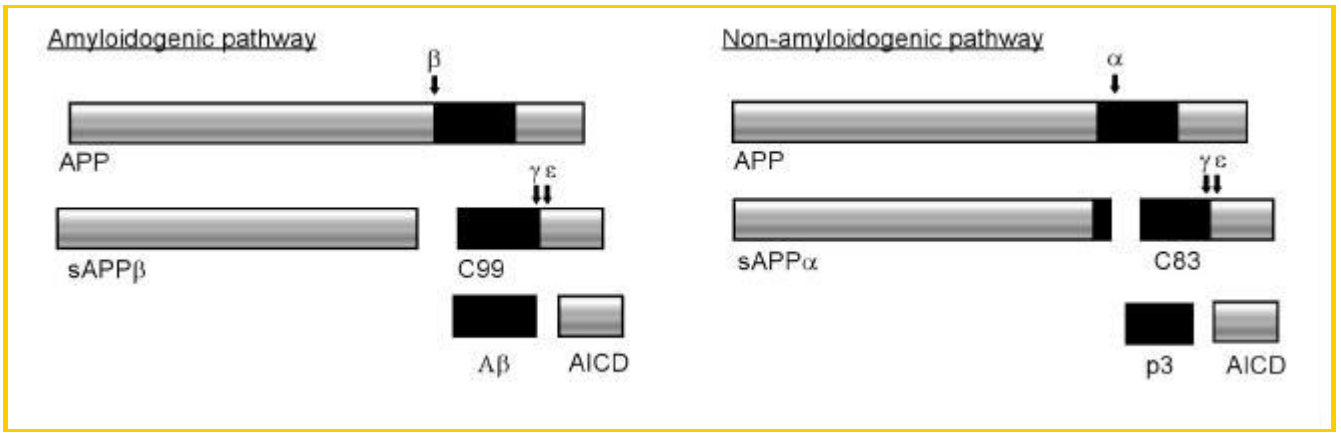

Figure 1. Illustration of the amyloidogenic and non amyloidogenic processing pathways of APP. In the amyloidogenic pathway soluble sAPPb and Ab is generated. In the non-amyloidogenic pathway soluble sAPPa and the peptide fragment p3 is generated. The p3 peptide cannot form plaques. In both processing pathways the APP intracellular domain (AICD) is formed via the e-cleavage.

The Presenilins

The gene encoding presenilin (PS) 1 was identified in 1995 by genetic linkage of an autosomal dominant form of hereditary early-onset Alzheimer's disease to chromosome 14[19]. The PS2 gene was found on chromosome 1 by homology searches of databases for amino acid sequences with high similarity to PS1[20]. At present, more than 120 mutations causing familial Alzheimer's disease have been identified in PS1, while only eight mutations leading to hereditary Alzheimer's disease have been found in PS2 (http://molgenwww.uia.ac.be/ADMutations/). The PS proteins are multipass transmembrane proteins. The most favored topological model suggests an eight transmembrane topology, where the N and C termini are located in the cytoplasm[21] (fig. 2), while other studies indicate a seven transmembrane topology[22]. Both topological models predicts a cytoplasmic loop in which PS

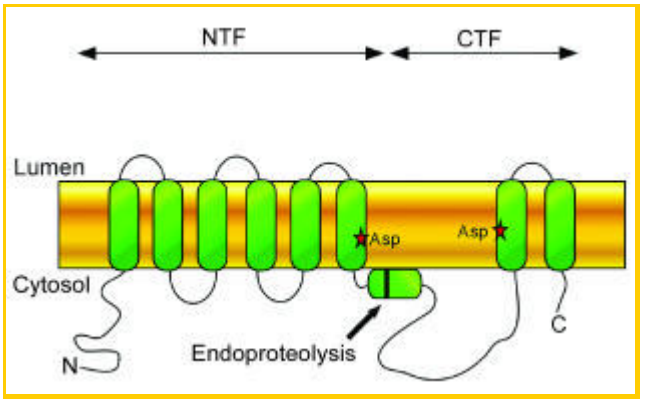

Figure 2. Illustration of the PS protein with eight transmembrane spanning domains. The PS protein undergoes endoproteolysis in the hydrophobic part of the large cytolplasmic loop and form a stable heterodimer consisting of an N -and C- terminal fragment (NTF and CTF). The critical aspartate residues for γ-secretase activity in TMD 6 and 7 are indicated with the red stars.

Recently, several type I proteins have been reported to undergo a PS-dependent cleavage within their transmembrane domains, in a process referred to as regulated intramembrane processing, or RIP[28]. Among the proteins undergoing RIP are APP, APLP1 and 2, CD44, ErbB-4, E-cadherin, Notch 1-4 and the Notch ligands Delta and Jagged[29-34]. The list of PSdependent RIP-substrates is currently growing and it appears that this proteolytic event, strange as it may seem since it occurs in the anhydrous environment of the lipid bilayer, is a conserved and general processing pathway in the cell.

Other γ-secretase complex components: nicastrin, Aph-1 and Pen-2

Nicastrin is a type I membrane protein found in the same protein complex as PS[35] and is required for γ-cleavage to occur[36-39]. The 709-residue nicastrin protein is a glycoprotein with a large ectodomain and a short cytoplasmic tail (fig. 3). Its function is not known, but it has been suggested that nicastrin brings the substrate and γ-secretase together. PS1 is required for the correct maturation of nicastrin[40-43], however the maturation status of nicastrin does not seem to affect γ-secretase activity per se[44].

Recently, two new proteins required for γ-cleavage were identified[45,46] (fig. 3). The Aph-1 and Pen-2 proteins were isolated in a genetic *Caenorhabditis elegans* screen for phenotypes resembling PS homologue deficiency. The seven TMD Aph-1 protein has two mammalian homologues, Aph-1a and Aph-1b[45]. Aph-1a also exists in two major splice variants, L and S[46]. Pen-2 has two predicted TMDs and one human homologue has been found[46]. A Pen-2 knockout mouse model has been developed which displays a phenotype similar to Notch-deficient mice; the embryos die before birth at E9-10[47].

The functional impact of these two novel γ-secretase components is just starting to emerge. Pen-2 has been reported to be essential for the endoproteolytic cleavage of PS148. In the absence of PS proteins, Pen-2 is rapidly degraded while the stability of Aph-1 is not affected in an appreciable way[49]. Aph-1 has been suggested to stabilize the full length PS1 protein[48].

Overexpression of either one of the four γ-secretase complex components do not lead to an increased Aβ generation, while overexpressing all four proteins facilitate γ-processing of APP[50-52]. Under these conditions PS1 processing into the stable heterodimer is increased, and more fully glycosylated nicastrin protein is produced. Moreover, overexpression of all four components in yeast cells that cannot process APP, restore γ-secretase processing and Aβ/AICD formation[51]. *In vitro* reconstitution of γ-secretase activity from purified components is yet to be performed.

The γ-secretase complex have been isolated under non-denaturing conditions and the estimated molecular weight reported is ranging from 200 kDa[52] to 500 kDa[42]. Much interest is now focused on determining the stoichiometry of the complex, and if different complexes exist. One can envision that there are complexes containing different homologues of the components, which possibly could influence substrate specificity. Most evidence suggests that PS is the enzyme component of the complex and that the conserved aspartates in TMD 6 and 7 provide the catalytic residues. The loss of enzyme activity in the

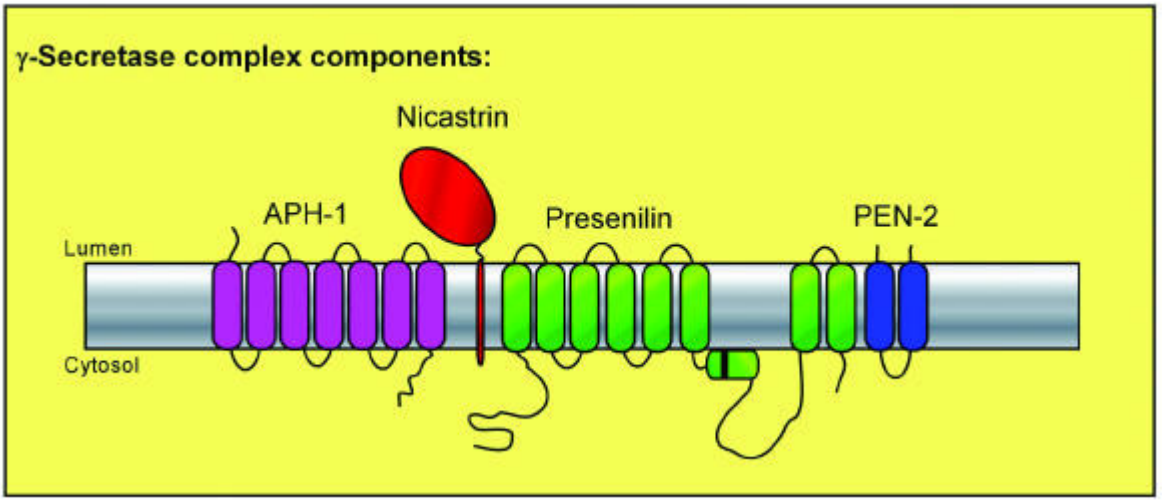

Figure 3. A schematic illustration of the identified components of the γ-secretase complex: PS with 8 TMDs, nicastrin- a type I integral membrane glycoprotein, Aph-1 with 7 TMDs and Pen-2 with 2 TMDs.

abscence of these residues implies that PS belongs to the aspartyl protease protein family, and the homology of PS to signal peptide peptidase family of aspartyl proteases further corroborates this hypothesis [53].

Not much is known how the γ-complex is assembled and what the ratios of the individual components in the complex are. It is also likely that there are additional modulatory proteins that are part of the active γ-secretase complex. These are important questions for understanding the exact mechanism of γ-secretase processing and the answers to these questions will be essential for the process of developing drugs targeting the activity of the γ-secretase complex. It will be a pharmaceutical challenge to target γ-secretase and create an Aβ lowering drug, while not affecting Notch signaling, or intramembrane processing of other RIP substrates. However, it is encouraging that non-peptide inhibitors reduce Aβ generation while having little effect on S3-processing of Notch[54]. In addition, lithium has been shown to reduce γ-secretase processing of APP in the absence of any effect on Notch processing[55]. It is also promising that the BACE knockout mice and the heterozygous nicastrin mice display a normal phenotype. Aβ formation is completely abrogated in BACE$^{-/-}$ mice[56] and reduced in the nicastrin$^{+/-}$ mice[57], making these proteins attractive targets for diminishing Aβ production. The crystal structure of BACE suggests that the enzyme has a large catalytic pocket[58], making the task of specifically targeting this pepsin protease family member with small molecular weight compounds difficult. The intense research efforts of the last couple of years have greatly increased our knowledge about the proteolytic activities generating Aβ, and we now face exciting times when studies of how to inhibit the detrimental production of Aβ, without causing adverse side-effects, will be initiated and executed.

References

1. Selkoe D.J., Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev*. 2001; 81: 741-66.
2. Zheng H., Jiang M., Trumbauer M.E., Sirinathsinghji D.J., Hopkins R., Smith D.W., Heavens R.P., Dawson G.R., Boyce S., Conner M.W., et al., β-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell*. 1995; 81: 525-31.
3. Heber S., Herms J., Gajic V., Hainfellner J., Aguzzi A., Rulicke T., von Kretzschmar H., von Koch C., Sisodia S., Tremml P., Lipp H.P., Wolfer D.P., Muller U., Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. *J Neurosci*. 2000; 20: 7951-63.
4. Schubert D., Jin L.W., Saitoh T., Cole G., The regulation of amyloid beta protein precursor secretion and its modulatory role in cell adhesion. *Neuron*. 1989; 3: 689-94.
5. Kamal A., Almenar-Queralt A., LeBlanc J.F., Roberts E.A., Goldstein L.S., Kinesin-mediated axonal transport of a membrane compartment containing β-secretase and presenilin-1 requires APP. *Nature*. 2001; 414: 643-8.
6. Buxbaum J.D., Liu K.-N., Luo Y., Slack J.L., Stocking K.L., Peschon J.J., Johnson R.S., Castner B.J., Cerretti D.P., Black R.A., Evidence that Tumor necrosis factor á converting enzyme is involved in regulated α-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem*. 1998; 273: 27765-67.
7. Lammich S., Kojro E., Postina R., Gilbert S., Pfeiffer R., Jasionowski M., Haass C., Fahrenholz F., Constitutive and regulated á-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc Natl Acad Sci U S A*. 1999; 96: 3922-7.
8. Lin X., Koelsch G., Wu S., Downs D., Dashti A., Tang J., Human aspartic protease memapsin 2 cleaves the β-secretase site of β-amyloid precursor protein. *Proc Natl Acad Sci U S A*. 2000; 97: 1456-60.
9. Yan R., Bienkowski M.J., Shuck M.E., Miao H., Tory M.C., Pauley A.M., Brashier J.R., Stratman N.C., Mathews W.R., Buhl A.E., Carter D.B., Tomasselli A.G., Parodi L.A., Heinrikson R.L., Gurney M.E., Membraneanchored aspartyl protease with Alzheimer's disease β-secretase activity. *Nature*. 1999; 402: 533-7.
10. Vassar R., Bennett B.D., Babu-Khan S., Kahn S., Mendiaz E.A., Denis P., Teplow D.B., Ross S., Amarante P., Loeloff R., Luo Y., Fisher S., Fuller J., Edenson S., Lile J., Jarosinski M.A., Biere A.L., Curran E., Burgess T., Louis J.C., Collins F., Treanor J., Rogers G., Citron M., β-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science*. 1999; 286: 735-41.
11. Hussain I., Powell D., Howlett D.R., Tew D.G., Meek T.D., Chapman C., Gloger I.S., Murphy K.E., Southan C.D., Ryan D.M., Smith T.S., Simmons D.L., Walsh F.S., Dingwall C., Christie G., Identification of a novel aspartic protease (Asp 2) as β-secretase. *Mol Cell Neurosci*. 1999; 14: 419-27.
12. Selkoe D.J., Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature*. 1999; 399: A23-31.
13. Iwatsubo T., Odaka A., Suzuki N., Mizusawa H., Nukina N., Ihara Y., Visualization of Aβ 42(43) and Aβ 40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ 42(43). *Neuron*. 1994; 13: 45-53.
14. Sastre M., Steiner H., Fuchs K., Capell A., Multhaup G., Condron M.M., Teplow D.B., Haass C., Presenilin-dependent γ-secretase processing of β-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. EMBO *Rep*. 2001; 2: 835-41.
15. Yu C., Kim S.-H., Ikeuchi T., Xu H., Gasparini L., Wang R., Sisodia S.S., Characterization of a presenilin-mediated amyloid precursor carboxyl-terminal fragment γ. *J. Biol. Chem*. 2001; 276: 43756-60.
16. Weidemann A., Eggert S., Reinhard F.B.M., Vogel M., Paliga K., Baier G., Masters C.L., Beyreuther K., Evin G., A novel ε-cleavage within the transmembrane domain of the Alzheimer amyloid precursor protein demonstrates homology with Notch processing. *Biochemistry*. 2002; 41: 2825-35.
17. Cao X., Sudhof T.C., A transcriptively active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science*. 2001; 293: 115-20.
18. Leissring M.A., Murphy M.P., Mead T.R., Akbari Y., Sugarman M.C., Jannatipour M., Anliker B., Muller U., Saftig P., De Strooper B., Wolfe M.S., Golde T.E., LaFerla F.M., A physiologic signaling role for the γ-secretase derived intracellular fragment of APP. *Proc Natl Acad Sci U S A*. 2002; 99: 4697-702.
19. Schellenberg G.D., Bird T.D., Wijsman E.M., Orr H.T., Anderson L., Nemens E., White J.A., Bonnycastle L., Weber J.A., Alonso M.E., Potter H., Heston L.L., Martin G.M., Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science*. 1992; 258: 668-71.
20. Levy-Lahad E., Wasco W., Poorkaj P., Romano D.M., Oshima J., Pettingell W.H., Yu C.-e., Jondro P.D., Schmidt S.D., Wang K., Crowley A.C., Fu Y.-H., Guenette S.Y., Galas D., Nemens E., Wijsman E.M., Bird T.D., Schellenberg G.D., Tanzi R.E., Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science*. 1995; 269: 973-77.
21. Li X., Greenwald I., Membrane topology of the C. elegans SEL-12 presenilin. *Neuron*. 1996; 17: 1015-21.
22. Nakai T., Yamasaki A., Sakaguchi M., Kosaka K., Mihara K., Amaya Y., Miura S., Membrane topology of Alzheimer's disease-related presenilin 1. *J. Biol. Chem*. 1999; 274: 23647-58.
23. Donoviel D.B., Hadjantonakis A.-K., Ikeda M., Zheng H., St George-Hyslop P., Bernstein A., Mice lacking both presenilin genes exhibit early embryonic patterning defects. *Genes Devel*. 1999; 13: 2801-10.

abscence of these residues implies that PS belongs to the aspartyl protease protein family, and the homology of PS to signal peptide peptidase family of aspartyl proteases further corroborates this hypothesis [53].

Not much is known how the γ-complex is assembled and what the ratios of the individual components in the complex are. It is also likely that there are additional modulatory proteins that are part of the active γ-secretase complex. These are important questions for understanding the exact mechanism of γ-secretase processing and the answers to these questions will be essential for the process of developing drugs targeting the activity of the γ-secretase complex. It will be a pharmaceutical challenge to target γ-secretase and create an Aβ lowering drug, while not affecting Notch signaling, or intramembrane processing of other RIP substrates. However, it is encouraging that non-peptide inhibitors reduce Aβ generation while having little effect on S3-processing of Notch[54]. In addition, lithium has been shown to reduce γ-secretase processing of APP in the absence of any effect on Notch processing[55]. It is also promising that the BACE knockout mice and the heterozygous nicastrin mice display a normal phenotype. Aβ formation is completely abrogated in BACE$^{-/-}$ mice[56] and reduced in the nicastrin$^{+/-}$ mice[57], making these proteins attractive targets for diminishing Aβ production. The crystal structure of BACE suggests that the enzyme has a large catalytic pocket[58], making the task of specifically targeting this pepsin protease family member with small molecular weight compounds difficult. The intense research efforts of the last couple of years have greatly increased our knowledge about the proteolytic activities generating Aβ, and we now face exciting times when studies of how to inhibit the detrimental production of Aβ, without causing adverse side-effects, will be initiated and executed.

References

1. Selkoe D.J., Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev*. 2001; 81: 741-66.
2. Zheng H., Jiang M., Trumbauer M.E., Sirinathsinghji D.J., Hopkins R., Smith D.W., Heavens R.P., Dawson G.R., Boyce S., Conner M.W., et al., β-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell*. 1995; 81: 525-31.
3. Heber S., Herms J., Gajic V., Hainfellner J., Aguzzi A., Rulicke T., von Kretzschmar H., von Koch C., Sisodia S., Tremml P., Lipp H.P., Wolfer D.P., Muller U., Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. *J Neurosci*. 2000; 20: 7951-63.
4. Schubert D., Jin L.W., Saitoh T., Cole G., The regulation of amyloid beta protein precursor secretion and its modulatory role in cell adhesion. *Neuron*. 1989; 3: 689-94.
5. Kamal A., Almenar-Queralt A., LeBlanc J.F., Roberts E.A., Goldstein L.S., Kinesin-mediated axonal transport of a membrane compartment containing β-secretase and presenilin-1 requires APP. *Nature*. 2001; 414: 643-8.
6. Buxbaum J.D., Liu K.-N., Luo Y., Slack J.L., Stocking K.L., Peschon J.J., Johnson R.S., Castner B.J., Cerretti D.P., Black R.A., Evidence that Tumor necrosis factor á converting enzyme is involved in regulated α-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem*. 1998; 273: 27765-67.
7. Lammich S., Kojro E., Postina R., Gilbert S., Pfeiffer R., Jasionowski M., Haass C., Fahrenholz F., Constitutive and regulated á-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc Natl Acad Sci U S A*. 1999; 96: 3922-7.
8. Lin X., Koelsch G., Wu S., Downs D., Dashti A., Tang J., Human aspartic protease memapsin 2 cleaves the β-secretase site of β-amyloid precursor protein. *Proc Natl Acad Sci U S A*. 2000; 97: 1456-60.
9. Yan R., Bienkowski M.J., Shuck M.E., Miao H., Tory M.C., Pauley A.M., Brashier J.R., Stratman N.C., Mathews W.R., Buhl A.E., Carter D.B., Tomasselli A.G., Parodi L.A., Heinrikson R.L., Gurney M.E., Membraneanchored aspartyl protease with Alzheimer's disease β-secretase activity. *Nature*. 1999; 402: 533-7.
10. Vassar R., Bennett B.D., Babu-Khan S., Kahn S., Mendiaz E.A., Denis P., Teplow D.B., Ross S., Amarante P., Loeloff R., Luo Y., Fisher S., Fuller J., Edenson S., Lile J., Jarosinski M.A., Biere A.L., Curran E., Burgess T., Louis J.C., Collins F., Treanor J., Rogers G., Citron M., β-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science*. 1999; 286: 735-41.
11. Hussain I., Powell D., Howlett D.R., Tew D.G., Meek T.D., Chapman C., Gloger I.S., Murphy K.E., Southan C.D., Ryan D.M., Smith T.S., Simmons D.L., Walsh F.S., Dingwall C., Christie G., Identification of a novel aspartic protease (Asp 2) as β-secretase. *Mol Cell Neurosci*. 1999; 14: 419-27.
12. Selkoe D.J., Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature*. 1999; 399: A23-31.
13. Iwatsubo T., Odaka A., Suzuki N., Mizusawa H., Nukina N., Ihara Y., Visualization of Aβ 42(43) and Aβ 40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ 42(43). *Neuron*. 1994; 13: 45-53.
14. Sastre M., Steiner H., Fuchs K., Capell A., Multhaup G., Condron M.M., Teplow D.B., Haass C., Presenilin-dependent γ-secretase processing of β-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. EMBO *Rep*. 2001; 2: 835-41.
15. Yu C., Kim S.-H., Ikeuchi T., Xu H., Gasparini L., Wang R., Sisodia S.S., Characterization of a presenilin-mediated amyloid precursor carboxyl-terminal fragment γ. *J. Biol. Chem*. 2001; 276: 43756-60.
16. Weidemann A., Eggert S., Reinhard F.B.M., Vogel M., Paliga K., Baier G., Masters C.L., Beyreuther K., Evin G., A novel ε-cleavage within the transmembrane domain of the Alzheimer amyloid precursor protein demonstrates homology with Notch processing. *Biochemistry*. 2002; 41: 2825-35.
17. Cao X., Sudhof T.C., A transcriptively active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science*. 2001; 293: 115-20.
18. Leissring M.A., Murphy M.P., Mead T.R., Akbari Y., Sugarman M.C., Jannatipour M., Anliker B., Muller U., Saftig P., De Strooper B., Wolfe M.S., Golde T.E., LaFerla F.M., A physiologic signaling role for the γ-secretase derived intracellular fragment of APP. *Proc Natl Acad Sci U S A*. 2002; 99: 4697-702.
19. Schellenberg G.D., Bird T.D., Wijsman E.M., Orr H.T., Anderson L., Nemens E., White J.A., Bonnycastle L., Weber J.A., Alonso M.E., Potter H., Heston L.L., Martin G.M., Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science*. 1992; 258: 668-71.
20. Levy-Lahad E., Wasco W., Poorkaj P., Romano D.M., Oshima J., Pettingell W.H., Yu C.-e., Jondro P.D., Schmidt S.D., Wang K., Crowley A.C., Fu Y.-H., Guenette S.Y., Galas D., Nemens E., Wijsman E.M., Bird T.D., Schellenberg G.D., Tanzi R.E., Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science*. 1995; 269: 973-77.
21. Li X., Greenwald I., Membrane topology of the C. elegans SEL-12 presenilin. *Neuron*. 1996; 17: 1015-21.
22. Nakai T., Yamasaki A., Sakaguchi M., Kosaka K., Mihara K., Amaya Y., Miura S., Membrane topology of Alzheimer's disease-related presenilin 1. *J. Biol. Chem*. 1999; 274: 23647-58.
23. Donoviel D.B., Hadjantonakis A.-K., Ikeda M., Zheng H., St George-Hyslop P., Bernstein A., Mice lacking both presenilin genes exhibit early embryonic patterning defects. *Genes Devel*. 1999; 13: 2801-10.

24. Herreman A., Serneels L., Annaert W., Collen D., Schoonjans L., De Strooper B., Total inactivation of γ-secretase activity in presenilin-deficient embryonic stem cells. *Nat Cell Biol.* 2000; 2: 461-2.
25. Zhang Z., Nadeau P., Song W., Donoviel D., Yuan M., Bernstein A., Yankner B.A., Presenilins are required for γ-secretase cleavage of γ-APP and transmembrane cleavage of Notch-1. *Nat Cell Biol*. 2000; 2: 463-5.
26. Wolfe M.S., Xia W., Ostaszewski B.L., Diehl T.S., Kimberly W.T., Selkoe D.J., Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature*. 1999; 398: 513-17.
27. Thinakaran G., Harris C.L., Ratovitski T., Davenport F., Slunt H.H., Price D.L., Borchelt D.R., Sisodia S.S., Evidence that levels of Presenilins (PS1 and PS2) are coordinately regulated by competition for limiting cellular factors. *J. Biol. Chem*. 1997; 272: 28415-22.
28. Brown M.S., Ye J., Rawson R.B., Goldstein J.L., Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. *Cell*. 2000; 100: 391-8.
29. Scheinfeld M.H., Ghersi E., Laky K., Fowlkes B.J., D'Adamio L., Processing of β-amyloid precursor-like protein-1 and -2 by γ-secretase regulates transcription. *J Biol Chem*. 2002; 277: 44195-201.
30. Okamoto I., Kawano Y., Murakami D., Sasayama T., Araki N., Miki T., Wong A.J., Saya H., Proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway. *J. Cell Biol*. 2001; 155: 755-62.
31. Ni C.Y., Yuan H., Carpenter G., Role of the ErbB-4 carboxyl terminus in γ-secretase cleavage. *J Biol Chem.* 2003; 278: 4561-5.
32. Ikeuchi T., Sisodia S.S., The Notch ligands, Delta1 and Jagged2, are substrates for Presenilin-dependent "γ -secretase" cleavage. *J Biol Chem.* 2003; 278: 7751-4.
33. Marambaud P., Shioi J., Serban G., Georgakopoulos A., Sarner S., Nagy V., Baki L., Wen P., Efthimiopoulos S., Shao Z., Wisniewski T., Robakis N.K., A presenilin-1/γ-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions. Embo J. 2002; 21: 1948-56.
34. De Strooper B., Annaert W., Cupers P., Saftig P., Craessaerts K., Mumm J.S., Schroeter E.H., Schrijvers V., Wolfe M.S., Ray W.J., Goate A., Kopan R., A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. *Nature.* 1999; 398: 518-22.
35. Yu G., Nishimura M., Arawaka S., Levitan D., Zhang L., Tandon A., Song Y.Q., Rogaeva E., Chen F., Kawarai T., Supala A., Levesque L., Yu H., Yang D.S., Holmes E., Milman P., Liang Y., Zhang D.M., Xu D.H., Sato C., Rogaev E., Smith M., Janus C., Zhang Y., Aebersold R., Farrer L.S., Sorbi S., Bruni A., Fraser P., St George-Hyslop P., Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and βAPP processing. *Nature*. 2000; 407: 48-54.
36. Goutte C., Hepler W., Mickey K.M., Priess J.R., aph-2 encodes a novel extracellular protein required for GLP-1-mediated signaling. *Development.* 2000; 127: 2481-92.
37. Hu Y., Ye Y., Fortini M.E., Nicastrin is required for γ-secretase cleavage of the Drosophila Notch receptor. *Dev Cell*. 2002; 2: 69-78.
38. Lopez-Schier H., St Johnston D., Drosophila nicastrin is essential for the intramembranous cleavage of notch. Dev Cell. 2002; 2: 79-89.
39. Chung H.M., Struhl G., Nicastrin is required for Presenilin-mediated transmembrane cleavage in Drosophila. *Nat Cell Biol*. 2001; 3: 1129-32.
40. Leem J.Y., Vijayan S., Han P., Cai D., Machura M., Lopes K.O., Veselits M.L., Xu H., Thinakaran G., Presenilin 1 is required for maturation and cell surface accumulation of nicastrin. J Biol Chem. 2002; 277: 19236-40.
41. Yang D.S., Tandon A., Chen F., Yu G., Yu H., Arawaka S., Hasegawa H., Duthie M., Schmidt S.D., Ramabhadran T.V., Nixon R.A., Mathews P.M., Gandy S.E., Mount H.T., St George-Hyslop P., Fraser P.E., Mature glycosylation and trafficking of nicastrin modulate its binding to presenilins. *J Biol Chem*. 2002; 277: 28135-42.
42. Edbauer D., Winkler E., Haass C., Steiner H., Presenilin and nicastrin regulate each other and determine amyloid β-peptide production via complex formation. *Proc Natl Acad Sci U S A.* 2002; 99: 8666-71.
43. Tomita T., Katayama R., Takikawa R., Iwatsubo T., Complex N-glycosylated form of nicastrin is stabilized and selectively bound to presenilin fragments. *FEBS Lett*. 2002; 520: 117-21.
44. Herreman A., Van Gassen G., Bentahir M., Nyabi O., Craessaerts K., Mueller U., Annaert W., De Strooper B., γ-Secretase activity requires the presenilindependent trafficking of nicastrin through the Golgi apparatus but not its complex glycosylation. *J Cell Sci.* 2003; 116: 1127-36.
45. Goutte C., Tsunozaki M., Hale V.A., Priess J.R., APH-1 is a multipassmembrane protein essential for the Notch signaling pathway in Caenorhabditis elegans embryos. *Proc Natl Acad Sci U S A.* 2002; 99: 775-9.
46. Francis R., McGrath G., Zhang J., Ruddy D.A., Sym M., Apfeld J., Nicoll M., Maxwell M., Hai B., Ellis M.C., Parks A.L., Xu W., Li J., Gurney M., Myers R.L., Himes C.S., Hiebsch R., Ruble C., Nye J.S., Curtis D., aph-1 and pen-2 are required for Notch pathway signaling, γ-secretase cleavage of βAPP, and presenilin protein accumulation. *Dev Cell*. 2002; 3: 85-97.
47. Li J., Myers R., Shuang R., Carter D., McKinley D., Hiebsch R., Ellerbrock B., Fleck T., Himes C., Ruble C., Carozza M., Drong R., Pauley A., Schellin K., Seaver L., Slightom J., Nye J.S. Presenilin enhancers PEN-1 and PEN-2 and their roles in Aβ production. In: *the 8th International Conference on Alzheimer's Disease and Related Disorders*. Stockholm; 2002.
48. Luo W.J., Wang H., Li H., Kim B.S., Shah S., Lee H.J., Thinakaran G., Kim T.W., Yu G., Xu H., PEN-2 and APH-1 coordinately regulate proteolytic processing of Presenilin 1. *J Biol Chem*. 2003; 278: 7850-54.
49. Steiner H., Winkler E., Edbauer D., Prokop S., Basset G., Yamasaki A., Kostka M., Haass C., PEN-2 is an integral component of the ã-secretase complex required for coordinated expression of presenilin and nicastrin. *J Biol Chem*. 2002; 277: 39062-5.
50. Takasugi N., Tomita T., Hayashi I., Tsuruoka M., Niimura M., Takahashi Y., Thinakaran G., Iwatsubo T., The role of presenilin cofactors in the γ-secretase complex. *Nature.* 2003; 422: 438-41.
51. Edbauer D., Winkler E., Regula J.T., Pesold B., Steiner H., Haass C., Reconstitution of ã-secretase activity. *Nat Cell Biol.* 2003; 5: 486-8.
52. Kimberly W.T., LaVoie M.J., Ostaszewski B.L., Ye W., Wolfe M.S., Selkoe D.J., γ-Secretase is a membrane protein complex comprised of presenilin, nicastrin, aph-1, and pen-2. *Proc Natl Acad Sci U S A.* 2003; 100: 6382-87.
53. Weihofen A., Binns K., Lemberg M.K., Ashman K., Martoglio B., Identification of signal peptide peptidase, a presenilin-type aspartic protease. *Science*. 2002; 296: 2215-8.
54. Petit A., Bihel F., Alves da Costa C., Pourquie O., Checler F., Kraus J.-L., New protese inhibitors prevent γ-secretase-mediated production of Aβ40/42 without affecting Notch cleavage. *Nat. Cell Biol.* 2001; 3: 507-11.
55. Phiel C.J., Wilson C.A., Lee V.M., Klein P.S., GSK-3α regulates production of Alzheimer's disease amyloid-β peptides. *Nature*. 2003; 423: 435-9.
56. Luo Y., Bolon B., Kahn S., Bennett B.D., Babu-Khan S., Denis P., Fan W., Kha H., Zhang J., Gong Y., Martin L., Louis J.C., Yan Q., Richards W.G., Citron M., Vassar R., Mice deficient in BACE1, the Alzheimer's β-secretase, have normal phenotype and abolished β-amyloid generation. Nat Neurosci. 2001; 4: 231-2.
57. Li T., Ma G., Cai H., Price D.L., Wong P.C., Nicastrin is required for assembly of presenilin/γ-secretase complexes to mediate Notch signaling and for processing and trafficking of β-amyloid precursor protein in mammals. J Neurosci. 2003; 23: 3272-7.
58. Hong L., Koelsch G., Lin X., Wu S., Terzyan S., Ghosh A.K., Zhang X.C., Tang J., Structure of the protease domain of memapsin 2 (β-secretase) complexed ith inhibitor. *Science*. 2000; 290: 150-3.

Risk Factors for Early Readmission of Alzheimer Patients to an Acute Care Unit

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Abstract

Objective: To determine the factors associated with early readmission (≤1 month) of patients with Alzheimer disease to an acute care unit specialized in dementia.

Design: Retrospective review.

Setting: An acute care unit for Alzheimer patients in a teaching hospital.

Participants: 143 patients followed between December 1998 and February 2000.

Measurements: Using bivariate analysis, we compared data relating to patients who were rapidly readmitted to the unit (≤1 month) (N = 42) with data of patients readmitted after a longer period (> 1 month) (N = 101). Comparison was based on the standard gerontological evaluation of each patient carried out at the time of first admission. Multivariate analysis was then carried out to take into account potential confounding factors.

Results: Bivariate analysis identified three factors associated with the risk of rapid readmission to the unit: male gender (P = 0.006), initial living arrangements (at home with assistance) (P = 0.046) and unsatisfactory social situation (P = 0.017).

Multivariate analysis showed that the risk of readmission in the short term was multiplied by 2.8 (95% CI = 1.27-6.36, P = 0.011) when the social situation was unsatisfactory, whereas two factors appeared to decrease the risk: female gender (odds ratio [OR] = 0.36, 95% CI = 0.16-0.78, P = 0.010) and independence in the basic activities of daily living, which was at the limits of significance (OR = 0.40, 95% CI = 0.16-1.01, P = 0.053).

Conclusion: Our analysis of 143 patients demonstrates the need for better management of the families of Alzheimer patients and also of professional carers in order to decrease the risk of early readmission.

Keywords: Alzheimer disease, hospital readmission, acute care unit

Introduction

Alzheimer disease (AD) is a major problem of public health. It affects more than 5% of persons aged over 65 years and 25% of those aged over 90. It is estimated that in France 600,000 persons suffer from AD, a figure which is indicative of the importance of the problem for all actors in society: families, care teams, health and social authorities. AD is a chronic, progressive pathology associated with numerous complications which lead to repeated hospital admissions. In recent years, various works have studied the characteristics and specific nature of the reasons for hospital readmission of Alzheimer patients (1-4). Some studies found that readmissions were more numerous and duration of hospital stay was longer in these patients (3-5). In order to improve their management, acute care units specialized in dementia were created, first in retirement homes and then in hospitals (6). Numerous publications have described the characteristics of these specialized units, in particular the manual "Key Elements of Dementia Care"

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which addresses the various aspects of dementia management, with particular emphasis on the development of care plans, the environment and human resources (7). The Alzheimer Acute Care Unit of Toulouse University Hospital meets the various criteria defining such a unit (8). The number of dementia patients admitted increases every year. Nevertheless, in spite of optimized management in a dedicated facility, many patients are rapidly readmitted after discharge from the unit. This being so, we believed it would be useful to try to identify the factors associated with early readmission.

Material and methods

1. Patient selection

We included in our study all patients hospitalized in the Alzheimer acute care unit in Toulouse between December 25, 1998 and February 15, 2000 and who underwent standardized gerontological assessment at that time. Only patients who were readmitted to the unit during the year following their previous admission, and for whom a year's follow-up was available, were analyzed.

We distinguished two groups of patients according to time to readmission:

– patients readmitted within a very short time (≤ 1 month)

– patients readmitted after a longer period (> 1 month ≤ 1 year).

2. Data collection

The following data were collected when the patient was first admitted:

a. Social and economic characteristics

Age, gender, living arrangements before hospitalization (retirement home, sheltered housing, own home) and outcome after hospitalization.

b. Reason for hospitalization

Behavioral problems, deterioration of general health status, cognitive evaluation, aggravation of neurocognitive problems, associated disorder, falls and difficulty in walking, temporary institutionalization

c. Standardized gerontological evaluation (EGS)

We took into consideration the principal elements of this evaluation:

– cognitive evaluation using the Mini-Mental State (MMS) (9)

– independence using the Activities of Daily Living score (ADL) (10)

– nutritional status with the Mini Nutritional Assessment (MNA) (11)

– postural problems and risk of falls assessed by one-leg balance (12)

– presence or absence of difficulties in walking

– risk of pressure sores using Norton's scale (13)

– sphincter function (urinary or mixed incontinence, occasional or chronic)

– social situation: satisfactory or unsatisfactory, taking into consideration the social context, availability of family and friends and presence of a principal caregiver (assessed subjectively by the nurse during the initial interview).

The following elements were also analyzed:

Laboratory tests (normal or abnormal), weight, serum albumin level, food intake (normal or abnormal), diagnosis established at the first admission to hospital (dementia of Alzheimer type, other dementia syndrome, other diagnoses).

3. Data analysis

Bivariate analysis was carried out using Epi Info software (Centers for Disease Control and Prevention, Atlanta, GA, USA). We compared data relating to patients readmitted in the short term (≤ 1 month) and in the longer term (> 1 month). Qualitative data was analyzed using the X2 test (or Fisher's test). Quantitative data was analyzed by means comparison (Student's t test or non-parametric test). Multivariate analysis was carried out using Egret software (Cytel Software Corporation, Cambridge, MA, USA) in order to take into account potential confounding factors. The dependent variable was readmission in the short term (yes/no) and the explanatory variables were those related to the 25% threshold after bivariate analysis.

Results

1. Population characteristics

We analyzed the data of the 143 patients who met the selection criteria. Their mean age was 79 years. The great majority were at risk of malnutrition as shown by the MNA. A large number were also dependent in the activities of daily living, as only 30.8% of the study population were totally independent in these activities. Lastly, it is important to note that 72.3% of patients presented Alzheimer disease. The characteristics of the sample are summarized in table 1.

2. Factors associated with rapid readmission

In our population, 42 patients (29.4%) were readmitted in the short term and 101 (70.6%) were readmitted after a longer period. Mean time to readmission was 119.4 days (+101.2).

A. Results of bivariate analysis

We compared the characteristics of patients readmitted in the short term (N = 42) with those of patients readmitted in the longer term (N = 101). Three elements of bivariate analysis were identified as statistically significant (table 2).

Table 1. Patient characteristics (N = 143).

Mean age (years) (mean +/- SD)	78.9 (± 8.4)
Gender (% women)	58
Satisfactory social situation (%)	58
Principal reasons for hospitalization Cognitive evaluation (%) Behavioral problems (%)	 35.7 31.5
Living arrangements Own home (with or without assistance) (%)	76.9
Disturbed sphincter function (%)	48.2
Nutritional status (MNA)* (mean +/- SD) MNA >23.5: normal (%) MNA 17-23.5: risk of malnutrition (%) MNA <17: malnutrition (%)	19.9 (± 3.9) 22.8 58.3 18.9
Mean albumin (g/l) (mean +/- SD)	34.3 (± 4.8)
Mean weight (kg) (mean +/- SD)	62.6 (± 12.9)
Fall before hospital admission (%)	14.9
Normal one-leg balance (%)	17
Difficulty in walking (%)	39.7
Evaluation of independence (mean ADL +/- SD)**	3.93 (± 1.8)
Cognitive function (mean MMS +/- SD)***	12.8 (± 8)
Risk of pressure sores (mean Norton scale score +/- SD) ≤ 14: pressure sore risk (%)	14.9 (± 2.4) 20.3
Outcome Return to own home (without change in management) (%) Return to own home (with provision of or increase in assistance) (%) Institutionalization or return to institution (%) Temporary institutionalization (%)	 46.2 16.8 33.6 3.4

These were:

– Gender

Male gender was associated with risk of early readmission. Of the subjects readmitted in the short term, 59.5% were men whereas only 40.5% were women (P = 0.006).

– Living arrangements before admission

We found that residence in own home with assistance was associated with the risk of early readmission. In the population readmitted in less than 1 month, 69% lived at home with assistance, compared with 14.3% at home without assistance and 16.7% in an institution (P = 0.046).

– Social evaluation

A social situation perceived by the nurse as unsatisfactory (no principal caregiver, family rarely available) was associated with the risk of early readmission. In the population of patients readmitted in the short term, 57.1% had an unsatisfactory social situation compared with 35.6% of patients who were readmitted after 1 month (P = 0.017).

No other variables analyzed were statistically significant (age, ADL, MNA).

B. Results of multivariate analysis

Since several factors were associated with the risk of early readmission, we carried out multivariate analysis to identify the individual impact of each of these factors.

Logistic regression was used to analyze the variables which were significantly associated with early readmission (gender, living arrangements, social situation) and those related to the 25% threshold (laboratory test results, ADL, age and diagnosis).

The risk of early hospital readmission was found to be multiplied by 2.8 (95% CI = 1.27-6.36, P = 0.011) when the social situation was unsatisfactory, whereas two factors appeared to decrease the risk: female gender (OR = 0.36, 95% CI = 0.16-0.78, P = 0.010) and independence in basic activities of daily living, which was at the limit of significance (OR = 0.40, 95% CI = 0.16-1.01, P = 0.053).

Discussion

After bivariate analysis, we found that three factors increased the risk of early readmission: male gender, living arrangements (at home with assistance) and unsatisfactory social situation.

Other authors have also revealed the role of gender in hospital admission. For McCusker, male gender is a risk factor for multiple repeat visits in the elderly admitted to emergency departments (14). For Gambassi et al (15), women with AD are less frequently admitted to hospital and have a markedly lower mortality rate than men with the same disease. These authors hypothesized that the

Table 2. Comparison of patient characteristics according to time to readmission (results of bivariate analysis).

Characteristics	Time to readmission > 1 month (N = 101)	Time to readmission < 1 month (N = 42)	P
Mean age (years) +/- SD	78.4 + 8	80.4 + 9	0.110
Gender (% women)	65.3	40.5	0.006
Dependence for activities of daily living (ADL)* (%)	65.3	78.6	0.120
Mean MMS +/- SD**	13.3 + 8	11.5 + 7	0.157
Abnormal one-leg balance (%)	81.8	85.7	0.570
Difficulty in walking (%)	38	44	0.510
Mean MNA +/- SD***	19.9 + 3	19.9 + 4	0.840
Mean weight (kg) +/- SD	63.14 + 12	61.17 + 13	0.320
Sphincter dysfunction (%)	46.5	52.5	0.520
Sphincter dysfunction (%)	46.5	52.5	0.520
Initial living arrangements - Own home without assistance (%) - Own home with assistance (%) - Other (%)	 27.7 46.5 25.7	 14.3 69.0 16.7	0.046
Reason for admission - Alzheimer disease (%) - Behavioral problems (%) - Altered general status, falls and other reasons (%)	 44.2 30.5 25.3	 38.1 38.1 23.8	0.330
Unsatisfactory social situation (%)	35.6	57.1	0.017
Diagnoses at end of first admission Alzheimer disease and complications (%) - Other dementias (%) - Other diagnoses (%)	 78.8 14.1 7.1	 57.1 28.6 14.3	0.052

* ADL: Activities of Daily Living (max 6, min 0)
** MMS: Mini Mental State (max 30, min 0)
*** MNA: Mini Nutritional Assessment

prevalence of serious comorbid conditions (chronic obstructive lung disease, cardiac rhythm disorders, malignancies) was much higher in the male than in the female population. This greater vulnerability may be the source of the increased risk of hospital readmission, but it was not measured in our analysis.

In our study sample, we observed that assisted living at home was associated with the risk of early readmission. It should be noted that for the patients living at home (with or without professional assistance), we were not able to find out systematically whether they lived alone or with a family member. This is an important limitation. However, other authors found similar results to ours. Caplan reported that use of support services at home and in particular recourse to a community nurse was associated with a risk of hospital readmission (16). Similarly, in a study of risk factors for early readmission of elderly persons to emergency services, Chu associated previous use of visiting nurse services with

risk of early readmission (17). It is important to stress that these are fragile, increasingly dependent individuals and that they place considerable strain on family and friends and also on professional caregivers. Moreover, neither family and friends nor home helps or nurses are trained in the care and management of patients with neurocognitive disorders. Familiarity with Alzheimer disease and with its complications is essential for good management at home. Psychological and behavioral problems, for instance, are often poorly interpreted by those close to the patient and then become a reason for seeking hospital admission. The daily care of the patient regularly gives rise to abnormal reactions: washing and dressing can be misunderstood by the patient who may feel physically molested or that his or her privacy is invaded and who reacts by obstructive or aggressive behavior (18). This is regularly followed by a request for psychotropic medications, whose unwanted effects are their considerable impact on cognitive function and on the degree of dependence, increasing the difficulties experienced.

Lastly, in our study, a social situation considered as unsatisfactory was an independent factor of early readmission. The role of "impoverished" social support in patient outcome has been analyzed in numerous studies, and some have shown that it increases the risk of institutionalization (19-21). Others have demonstrated that an unfavorable family environment is a risk factor for hospital readmission (3, 22). This observation leads us to seek the reasons for the absence or inadequacy of family support. Certain patients have no immediate family network (unmarried without children, family living at a distance, etc). Others do have a family network but its members can no longer assume their supporting role because of the burden and difficulty of management of such patients. The accumulation of cognitive deficit, increasing dependence, permanent aggression by behavioral problems which are poorly understood and wrongly interpreted, lead progressively to family exhaustion and reactions of intolerance, as well as the impossibility of controlling the situation at home. All these factors contribute to the concept of the "burden of the principal caregiver". The burden felt is a function of the degree of dependence and the intensity of behavioral problems, but it varies according to the capacity for adaptation and the resilience of the principal caregiver. In addition, burden may have a damaging effect on the caregiver's health (23, 24). Lastly, it is responsible for sudden episodes of rejection, leading to readmissions of the care recipient. Whereas at the present time the private health care system and the structures aiming at keeping patients in their own home are perfectly adapted to management of physical dependence, on the other hand decompensation of the care relationship, sudden breakdowns, cannot be correctly managed by these ambulatory systems. Episodes of rejection thus lead to emergency hospital admissions which, if they are not averted, always result in deterioration of the patient's condition and reinforcement of the guilt feelings of family and friends. Numerous works have shown that the subjective burden felt by those close to the patient is one of the best predictors of institutionalization (25), hospitalization (26) and also of repeated transitions between the home and the emergency services (27). A recent study by Bertogliati addressed the setting up of a dual management strategy for the family and the patient, with the aim of optimizing the cognitive treatment of dementia patients through management of the families. From a global viewpoint, it appears that group management helps the caregiver to acknowledge his or her own distress and burden of work, thus allowing negative affects such as guilt, resentment or aggressiveness towards their relative to be expressed. Lastly, from a more specific viewpoint, providing increased knowledge about the disorder enables better interpretation of behavioral problems and reduces conflicts and the feeling of helplessness (28). This study thus suggests that fuller information to caregivers about Alzheimer disease and its potential complications would allow better control of management of the patient at home. The decreased frequency of episodes of rejection related to the feeling of helplessness could help to reduce hospital readmissions which generally are not medically justified.

Lastly, certain methodological limitations of our study must be mentioned. We decided to restrict our analysis to persons who were readmitted to the Alzheimer acute care unit without taking into account patients who were readmitted to another facility. Moreover, we should point out that certain patients are sometimes readmitted to the unit for a short period by request of the unit's physicians, for further investigation or by appointment for a test requiring a brief hospital stay. We were not able to exclude them from the study as there is no numerical code for medical information when data are entered and the examiner alone is free to select the reason for admission. Thus admission for further investigation is generally and wrongly entered as «cognitive evaluation». Another non-negligible bias concerns evaluation of the patient's social situation, carried out subjectively by the nurse when the patient is admitted to the unit. However, it should be noted that the nursing staff are particularly experienced and trained in this type of evaluation.

Conclusion

In our dementia patients, the factors usually associated in the literature with risk of hospital readmission, such as severe cognitive impairment (MMS) (14, 22, 29), loss of ability in the basic activities of daily living (ADL) (1, 14, 16, 22) or precarious nutritional status (30), were not correlated with this risk. However, as far as we are aware, few studies have addressed the question of early readmission as such. Moreover, the majority of studies of risk factors for hospital readmission concern elderly

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persons in general, and not as in our work a homogeneous population of patients with dementia.

Using multivariate analysis, we found that only an unsatisfactory social situation was associated with the risk of early readmission, whereas female gender and adequate independence (at the limit of significance) appeared to reduce this risk.

Above all, this study reveals the need for global management of the patient and his or her family. Prevention of early hospital readmissions demands better evaluation of the patient's family environment and fuller information on Alzheimer disease to all those involved in its management (principal caregivers, home helps, nurses in private practice). With this aim in mind, we plan to organize consultations for caregivers in our unit, in order to provide information and advice on the management of these patients.

References

1. Nourashémi F, Andrieu S, Sastres N et al. Descriptive analysis of emergency hospital admissions of patients with Alzheimer's disease. *Alz Dis Assoc Disorders* 2001; 15: 21-25.
2. Albert SM, Costa R, Merchant C et al. Hospitalization and Alzheimer's disease: Results from a community-based study. *J Gerontol A Biol Sci Med Sci* 1999; 54(5): 267-271.
3. Mercer GT, Molinari V, Kunik ME et al. Rehospitalization of older psychiatric inpatients: an investigation of predictors. *Gerontologist* 1999; 39: 91-598.
4. Fillenbaum G, Heyman A, Peterson B et al. Frequency and duration of hospitalization of patients with AD based on Medicare data: CERAD XX. *Neurology* 2000; 54: 740-743.
5. Torian L, Davidson E, Fulop G et al. The effect of dementia on acute care in a geriatric medical unit. *Int Psychogeriatr* 1992; 4: 231-239.
6. Holmes D, Teresi JA, Ory MG. Unités de Soins Alzheimer. Maladie d'Alzheimer, *Recherche et Pratique Clinique*, Vol. 4. Paris: Serdi Edition, 2000, pp 17-26.
7. Key Elements of Dementia Care. Chicago: *Alzheimer's Disease and Related Disorders Association*, Inc, 1997.
8. Holmes D, Vellas B. Unités de soins Alzheimer. Maladie d'Alzheimer, *Recherche et Pratique Clinique*, Vol. 4. Paris: Serdi Edition, 2000, pp 7-15.
9. Folstein MS, Folstein SE, M.C Hugh P.R Mini Mental State: a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189-198
10. Katz S, Downs TD, Cash HR et al. Progress in the development of ADL. *Gerontologist* 1970; 10: 20-30.
11. Guigoz Y, Vellas B, Garry PJ. Mini nutritional assessment: a practical assessment tool for grading the nutritional status of elderly patients. In: Albarede JL, Vellas B, Garry P, eds. *L'Année Gérontologique* suppl. 2: Facts and Research in Gerontology. Paris: Serdi Edition, 1994, pp 15-59.
12. Vellas BJ, Wayne SJ, Romero L et al. One-leg balance is an important predictor of injurious falls in older persons. *J Am Geriatr Soc* 1997; 45: 735-738.
13. Norton D. Norton scale for decubitus prevention. *Krankenpflege* (Frankf) 1980; 34: 16.
14. McCusker J, Healey E, Bellavance F et al. Predictors of repeat emergency department visits by elders. *Acad Emerg Med* 1997; 4: 581-588.
15. Gambassi G, Lapane KL, Landi F et al. Gender differences in the relation between comorbidity and mortality of patients with Alzheimer's disease. Systematic Assessment of Geriatric drug use via Epidemiology (SAGE) Study Group. *Neurology* 1999; 53: 508-516.
16. Caplan GD, Brown A, Croker WD et al. Risk of admission within 4 weeks of discharge of elderly patients from the emergency department - the DEED study. *Age Ageing* 1998; 27: 697-702
17. Chu LW, Pei CK. Risk factors for early emergency hospital readmission in elderly medical patients. *Gerontology* 1999; 45: 220-226.
18. Vellas B, Cayla F, Bocquet H et al. Prospective study of restriction of activity in old people after falls. *Age Ageing* 1987; 16: 189-193.
19. Barberger-Gateau P, Grolier L, Maurice S. L'hospitalisation en court séjour des personnes âgées: premier pas vers l'entrée en institution? *Rev Epidemiol Sante Publique* 1990; 38: 323-332.
20. Rudberg MA, Sager MA, Zhang J. Risk factors for nursing home use after hospitalisation for medical illness. *J Gerontol* 1996; 51A: 189- 194.
21. Kariger E, Blanchard F, Ennuyer B et al. Facteurs prédictifs du devenir à 6 mois des personnes de plus de 75 ans admises en urgence à l'hôpital. *Rev Epidemiol Sante Publique* 1996; 44: 47-56.
22. Di Iorio A, Longo AL, Mitidieri Costanza A et al. Characteristics of geriatric patients related to early and late readmissions to hospital. *Aging* (Milano) 1998; 10: 339-346.
23. Whitlatch CJ, Zarit SH, Von Eye A. Efficacy of interventions with caregivers: a reanalysis. *Gerontologist* 1991; 31: 9-14.
24. Schulz R, Beach SR. Caregiving as a risk factor formotaity: the Caregiver Health Effects Study. *JAMA* 1999; 282: 2215-2219.
25. Zarit S, Reever KE, Bach-Peterson J. Relatives of the impaired elderly: correlates of feelings of burden. *Gerontologist* 1980; 20: 649-655.
26. Knight B, Lutzky S, Macofsky-Urban F. A meta-analytic review of interventions for caregivers distress: recommendations for future research. *Gerontologis*t 1993; 33: 240-248.
27. Cohen CA, Pushkar D. Transitions in care: Lessons learned from a longitudinal study of dementia care. *Am J Geriatr Psychiatry* 1999; 7: 139-146.
28. Bertogliati C, Lafont V, Averbeke H et al. Intérêt d'une prise en charge des familles dans le traitement cognitif des démences. *L'Année Gérontologique* 2001; 15: 183-190.
29. Saravay SM, Pollack S, Steinberg MD et al. Four-year follow-up of the influence of psychological comorbidity on medical rehospitalization. *Am J Psychiatry* 1996; 153: 397-403.
30. Sullivan DH. Risk factors for early hospital readmission in a select population of geriatric rehabilitation patients: the signifi - cance of nutritional status. *J Am Geriatr Soc* 1992; 40: 792-798.

Efficacy and Safety of Rivastigmine in Patients with Mild to Moderate Probable Alzheimer's Disease in a Community Setting

Hellenic Alzheimer's Disease study group. Participants (centre's order according to patient contribution):

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Abstract

The efficacy and safety of the acetylcholinesterase inhibitor rivastigmine (EXELON®) was evaluated in Alzheimer's Disease (AD) patients in an international multicentre, open-label study. The results from 104 patients who participated in the study in Greece are presented in this report. The population of the study consisted of patients ranging between 52 and 88 years of age, with mild to moderate probable Alzheimer's Disease. The duration of the study was 26 weeks. Patients received a starting dose of 3 mg/day rivastigmine, followed by escalating dose increases of 3 mg/day, until the highest tolerable dose was achieved, with maximum dosage: 12 mg/day. Results: There was an average improvement of 7.8% in the MMSE score after 12 weeks of treatment and a stabilisation of the MMSE after 26 weeks, demonstrating clinical efficacy. 97% of patients treated with rivastigmine improved or maintained their baseline level of performance in the Naming Objects and Fingers Test of the Alzheimer's Disease Assessment Scale - Cognitive subscale, (29% mean improvement, p=0.009). The disease severity and the mental state of the majority of patients showed either improvement or stabilisation during the 26 weeks of the study. More specifically, in 15% of the patients the Global Deterioration Scale assessment was improved and in 82% of the patients was stabilised. This indicates the efficacy of rivastigmine, as it is well established through several studies that untreated AD patients decline significantly in the same period of time. Safety- tolerability: 83% of patients completed the study and 62% tolerated the highest maintenance dose of 12 mg/day. Treatment with rivastigmine was not associated with any significant adverse events, or with any abnormalities in laboratory parameters, ECGs and cardiovascular vital signs. Conclusion: This study provides evidence of the efficacy and safety of rivastigmine in the treatment of patients with mild to moderate AD.

Introduction

Alzheimer's Disease (AD) is one of the most disabling conditions of old age, affecting about 5% of people aged over 65 years (Bachman et al. 1992). It is the most common type of dementia and affects about 17 to 25 million people worldwide (Brayne et al., 1995). The annual incidence of Alzheimer's disease increases with age, from about 1% in those aged 65 to 75 years to more than 8% in those > 85 years (Bachman et al., 1993; Hebert et al., 1995). As the elderly proportion of the population is increasing, the prevalence of AD is predicted to grow, making it one of the major public health concerns.

Although the underlying causes of AD are still unknown, the neuropathological changes occurring in the brains of AD are well documented (Yankner, 1996). In addition to the presence of plaques and tangles, AD is characterized by a progressive degeneration of specific groups of neurons. In particular, there is a substantial loss of cholinergic neurons (Morris et al., 1993). Loss of central cholinergic function in the brains of patients with AD has been correlated with deficits in memory and other aspects of cognition (Stern et al., 1994). This has led to the development of therapeutic strategies which involve cholinergic activation by utilising presynaptic (precursor loading), synaptic (acetylcholinesterase inhibitors) and postsynaptic (cholinergic agonists) approaches (Perry et al., 1978; Perry et al., 1986). To date, cholinesterase inhibition is the only approach proven to be effective in the treatment of AD symptoms (Enz et al., 1993).

Rivastigmine is a "dual" cholinesterase inhibitor, as it inhibits both acetylocholinesterace (AChE) and a butyrylcholinesterase (BuChE). (Enz et al., 1993; Cutler et al., 1998; Kennedy et al., 1999). Inhibition of BuChE by rivastigmine is important, in light of the fact that BuChE is increased in the brains of AD patients (Perry EK. et al., 1978; Weinstock M., 1999). There is emerging evidence of the role of BuChE in the cholinergic regulation and in the course of AD (Guillozet AL. et al., 1997; Giacobini E, et al., 1999).

Rivastigmine is a pseudo - irreversible inhibitor of the carbamate type that shows brain and brain-region selectivity, especially for the hippocampus and cortex areas which are associated with AD cholinergic dysfunction (Anand et al., 1996).

In contrast to other ChE inhibitors, the metabolism of rivastigmine is almost entirely independent of the hepatic cytochrome P450 system and occurs mostly via esterases (Nordberg et al., 1998; Polinsky, 1998) reducing thereby the potential for drug-drug interactions. A recent analysis of rivastigmine double-blind clinical trials has revealed no adverse pharmacodynamic drug interactions between rivastigmine and over twenty classes of medications (Grossberg et al.; 2000). The duration of AChE inhibition by rivastigmine is approximately 10 hours (Schneider et al., 1998).

The efficacy and safety of rivastigmine in the treatment of mild to moderately severe AD was demonstrated in a large phase III clinical program that included 3000 AD patients treated with rivastigmine and 1000 treated with placebo. It was shown that rivastigmine effectively improves the three key-symptom domains in AD: Cognition, Activities of Daily Living (ADL) and Behavioural Symptoms. It is also proved rivastigmine is well tolerated and safe for elderly patients (Corey-Bloom et al., 1998; Anand et al., 1996; Rosler et al., 1999; Sramek et al., 1996; McKeith et al, 2000).

The main focus of the international multicentre trial, part of which is presented here, was to study the use of rivastigmine 3 mg/day - 12 mg/day in a broader population of "real world" patients in the community setting.

The study had two objectives. The first objective was to evaluate the safety and tolerability of rivastigmine in patients with mild to moderate probable AD, including patients who were also receiving concomitant medications, or were suffering from other diseases such as psychiatric comorbidity (including depression), stable insulin dependent diabetes mellitus, stable cardiac disease and controlled seizure disorder.

A second objective was to evaluate the efficacy of rivastigmine in these patient populations.

Materials and Methods

Patients

Eligible patients were at least 50 years of age, not of childbearing potential, who fulfilled DSM-IV criteria for Alzheimer's type dementia (American Psychiatric Association, 1994), and had probable AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984).

Inclusion criteria of this study were broader compared to previous double-blind controlled trials. Severely demented patients with Mini-Mental State Examination (MMSE) score as low as 6 (MMSE 6-26 allowable range) were eligible for the study. In addition, patients with coexisting medical conditions were allowed to participate, provided that the illnesses were not both severe and unstable (i.e. severe and unstable cardiovascular disease; active uncontrolled seizure disorder; active peptic ulceration; acute or severe asthmatic conditions). Patients were also excluded if they had a total ischemic score of 5 or more on the Modified Hachinski Ischemic Score or a known hypersensitivity to ChEI. There were no restrictions on concomitant medications other than acetylcholine precursors and anticholinergic drugs.

Patients were community-based and for the purposes of this trial, "community setting" was defined as patients seen in an outpatient clinic. Each patient had a responsible caregiver and patients along with their caregivers provided written informed consent. Patients who had CT or MRI scan findings indicating a type of dementia other than AD, were excluded from the study.

All procedures followed in this study were in accord with NOVARTIS standards that meet the regulations of Good Clinical Practice (GCP). These standards respect GCP guidelines, the US code of Federal Regulations dealing with clinical studies and the Declaration of Helsinki ("Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects", Helsinki 1964, amended Tokyo 1975, Venice 1983, and Hong Kong 1989). The protocol, its amendments and the informed consents were reviewed and approved by the Institutional Review Boards of each participating hospital and the local Health Authorities.

Study design and treatments

This study was conducted in the outpatient clinics of 9 hospitals in Greece. All investigators involved were neurologists or psychiatrists. The study utilised a prospective, multicentre, open-label approach. Following screening and baseline assessments, patients began treatment with rivastigmine for 26 weeks. Throughout the study the patients were instructed to take two capsules per day with a full meal, one capsule with breakfast and one capsule with supper.

All patients began treatment at a dose of 1.5 mg b.i.d. (3 mg/day) and were scheduled to have dose increases of 1.5 mg b.i.d every two weeks until signs of intolerance were encountered, or the maximum dose of 6 mg b.i.d. (12 mg/day) was achieved. This design included dosing flexibility allowing longer intervals between dose-increases if signs of low tolerability were observed. In a case like this, the investigator could also instruct the patient to skip doses (no more than 3 consecutive doses in a week); prescribe an antiemetic; or titrate the patient's dose downward by one dose.

Safety evaluations

Safety evaluations were performed at the end of Weeks 2, 4, 12, 18 and 26 and included assessment of the following variables: physical / neurological examination, vital signs, clinical laboratory tests (including haematology, blood chemistry and urinalysis), and electrocardiogram. Adverse events were assessed by physician's questioning of patients and carers for newly imerged symptoms at each visit. Prior and concomitant medications and therapies were also documented throughout the study.

Efficacy evaluations

Evaluation of efficacy was performed at the end of Weeks 12, 18 and 26. Efficacy measures were chosen to fulfil the FDA's dual efficacy requirement for AD clinical trials: improvement on a performance-based cognitive instrument and demonstration that the improvement is clinically meaningful (F-D-C reports, 1992). Efficacy variables used were as follows; change from the baseline in the total score of the Mini-Mental State Examination (MMSE), change

Table 1. Stages of GDS.

GDS stage	Clinical Phase	Clinical Characteristic
1	Normal	
2	Normal	Subjective forgetfulness but normal examination
3	Mild cognitive impairment	Difficulty at work, in speech or when travelling in unfamiliar areas, detectable by family; subtle memory deficit on examination
4	Mild dementia	Decreased ability to travel, count or remember current events
5	Moderate dementia	Needs assistance to choose clothes, disorientation to time and place, decreased recall of name of grandchildren
6	Moderately severe dementia	Needs supervision for eating and toileting, may be inconti nent, disorientated to time, place and possible person
7	Severe dementia	Severe speech loss, incontinence and motor stress

*Adapted from Reisberg et al., 1982.

from baseline in the Global Deterioration Scale (GDS), change from baseline in the (Modified) ADAS-Cog (Naming Objects and Fingers subtest) and the Digit Symbol Substitution Task (DSST).

The Mini-Mental State Examination and the Global Deterioration Scale were used as staging measures at baseline and at the end of weeks 12 and 26. The MMSE is the most widely used mental test in AD drug studies and assesses recent memory, attention, concentration, naming, repetition, comprehension, visuospatial function (Folstein et al., 1975). Several studies have shown that the annual average deterioration of untreated AD patients is between 3 and 4 MMSE points. (Olichney et al., 1998; Thal et al. 2000).

The Global Deterioration Scale is a 7-point rating scale used to identify the level of the patient's cognitive and functional state, where a rating of 1 corresponds to no decline while a rating of 7 to a very severe decline. The patient's overall state is rated using information obtained by both the patient and the caregiver in the areas of memory functioning, self care and activities of daily living (Reisbeg et al., 1982) (see Table 1).

(Modified) ADAS-Cog: The Alzheimer's Disease Assessment Scale (ADAS) is a performance-based test that measures cognitive and behavioral dysfunction in patients with Alzheimer's disease (Rosen et al., 1984). The Naming Objects and Fingers subtest of the ADAS-Cog was used in this study in combination with a test that assesses patient's attention and concentration i.e. the Digit Symbol Substitution Task (DSST) (Rosen et al., 1984).

Table 2 identifies the efficacy scales used, symptoms and domains measured, evaluation sources, and the interpretation of rating scores.

Statistical methods

The study sample population consisted of 109 patients who met all inclusion/ exclusion criteria at Visit 0 (screening). 104 patients performed Visit 1 (Baseline) assessments. Of the 93 patients that attended visit 2 (end of Week 12), 90 completed the assessments, while of the 90 that participated in Visit 3 (end of week 26), 86 completed the assessments. For all efficacy variables the following descriptive statistics were reported at each visit where assessments took place: sample size, mean, median, standard deviation, minimum and maximum.

The primary efficacy endpoints were defined as follows:

· change from baseline in the total score of MMSE assessed by paired t-tests at weeks 12 and 26

· change from baseline in the Global Deterioration Scale assessed by Wilcoxon rank test at weeks 12 and 26

The secondary variables were:

· change from baseline in the Digit Symbol Substitution Task (DSST) assessed by paired t-tests at weeks 12 and 26

· change from baseline in the score of total incorrect items in the Naming Objects and Fingers test assessed by both paired t-tests and Wilcoxon rank test at weeks 12 and 26

In addition to the analysis of the pooled data, data were also stratified according to varying severity of Alzheimer's Disease. This was determined on the basis of the MMSE score, (ie. mild = MMSE total score of 19 to 26, moderate = MMSE total score of 10 to 18). Furthermore data were also summarised for patients who were receiving any other psychotropic medication and those who were not receiving any. Lastly data were also stratified according to different rivastigmine dose levels.

Table 2. Efficacy Measurements.

Test	Symptoms or domains assessed	Source of information	Range of scale and interpretation
MMSE	Overall mental state	Patient	0-30 points <24 = cognitive decline 0-10 = severe impairment
GDS	Staging disease severity Activities of daily living	Clinician based on information from patient and caregiver	1-7 points 1 = no cognitive decline 7 = severe cognitive decline
DSST	Achieving a complex task: cognition correlation	Patient	0-75 points 0 = worst performance 75 = best performance
Naming Objects and Fingers	Cognition Memory Language	Patient	0-5 points 0 = no decline 5 = severe decline

Results

The study population and its progress throughout the study are presented in Figure 1. A total of 109 patients met inclusion/ exclusion criteria while 104 of these were exposed to at least one dose of study medication. The mean age of the study population was 69.7 (range 52 to 88 years), more patients were female (56%) while all patients were Caucasian. The mean duration of dementia was 33.1 months (range 6 to 216 months).

Dosing

Of the 104 patients who were exposed to at least one dose of study medication 86 completed treatment. This corresponds to 83% of the patient population. The vast majority of those patients (53 out of 86, 62%) tolerated the maximum dose of 6 mg b.i.d. rivastigmine (12 mg/day). Another 19 patients (22%) received 4.5 mg b.i.d. (9 mg/day) of study treatment whereas for 12 patients (14%) dose escalation stopped at 3 mg b.i.d (6 mg/day). Only 2 patients (2%) were unable to tolerate a dose greater than 1.5 mg b.i.d (3 mg/day).

Current medical conditions and concomitant medications

50% of patients reported prior or current medical conditions, or both. These were most commonly cardiovascular (25%), whereas another 12% of the study population suffered from metabolic and nutritional disorders. 60% of patients were taking concomitant drug treatment at baseline. The most common medications taken included drugs for the following disorders: central nervous system (35%), cardiovascular (34%), alimentary tract and metabolism (17%). 13% of the study population received selective serotonin reuptake inhibitors and 11% received salicylic acid or derivatives.

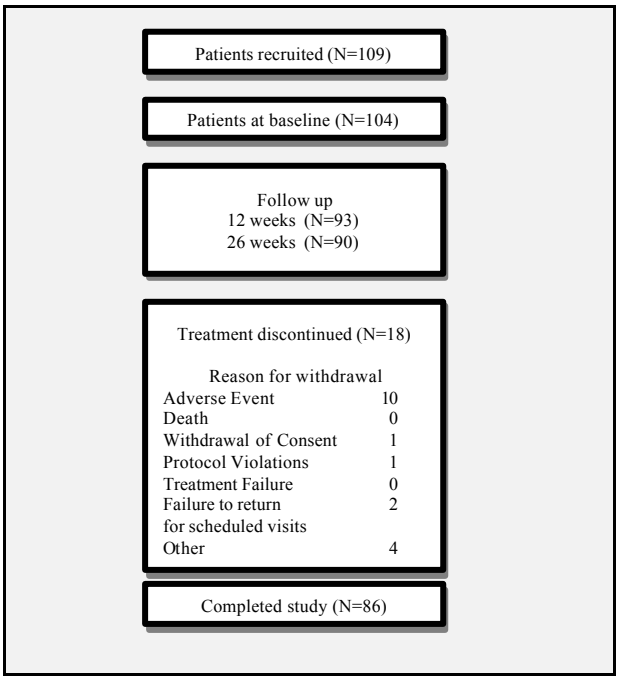

Figure 1. Patient disposition and reasons for withdrawal

Mini-Mental State Examination

From a total of 104 patients at baseline, MMSE data were available for 90 at week 12 and for 86 at week 26. Therefore for statistical purposes data of patients who completed both baseline and a subsequent visit MMSE assessment, were used for the comparison.

A significant increase in the MMSE score was observed at week 12 compared to baseline, whereas at the end of the 26 weeks (Visit 3) the MMSE score was stabilised close to baseline level (see Table 3). More specifically, in the total population, an increase of 0.7 (5.3%) in the MMSE score is observed at week 12 when compared to baseline, p=0.014 (see Figure 2). This increase is more significant in the group of the moderate AD patients (N=56), who achieved at week 12 an improvement of 1 unit in their MMSE score compared to baseline, corresponding to 7.8% improvement, p=0.018 (see Figure 2, table 4).

Table 3. Summary Statistics of Change in MMSE score in total population.

MMSE score, change from baseline

	week 12	week 26
N	90	86
Mean	0.7	-0.3
Std Dev	2.7	3.1
Min	-8	-9
Max	7	8

Another aspect that came up from the analysis of this assessment is that patients who were not receiving psychotropic medication showed a better response to treatment with rivastigmine. In detail, in this certain group of patients there was an increase of 0.8 (5.7%) in the MMSE score at week 12 compared to baseline, p=0.020 (see figure 3).

Table 4. Summary Statistics of Mean Change in MMSE score at week 12 (V2) and week 26 (V3) in comparison to baseline (V1).

Total population	0.7	-0.3
Mild Alzheimer's Disease patients	0.2	-0.4
Moderate Alzheimer's Disease patients	1	-0.2
Patients receiving psychotropic medication	0.6	-0.1
Patients not receiving psychotropic medication	0.8	-0.3

*V1 (Visit 1 = baseline), V2 (Visit 2 = week 12, V3 (Visit 3 = week 26)

Global Deterioration Scale

At baseline, the mean score of the 104 patients who were assessed by GDS was 4 (min. 2 max. 5).

At week 12, 90 patients had available data for the GDS assessment. Their mean score was 3.9, which corresponds to a statistically significant improvement in disease severity in comparison to baseline (0.1 change in the score, p=0.018). Specifically, 13 out of 90 patients improved their GDS score, while only 4 out of 90 deteriorated.

At week 26, 86 patients completed the GDS assessment. Their mean score remained 3.9, which indicates improvement compared to baseline and stabilisation compared to week 12 (see table 5).

Table 5. Summary Statistics of Change in GDS score in total population.

	Change in GDS score at week 12	Change in GDS score at week 26
N	9	8
Mean	-0.1	-0.1
Std Dev	0.5	0.8
Min	-2	-2
Max	1	3

Digit Symbol Substitution Task

This task appeared to be quite difficult to be handled by the participants of this study. 59 out of 104 patients managed to complete the test at baseline whereas at week 12 and 26 the number of patients that completed the test were 58 and 55

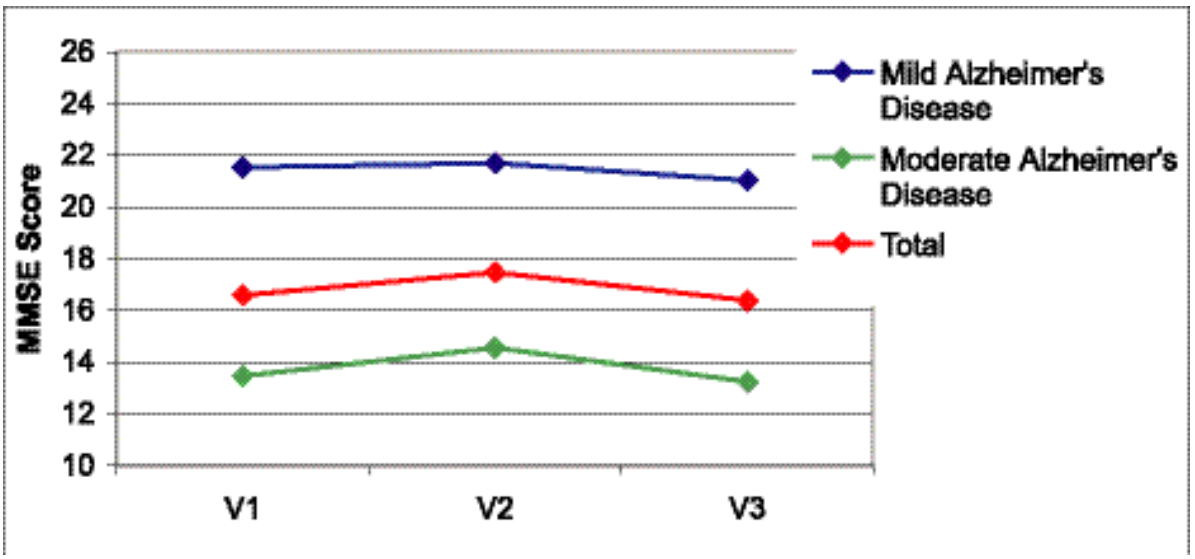

Figure 2. Graphical representation of the mean percentage difference in MMSE score in the three visits.

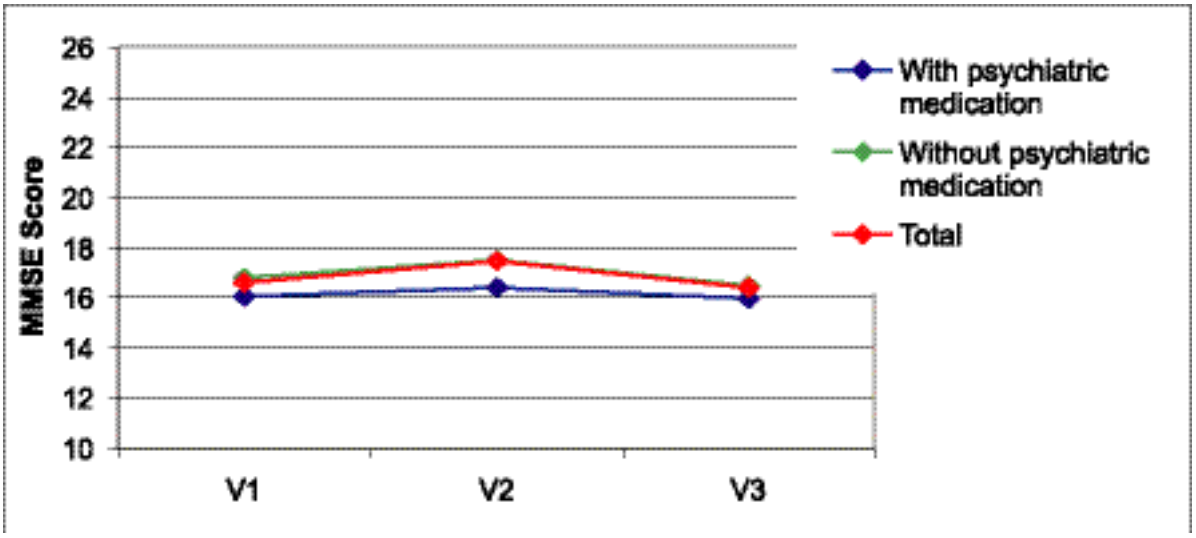

Figure 3. Graphical representation of MMSE score in the three visits adjusted for psychiatric medication they receive.

respectively. The low compliance with the test procedure render results less meaningful than the other assessments. It was concluded that there was not a statistically significant change observed between baseline and subsequent visits.

ADAS-Cog subscales: Naming Objects and Fingers test

In the mild Alzheimer's Disease patient population group (N=34) there was a decrease in the total incorrect score of the Naming Objects and Fingers test by 29%, p=0.009, indicating improvement of cognitive function. More specifically, in 9 out of 34 patients there was a decrease in the total incorrect score and only 1 patient in the same subpopulation showed an increase. The relevant statistics are summarised in table 6.

In all other patient subpopulations a statistically significant difference was not observed and the rivastigmine treatment can therefore be considered as having a stabilising effect as far as this particular test can assess (see figure 4).

Table 6. Summary Statistics of Change in total incorrect score of the Naming objects and fingers tests, in the mild AD patients.

	Change in total incorrect score at week 12	Change in total incorrect score at week 26
N	34	34
Mean	-0.3	-0.1
Std Dev	0.7	0.9
Min	-2	-2
Max	1	2

Safety

Treatment with rivastigmine was not associated with any mortality, serious adverse events, effects on laboratory parameters, on ECGs, or cardiovascular vital signs. Although adverse events were reported in 58 of the 104 patients that participated in the study (57%), the majority were mild to moderate and were resolved without any treatment. The most common adverse events were gastrointestinal system disorders (39%), mainly nausea (25%), vomiting (14%) and diarrhoea (7%). Other reported adverse events included dizziness (8%), fatigue (4%), headache (3%), somnolence (3%), agitation (3%), tremor (2%), aggressive reaction (2%), depression (2%), nervousness (2%), rash (2%) and hypertension (4%). There were only 10 adverse event cases that lead to discontinuation from the study, of which 5 were reported as significant (pneumonia / pulmonary carcinoma, accidental trauma, nausea, confusion and cerebrovascular disorder). Of these 10 adverse events only 2 were characterised as related to study treatment (vomiting/diarrhoea, dizziness). 3 were reported as probably related (nausea, accidental trauma, vomiting), 3 as possibly related (vomiting, confusion, diarrhoea) and 2 as not related (cerebrovascular disorder, pneumonia / pulmonary carcinoma).

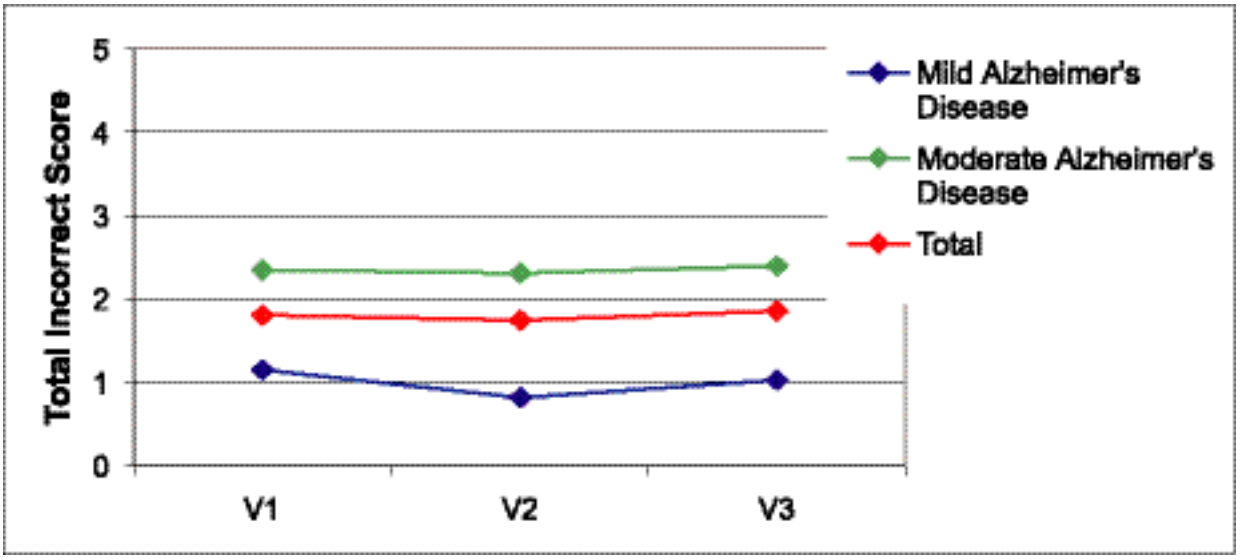

Figure 4. Graphical representation of the total incorrect score change in the three visits.

Discussion

In an effort to evaluate further the safety and efficacy of rivastigmine in a broader and more "real world" population, this open-label trial has focused on patients living in the community, including those with severe dementia or suffering from coexisting illnesses, as long as these were not considered both severe and unstable.

Efficacy

Patients who received rivastigmine treatment demonstrated a statistically significant increase in their MMSE score at week 12 while the MMSE score was stabilised by the end of the 26 week treatment period. The improvement in the MMSE score corresponds to improvement in a number of cognitive functions. Cognitive function in patients with Alzheimer's Disease who are not treated is expected to deteriorate by an average of 3.5 MMSE points in a year. (Olichney et al., 1998; Thal et al., 2000; Rosler et al., 1999). The cognitive improvement or stabilisation observed in this study in patients taking rivastigmine over 6 months can therefore be characterised as highly significant and relevant to clinical practice. These results are in accordance with results of the Phase III controlled studies with rivastigmine.

Patients who received treatment with rivastigmine for 12 weeks showed a statistically significant improvement in disease severity as this was assessed by the Global Deterioration Scale. Improvement was observed in 15% of the patients whereas 82% remained stable. This is also of great clinical relevance since patients of this age group and dementia stage, are expected to deteriorate substantially due to the progressive nature of the disease (Reisberg et al., 1997).

In the ADAS-Cog subscale (the Naming Objects and Fingers Test) patients treated with rivastigmine were able to maintain their level of performance throughout the 26-week treatment. In particular, 97% of the mild AD patients demonstrated either improvement or no change from baseline at 12 weeks. These results clearly show the beneficial effect of rivastigmine, taking in consideration that AD patients decline significantly on the ADAS-Cog subscale over the same period as shown in several clinical studies (Solomon et al., 1996).

Although the Digit Symbol Substitution Task did not provide any statistically significant results, perhaps due to its complexity and difficulty for completion, it still provides evidence for the efficacy of rivastigmine: patients who managed to complete the assessment at baseline, were still able to do so after 12 and 26 weeks. This is of clinical relevance since it would be expected that fewer AD patients could carry out the assessment after 6 months of disease progression.

Safety and Tolerability

Rivastigmine treatment was not associated with any changes in the physical examination, vital signs laboratory values and ECG. In particular there was no change in transaminase levels and no evidence that the treatment compromised cardiovascular function. This is a major advantage of rivastigmine, since cardiovascular diseases

were the most common concurrent medical conditions in the study population.

The fact that the vast majority of the patients who completed protocol treatment received the highest allowed dose of rivastigmine (12 mg/day) suggests that the drug is highly tolerable and that adverse events are easily manageable. Adverse events were generally mild to moderate and in most cases resolved without treatment.

Conclusion

This study evaluated the safety and efficacy of rivastigmine in a broad "real world" population, in the community setting. The positive outcome of this study is in accordance with the results coming out of other clinical studies with rivastigmine, including the ADENA program -the largest multicentre-multinational clinical trial conducted to date for an antidementia medication (3000 patients) (Schneider et al.,1998).

In conclusion rivastigmine is an effective, well tolerated, and safe treatment which provides clinically significant cognitive benefits and enhances global functioning of patients with mild to moderate Alzheimer's Disease.

Abbreviations

AChE - Acetylcholinesterase
AD - Alzheimer's Disease
ADENA - Alzheimer's Disease Treatment with ENA-713
ADL - Activities of Daily Living
ADAS - Alzheimer's Disease Assessment Scale
ADAS-Cog -ADAS cognitive subscale
ADRDA - Alzheimer's Disease and Related Disorders Association
BuChE - butyrylcholinesterase
CT - Computerised Tomography
DSST - Digit Symbol Substitution Task
ECG - Electrocardiogram
GCP - Good Clinical Practice
MRI - Magnetic Resonance Imaging
NINCDS - National Institute of Neurological and Communicative Disorders and Stroke
MMSE - Mini-Mental State Examination

References

1. Anand R, Gharabawi G, Enz A (1996). Efficacy and safety results of the early phase studies with EXELON® (ENA-713) in Alzheimer's disease: an overview. *Journal of Drug Development in Clinical Practice*. 8; 109-116.
2. American Psychiatric Association (1994). Diagnostic and statistical Manual of Mental Disorders 4th Edition (DSM-IV), *American Psychiatric Press*: Washington DC 1994.
3. Bachman DL, Wolf PA, Linn R et al. (1992). Prevalence if dementia and probable senile dementia of the Alzheimer type in the Framingham study. *Neurology*. 42; 115-119.
4. Brayne C, Gill C, Huppert FA (1995). Incidence of clinically diagnosed subtypes of dementia in an elderly population. Cambridge Project for later life. *British Journal of Psychiatry*. 167; 255-262.
5. Corey-Bloom J, Anand R, Veach MS (1998). A randomised trial evaluating the efficacy and safety of ENA-713, a new acetylcholinesterase inhibitor in patients with mild to moderately severe Alzheimer's disease. *International Journal of Geriatric Psychopharmacology*. 1; 55-65.
6. Cutler NR, Polinsky RJ, Sramek JJ, Enz A, Jhee SS, Mancion L, Hourani J, Zolnouni P (1998). Dose-dependent CSF acetylcholinesterase inhibition by SDZ ENA 713 in Alzheimer's disease. *Acta Neurol Scand*. 97: 244-50.
7. Enz A, Amstutz R, Boddeke H, Gmelin G, Malanowski J (1993). Brain selective inhibition of acetylcholinesterase: a novel approach to therapy for Alzheimer's disease. Prog Brain Res. 98: 431-8.
8. Enz A, Floersheim P (1996). Cholinesterase inhibitors: an overview of their mechanisms of action in *Alzheimer's Disease. Alzheimer's Disease: from molecular biology to therapy.* Boston: Birkh publisher, 211-5.
9. Enz A, Amstutz R, Boddeke H et al. (1993). Brain selective inhibition of acetylcholinesterase: a novel approach to therapy for AD. *Progresses in Brain Research* 98; 431-438
10. F-D-C reports (1992) Inc. FDA guidance of Alzheimer's drug clinical utility assessments. FDC reports ("The Pink Sheet") 54: 13-15.
11. Folstein MF, Folsetin SE, McHugh PR (1975). "Mini-Mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*. 12: 189-198.
12. Grossberg G, Stahelin HB, Messina JC, Anand R, Veach J (2000). Lack of adverse pharmacodynamic drug interactions with rivastigmine and twenty-two classes of medications. *Int J Geriatr Psychiatry* 15: 242-7.
13. Giacobini E, Griffini PL, Maggi T, Mascellani G, and Mandelli R (1999). Butyrylcholinesterase: Is it important for cortical acetylcholine regulation? *Soc. Neurosci*. Abstr. 84.18.
14. Guillozet AL, Smiley JF, Mash DC, Mesulam M (1997). Butyrylcholinesterase in the life cycle of amyloid plaques. *Ann Neurol*, 42:909-918.

15. Hebert LE, Scherr PA, Beckett LA, Albert MS, Pilgrim DM, Chown MJ, Funkenstein HH, Evans DA (1995). Age-specific incidence of Alzheimer's disease in a community population. *JAMA*; 273: 1354-9.
16. Kennedy JS, Polinsky RJ, Johnson B, Loosen P, Enz A, Laplanche R, Schmidt D Mancione LC, Parris WC, Ebert MH (1999). Preferential cerebrospinal fluid acetylcholinesterase inhibition by rivastigmine in humans. *J Clin Psychopharmacol*; 19: 513-21.
17. Knapp KJ, Knopman DS, Solomon PR (1994). A 30 week randomised controlled trial of high-dose tacrine in AD patients JAMA 271: 985-991.
18. McKeith I, del Ser T, Spano PF et al. (2000). Efficacy of rivastigmine in dementia with Lewy Bodies: results of a randomised placebo controlled international study. *Lancet*; 356: 2031-2036.
19. MacKhann G, Drachman D, Folstein M et al. (1984). Clinical diagnosis of AD: report of the NINCDS-ADRDA Work Group under the auspices of the Department of health and Human Services Task Force on AD. *Neurology*. 34: 939-944.
20. Morris JC, Edland S, Clark C et al. (1993). Corsortium to establish a registry for Alzheimer's Disease (CERAD). Part IV. Rates of Cognitive change in the longitudinal assessment of probable Alzheimer's disease. *Neurology*. 43: 2457-2465.
21. Nordberg A, Svensson AL (1998). Cholinesterase inhibitors in the treatment of Alzheimer's disease: a comparison of tolerability and pharmacology. *Drug-Saf*.; 19(6): 465-80.
22. Olichney JM, Galasko D, Salmon DP et al. (1998). Cognitive decline is faster in Lewy body variant than in Alzheimer's disease. *Neurology*; 51:351-357.
23. Perry EK (1996). The cholinergic hypothesis. Br *Med Bull*; 42: 63-9.
24. Perry EK, Perry RH, Blessed G and Tomlinson BE (1978). Changes in brain cholinesterases in senile dementia of Alzheimer type. *Neuropathol and Applied Neuorobiol*; 4:273-277.
25. Perry EK, Tomlinson BE, Blessed G et al. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *British Medical Journal*; 2; 1457-1459 .
26. Polinsky RJ (1998). Clinical pharmacology of rivastigmine: a new-generation acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. *Clin Ther*; 20: 634-47.
27. Reisberg B, Ferris SH, DeLeon MJ, Crook T (1982). The Global Deterioration Scale for assessment of primary degenerative dementia. *Am J Psychiatry*; 139: 1136-1139.
28. Reisberg B, Schneider L, Doody R, Anand R, (1997). Clinical global measure of dementia. Position paper from the *International Working group on Harmonisation of Dementia Drug Guideline. Alzheimer's Disease Associated Disorders* 11s3; 8-18.
29. Rosen WG, Mohs RC, Davis KL, (1984). A new rating scale for Alzheimer's disease. American Journal of Psychiatry 141: 1356-1364
30. Rosler M, Anand R, Cicin-Sain A, Gauthier S, Agid Y, Dal-Bianco P, Stahelin HB, Hartman R, Gharabawi M (1999). Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. *British Medical Journal* 318; 633-638
31. Schneider LS, Anand R and Farlow MR (1998). Systematic review of the efficacy of rivastigmine for patients with AD. *International Journal of Geriatric Psychopharmacology*; 1 S1; 26-34.
32. Solomon PR, Knapp MJ, Gracon SI (1996). Long-term tacrine treatment in patients with Alzheimer's disease. Lancet; 348; 275-276.
33. Sramek JJ, Anand R, wardle TS (1996). Safety/ tolerability trial of SDZ ENA 713 in patients with probable AD. *Life Sciences*; 58; 1201-1207
34. Stern RG, Mohs RC, Davidson M et al., (1994). A longitudinal study in Alzheimer's Disease: measurement, rate and predictors of cognitive deterioration. *American Journal of Psychiatry*; 151; 390-396
35. Thal LJ, Calvani M, Amato A, et al (2000). A 1-year controlled trial of acetyl-l-carnitine in early-onset AD. *Neurology*; 55(6): 805-810
36. Weinstock M. Selectivity of cholinesterase (1999). Clinical implications for the treatment of Alzheimer's disease. *CNS Drugs*; 12: 307-323.
37. Yankner BA (1996). Mechanisms of neuronal degeneration in Alzheimer's disease. *Neuron*; 16: 921-32.

Vitamin B_{12}, Folate and Homocysteine in Persons with Cognitive Impairment

A comparative study of serum levels among persons living single or in a family

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Abstract

A combination of both internal and external factors can influence the levels of vitamin B_{12} and folate in elderly persons. In this study we compared the serum levels of folate, vitamin B_{12}, and homocysteine (Hcy) in persons with cognitive impairment living either single or in a family setting. The survey comprised 120 persons, sixty men and sixty women. In each group half of the patients lived in a family, whereas the other half lived single. From patient journals between 1995-99, age, sex, weight, diagnosis, form of habitation, cobalamin, folate, Hcy and creatinine in serum, as well as results of Mini Mental Test at the first visit to the investigation units were recorded. The mean age for both men and women in a family was 72. Mean ages of men and women living single were 70 and 75 years, respectively.

All groups had negative correlation vitamin B_{12} - Hcy. There was a significant negative correlation vitamin B_{12} - Hcy in the group single living men ($p<0.05$). There was a stronger negative correlation between folate - Hcy than for vitamin B_{12} - Hcy and for men in a family it was significant ($p<0.05$). Single living men had significantly higher Hcy levels ($p<0.01$) compared to men living in a family.

The results of this study suggest that men living single run a greater risk of developing vitamin B_{12} and folate deficiency than men living in a family. A higher degree of awareness and an early diagnosis of deficiency of these vitamins are of great importance, since the best results in treatment of a deficiency with vitamin B_{12}/folate have been reported when treatment is started within six months from detection of cognitive disturbances.

Keywords: Vitamin B_{12}, folate, homocysteine, cognitive impairment

Background

Several studies have shown a connection between low levels of serum folate and vitamin B_{12} and the risk of developing dementia (1-7). Deficiency in these vitamins disturbs DNA and RNA synthesis in cells and reduces their capacity for repair. The tissues afflicted are primarily those with a high-speed production of new cells, such as bone marrow, resulting in anaemia. Elderly persons with vitamin deficiency show central nervous system symptoms that can occur isolated and earlier than symptoms from blood producing organs (8).

The serum levels of folate and vitamin B_{12} depend on a number of different factors, including the amounts available in food, the absorption capacity of the digestion and intestinal canals, as well as transport and metabolism. This study is aimed at a social-medical aspect, in which we compared the serum levels of folate, vitamin B_{12}, and

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homocysteine in persons with cognitive impairment living either single or in a family setting. The Ethical Committee of Gävle-Dala Sweden approved the study.

Vitamin B_{12}

Cobalamin or vitamin B_{12} consists of a group of closely related chemical compounds, which cannot be synthesized in the body and must therefore be taken up through food. The most important food sources of vitamin B_{12} include liver, egg, meat, and milk products. The vitamin occurs as methyl-, hydroxo- and deoxyadenosylcobalamin forms in food and is mostly bound to proteins.

Cobalamin in food is set free by cooking and also by digestion and intestinal enzymes. Saliva contains a protein, haptocorrin (HC) that binds B_{12}. This complex passes through the ventricle and the vitamin is set free by the action of pancreatic enzyme. Free vitamin then binds to a glycoprotein, intrinsic factor (IF) transport protein produced by the parietal cells of the ventricle. IF is tightly bound to the vitamin and provides protection against proteolysis. The cobalamin-IF complex is then absorbed via special cubilin receptors in the mucous membrane of the distal ileum.

Normal food provides a daily intake of 7-15 nmol cobalamin. Of this, 3-4 nmol is absorbed in the ileum. Our bodies contain a total of 4 mg cobalamin, of which 30% is found in liver and kidneys. The body loses cobalamin through the bile and urine. Strict vegans acquire a vitamin B_{12} deficiency due to their diet. However, the most common causes of cobalamin deficiency include too low content of IF and intestinal diseases affecting the ileum. It should be noted, especially with regard to the elderly, that microwave heating of food causes a 30-40% loss of vitamin B_{12} (9).

Due to the difficulties in accurately measuring levels of B_{12} in the body, interest has focused on metabolic processes where cobalamin is active so as to find suitable markers of B_{12} deficiency. The metabolites methylmalonate and homocysteine have been used as suitable and sensitive, albeit non-specific markers of B_{12}.

Folate

Folates are the collective name of water-soluble vitamins in the B vitamin group. They are found mostly in fruit, fresh vegetables and cereals. The daily requirement is an approximate 400q and the body's store of folates can last for 3-4 months during reduced intake. Folates in the food are absorbed in a reduced form in the proximal jejunum. This occurs with the help of proteins and releasing enzyme in the mucosa of the duodenum and jejunum. A number of studies have shown that elderly people have an increased risk of developing folate deficiency (7, 10). It has also been noticed that disturbed transport and metabolism may cause both vitamin B_{12} and folate deficiency in the elderly (11, 12). Low levels of B_{12} in combination with folate deficiency have been shown to cause cognitive disturbances in elderly persons (6). Folate deficiency without low cobalamin levels may also cause neurological damage (13). A study by Wang et al. of 370 non-demented elderly people showed that low serum levels of folates and/or cobalamin resulted in a doubled risk of developing Alzheimer dementia during a three-year follow-up (3).

Several theories have been presented to explain the origin of folate deficiency. One of these is the so-called "low-intake" hypothesis, as studied by Levitt and Karlinsky among others (5). This hypothesis proposes that people with a reduced cognitive ability have a reduced capacity to cope with their nutrition. Another reason is prolonged heating of foods, which spoils folate, vitamin B_6 and B_{12} (14, 15). It is also possible that a diet with low intake of little fresh fruit and vegetables can lead to a loss of folate and an increased amount of homocysteine (16).

Homocysteine

Both vitamin B_{12} and folate belong to the methyl cycle (Fig 1) which is important for the production of enzymes, membranes and neurotransmitters in the brain. To ensure a normal folate function, it is necessary that methylcobalamin is available.

Vitamin B_{12} is a co-enzyme in the normal renewal of folates. In the case of cobalamin deficiency, DNA synthesis is restricted due to a relative lack of tetrahydrofolate.

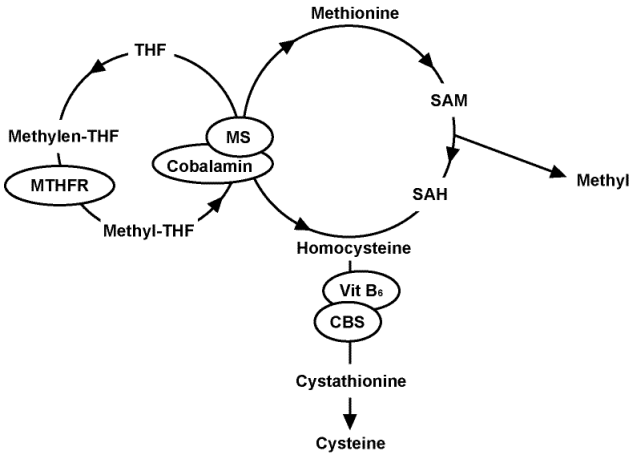

Figure 1. Homocysteine (Hcy) metabolism.
Hcy is formed by demethylation of methionine. The methyl group in mehtionine is activated by conversion to S-adenosyl-methionine (SAM), which is demethylated to S-adenosyl-homocysteine (SAH). SAH is hydrolysed to Hcy. Methionine synthase (MS) remethylates Hcy, vitamin B12 is a cofactor and methyltetrahydrofolate the substrate in the reaction. MTHFR = 5,10 Methylentetrahydrofolate reductase; CBS = cystathionine-synthase.

Without vitamin B_{12}, the cells cannot absorb folates in a normal way. Consequently, when there is a B_{12} deficiency the supply of intracellular folate is smaller than normal, whereas serum folate is either normal or increased.

SAM is the most important methyl donor of the body. Methyl groups are essential for many processes including the myelineation of neurons and for neuotransmitter function. A SAM deficiency is regarded to cause neuropsychiatric disruptions such as reduced memory function in elderly people. An increased homocysteine will result from a deficiency in methyl donor and co-enzyme.

Homocysteine in plasma is mostly bound to protein. A small amount is unbound and contains a very reactive sulphydryl group that may have toxic effects, for example by producing oxygen radicals (17). Unbound homocysteine may affect vessel endothelium and can injure the plexus choroideus (18). Homocysteine levels are enhanced in both cobalamin and folate deficiencies (4,19-21). Homocysteine is a broad marker of both malnutrition and malabsorbation, with significance for the absorption of folate, cobalamin and vitamin B_6 (22).

In the above-mentioned studies on cobalamin and folate deficiency and increased homocysteine levels in persons with cognitive impairment, values were compared with those of a healthy control group of the same age. In order to examine more specifically the influence of food on the B_{12} and folate status, we in this study compared the relevant values in two groups of persons that probably differ as to food and nutrition intake, namely persons living in a family and persons living single.

Method and material

This survey comprised 120 persons and was performed at the Department of Geriatrics-Rehabilitation, Falun Hospital, and at the Geriatric Department, Huddinge University Hospital. Persons were recruited from memory investigation units at the departments in question, where general practitioners or equivalent doctors referred the patients during 1995-1999. In each group half of the patients lived in a family, whereas the other half lived single. Forty-one men and 34 women were from Falun. Nineteen men and 26 women were from Huddinge.

From the patient case books we obtained information as to age, sex, weight, diagnosis, form of habitation, cobalamin, folate, homocysteine and creatinine in serum, as well as results of Mini Mental Test at the first visit to the investigation units (23). The mean age for both men and women in a family was 72. For mean ages of men and women living single were 70 and 75 years, respectively.

Statistics

Student´s t-test was used for differences between groups and Pearson´s correlation test was used for studying the correlation of variables. All statistics were performed in the Excel program of Microsoft Software.

Results

No significant difference was seen as to age between persons in a family and those living single, although women living alone had, as could be expected, the highest mean age (Table 1). Men and women living in a family weighed more than those living single. The mean values from the Mini Mental Test showed no significant differences between the groups. Both groups of women and the group of men living in a family contained persons who were not able to do the MMT (women in a family: one person, single living women: three persons, and men in a family: two persons,

Table 1. Investigated parameters for patients of different social constellations.

	Men in a family	n	Men single	n
Age	72±7.2	30	70±11	3
Weight	77±10.8	25	73.9±12.1	26
MMT	23.1±3.9	28	22.8±6	30
B_{12} (170-600 pmol/l)	264±112	29	248±102	30
Folate (>4 nmol/l)	10.9±3.6	28	11±9.3	29
Homocysteine (m<20, mol/l)	15.5± 6.1 *	30	21.9 ±8.3 *	30
Creatinine (<110 mol/l)	96.7±14.7	29	98.7±18.3	27

* = p<0.01 Hcy men in family/men single

Table 1. (cont.)

	Women in a family	n	Women single	n
Age	72± 6.8	30	75±.8.5	30
Weight	65.5±8.8	30	61.7±10	27
MMT	22.3±5.6	29	23.4 ±4.7	27
B_{12} (170-600 pmol/l)	263±88	30	272 ±137	30
Folate (>4 nmol/l)	11.1±4.6	30	13.9 ±12.8	30
Homocysteine (w<16 mol/l)	18±5.5	30	19.3± 7.5	30
Creatinine (<110 mol/l)	83.9±13.7	30	79,6 11,9	30

respectively), and they were excluded in the statistical analyses. The mean values for vitamin B_{12} were in all groups within the lower part of the reference region. Men living single had the lowest values (248 pmol/l). There were no significant differences between the groups regarding vitamin B_{12} and folate levels. Women living alone had the highest level of folate (13.9 nmol/l).

All mean values for homocysteine were above the limit of the reference region, except for the values for men living in families. A significant ($p<0.01$) difference in homocysteine levels was found between men in families (15,5 mol/l) and men living single (21.9 mol/l). Creatinine levels were within the reference region for all groups.

Correlations were performed with homocysteine and age, weight, MMT, B-vitamins, and creatinine and the results are depicted in table 2.

Discussion

Absorption and metabolism of cobalamin and folate are extremely complex and depend on many factors. This survey concentrated on social factors as the cause of vitamin deficiency. Since the study was retrospective and aimed at patients with cognitive impairment, we have not been able to take into account anamnestic food factors. Nor has it been possible to examine the function of the ventricle, which would have been preferable with regard to the absorption of vitamin B_{12}. Moreover, data about the person's height would have been valuable as it then would have been possible, in combination with weight data, to determine BMI (Body Mass Index). Several studies have shown that elderly people's intake of food is insufficient (24-26). This could result in a folate/ B_{12} deficiency and later on impaired cell functions.

Table 2. Correlations of Hcy and investigated parameters in patients of different social constellations.

Correlations, r =		Men in a family	n	Men single	n
Hcy/age	above median (73)	0.503	15 (73)	-0.129	15
	below median (73)	-0.019	15 (73)	0.388	15
	total	0.217	30	-0.032	30
Hcy/weight		0.065	25	0.194	26
Hcy/MMT		-0.055	28	0.097	30
Hcy/B_{12}		-0.098	29	-0.361	3
Hcy/Folate		-0.388	28	-0.110	29
Hcy/Creatinine		0.234	29	0.326	27

Table 2. (cont.)

Correlations,	r =	Women in a family	n	Women single	n
Hcy/age	above median (72,5)	-0.069	15 (76,5)	-0.059	15
	below median (72,5)	-0.432	15 (76;5)	-0.360	15
	total	-0.003	30	0.440*	30
Hcy/weight		-0.110	30	0.456 *	27
Hcy/MMT		0.322	29	0.115	27
Hcy/B_{12}		-0.079	30	-0.290	30
Hcy/Folate	-0.359	30	-0.260		30
Hcy/Creatinine	0.038	30	0.136		30

* = $p < 0.05$

The mean values of vitamin B_{12}, folate and creatinine showed no significant difference between persons in the two different forms of habitation. As serum creatinine levels were on average within the reference region it can be assumed that the increased levels of homocysteine are not caused by kidney dysfunction, which could otherwise be a cause. Men living in a family had a higher mean value of cobalamin than those living single, whereas the situation was opposite for the women. Men living single had the lowest mean value. One can easily understand that diet may not be sufficiently varied for this group. These men belonged to a generation where men were not particularly active in cooking. If interest is weak they are likely to choose pre-cooked food, which they keep warm, or heat in a microwave oven thus reducing folate, cobalamin, and vitamin B_6 (14, 15).

According to other authors serum cobalamin is not a reliable indicator of B12 deficiency and one cannot exclude B_{12} deficiency at values between 100 - 200 pmol/l (27, 28). The difficulties in estimating the functional levels of the vitamin are due to the fact that cobalamin, for its function, must pass a large number of membranes with the aid of receptors and carrier proteins. Moreover, the biologically active part of serum consists of only the 30% bound to holotranscobalamin. Of all the individuals in the survey 19% were below the bottom limit of the reference region (<170 pmol/l). Of the total number of examined persons 29% had values <200 pmol/l, of these 15 persons were living in a family and 20 single.

The mean values of folates were nearly identical for men in family and single living men. The high folate level in single living women (13.9 nmol/l) may be explained by women eating more fruit and vegetables than men.

There was a significant difference ($p<0.01$) in homocysteine levels in men living in a family compared to those living single. Diet can be the foremost reason for the difference, in spite of normal serum levels of B_{12}/folate. Overt deficiency is not yet manifest as could be seen by significantly reduced serum levels of the vitamins. As creatinine levels, which have an influence on homocysteine levels, were within the reference region for both groups, it is unlikely that Hcy was caused by kidney dysfunction. The women living single also had higher homocysteine levels than those living in a family. The number of men with increased homocysteine levels was 20 (5 in a family and 15 alone). Forty women (20 in each group) had increased homocysteine levels. Recently Bates et al reported that there may be important environmental factors that predict functional B_{12} status in older people (29). Subtle functional effects with neuropsychiatric symptoms may be consequences of early vitamin B_{12} deficiency (30). Out of the total number of examined persons in that study 50% had homocysteine values above the reference region. This percentage corresponds to that given in a study by Gottfries et al in 1998, where increased homocysteine values were found in 39% of patients with a slightly impaired cognitive capacity (19). The higher prevalence figures in our study may be influenced by the fact that a considerable number of the persons were diagnosed at a later stage of their illness. Previous studies have also shown an almost linear relation between the seriousness of cognitive impairment and homocysteine levels (4, 20)

Homocysteine was negatively correlated to age in all groups, except for men in a family. The correlation was significant ($r = -0.440$, $p<0.05$) for the group of women living single. This is an unexpected finding, as it is known that homocysteine levels increase with age (31). When performing partial correlations with age and homocysteine (above and below median value) these results are the same for all women and single men above median age. This might be due to the small number of patients and spurious chance phenomena. Among the women living single there was a significant positive relation ($r = 0.456$, $p<0.05$) between homocysteine and weight. Diet composition could

be a possible reason for this. Men in a family were the only group with a negative correlation between homocysteine and MMT

(r = -0.055). Of the other groups, women in a family had the highest positive correlation

(r = 0.322). These positive findings are opposed to the established results of other studies (1,4) and could probably be explained by chance due to the small number of patients.

All groups had negatively correlated homocysteine and B_{12}. Of these, the group of men living single showed a significant correlation ($r = -0.361$, $p<0.05$). As expected, the strongest negative correlation was found between homocysteine and folate, owing to the importance of folate in the methylation cycle. In this study this was most evident in the group of men living in a family ($r = -0.388$, $p<0.05$).

Positive, non-significant relations were found between homocysteine and creatinine for all groups.

Conclusion

A combination of both inner and outer factors can influence the levels of vitamin B_{12} and folate in elderly persons (26). Deficiency states of vitamin B_{12} or folate could sensitively, however non-specially, be diagnosed by Hcy, which correlates negatively with those vitamin levels.

The results of this study suggest that slightly cognitively impaired men living single run a greater risk of developing vitamin B_{12} and folate deficiency than men living in a family. A higher degree of awareness and an early diagnosis of deficiency of these vitamins are of great importance, since the best results in treatment of a deficiency with vitamin B_{12}/folate have been reported when treatment is started within six months from the detection of cognitive disturbances (32, 33).

References

1. Seshadri S et al. Plasma homocysteine as a risk factor for dementia and Alzheimer´s disease. *N Engl J Med*; *2002*; 346(7): 476-83
2. Wahlin Å et al. Effects of serum vitamin B12 and folate status in episodic memory performance in very old age: A population-base study. *Psychol Ageing*; 1996; 11:487-96
3. Wang HX, Wahlin Å, Basun H, Fastborn J, Winblad B, Fratiglioni L. Vitam in B12 and folat in relation to the development of Alzheimer´s disease. *Neurology*; 2001,56: 1188-94
4. Lehmann M, Gottfries CG, Regland B. Identification of Cognitive Impairment in the Elderly: Homocysteine is an Early marker. *Dement Geriatr Cogn Disord*; 1999; 10:12-20
5. Levitt AJ, Karlinsky H. Folate, vitamin B12 and cognitive impairment in patients with Alzheimer´s disease. *Acta Psychiatr Scand*; 1992; 86: 301-5.
6. Wahlin Å, Bäckman L, Hill RD, Winblad B. Measuring of folate and vitamin B12 in the elderly. Low serum levels cause reduced memory function. Lakartidningen (*J Swed Med Ass*) 1997; 94(23): 2177-82.
7. Joosten E, Lesaffre E, Riezler R, Ghekiere V, Dereymaeker L, Pelemans W, Dejaeger E. Is metabolic evidence for Vitamin B12 and folate deficiency more frequent in elderly patients with Alzheimer´s Disease? *J Gerontol*; 1997; 52A: 76-79
8. Gottfries CG. Alzheimer´s disease. Known and possible causes. *Hassle information*; Sweden. 1997; 7: 3-9.
9. Watanabe et al. Effects of microwave heating on the loss of vitamin B12 in foods. *J Agri Food Chem*; 1997; 45: 12: 4661-63.
10. Hanger HC et al. A community study of vitamin vitamin B12 and folate levels in elderly. *JAGS*; 1991; 39:1155-59.
11. Green R, Miller J. Folate deficiency beyond megaloblastic anemia: Hyperhomocysteinemia and other manifestations of dysfunctional folate status. *Semin Hematol*; 1999; 36: 47-64.
12. Björkegren K. & Svärdsudd K, Serum cobalamin, folate, metyl malonic acid and total homocysteine as vitamin B_{12} and folate tissue deficiency markers amongst elderly Swedes - a population-based study. *J Int Med*; *2001*; 249: 423-32.
13. Ravakhah K, West BC. Case report: subacute combined degeneration of the spinal cord from folat deficiency. *Am J Med Sci*; 1995; 310:214-6
14. Kilshaw et al. Effects of treatment of cow´s milk and hcy on the nutritional quality of antigenic properties. *Arch Dis Child*; 1982; 57:842-7
15. Mc Killop DJ et al. The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. *Br J Nutr*; 2002; 88:681-88.
16. Bolander-Gouaille C. Focus on homocysteine. Springer Verlag, France, 2000.
17. Engstedt L, Nilsson-Ehle H, Norberg B, Palmblad J (red). Controversies of vitamin B_{12}. Knowledge, competence, communication. Klippan, Pedagogförlaget, Sweden. 1998.
18. Spector R, Johanson C. The mammilian choroid plexus. *Scientific American Nov*; 1989:48-55.

19. Gottfries et.al. Early diagnosis of cognitive impairment in the elderly with focus on Alzheimer´s disease. *J Neural Transmission*; 1998; 105: 773-86.
20. Nilsson K, Gustafson L, Hultberg B. Optimal use of markers for cobalamin and folate status in psychogeriatric population. *Int J Geriatr Psychiatry*; 2002 Okt; 17(10):919-925.
21. Savage et.al. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiency. *Am J Med*; 1994:96:239-46.
22. Lökk J. Folate/cobalamin in the elderly - deficiency symptoms are common and difficult to catch. Lakartidningen, (*J Swed Med Ass*) 2001; 98(51-52): 5878-82.
23. Folstein MF, Folstein SE, McHugh PR. "Mini-Mental State". A practical method for grading the cognitive stat of patints by the clinician. *J Psychiatry Res*; 1975; 12:189-98.
24. de Groot CP, van den Broek T, van Staveren W. Energy intake and micronutrient intake in elderly Europeans: seeking the minimum requirment in the SENECA study. *Age Ageing*; 1999 Sep; 28(5): 469-74.
25. Haller J. The vitamin status and its adequacy in the elderly: an international overview. *Int J Vitam Nutr Res*; 1999 May; 69(3): 160-8
26. Irving GF, Olsson BA, Cerderholm T. Nutritional and cognitive status in elderly subjects living in service flats, and the effect of nutrition education on personnel. *Gerontology*; 1999 Jul-Aug; 45(4): 187-94.
27. Norberg B, Almay B, Ernerudh J, Fehling C, Johansson B, Link H et.al. Neurological departments suggest new lines of direction for dealing with suspect B12 -deficiency. Lakartidningen (*J Swed Med Ass*) 1992; 89 (22): 2011-12.
28. Nilsson K. et al. Plasma homocysteine is a sensitive marker for tissue deficiency of both cobalamins and folates in a psychogeriatric population. *Dement Geriatr Cogn Disord* ; 1999; 10: 476-82.
29. Bates CJ et al. Relationship between methylmalonic acid, homocysteine, vitamin B12 intake and status and socio-economic indices in a subset of participants in the British National Diet and Nutrition Survey of people aged 65 y and over. *Eur J Clin Nutr*; 2003; 57: 349-57).
30. Penninx BW et al. Vitamin B 12 deficiency and depression in physically disabled older women: epidemiologic evidence from the women´s health and aging study. *Am J Psych*; 2000; 157: 715-21.
31. Joosten E et al. Metabolic evidence that deficiencies of vitamin B12 (cobalamin) occur commonly in elderly people. *Am J Clin Nutr* 1993; 58:468-76
32. Martin DC, Francis J, Protetch J, Huff FJ. Time dependency of cognitive recovery with cobalamin replacement: report of a pilot study. *J Am Geriatr Soc*; 1992; 40: 168-72
33. Regland B, Gottfries CG. Vitamin B12- deficiency - also a neuropsychiatric problem. Lakartidningen, (*J Swed Med Ass*). 1992; 89(35): 2736-40.

Mild Cognitive Impairment with Subcortical Vascular Features: a Case Report

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Abstract

Brain small vessel disease is associated with cognitive impairment, parkinsonism, non-cognitive symptoms and a high incidence of adverse outcomes (cognitive and functional loss, institutionalization, and death). The case is reported of a 72-yearold hypertensive man with non-amnestic mild cognitive impairment, disproportionate dysexecutive disturbances on neuropsychological exam, gait disturbances with parkinsonian signs, anxiety, and signs of small vessel white matter damage and little medial temporal atrophy on CT and MR. Mild cognitive impairment with subcortical vascular features indicates prodromal subcortial vascular dementia and the condition can and should be recognized.

Keywords: mild cognitive impairment, small vessel disease, hypertension, subcortical vascular dementia, small vessel disease

Introduction

Mild cognitive impairment (MCI) refers to a transitional state between normal aging and dementia, where patients have cognitive impairment without impact on activities of daily living. MCI is a clinically heterogeneous syndrome comprising a number of conditions[1]. The one relatively more clearly defined is amnestic MCI (aMCI), which refers to individuals with memory deficit and preserved general cognition[2] who have early stage of Alzheimer's disease (AD)[3]. Theoretically, patients with other dementing disorders such as Lewy body dementia, frontotemporal dementia, and vascular dementia might pass through the pre-dementia stage and be captured before the full blown clinical picture has developed[1]. However, clinical descriptions of patients at this stage are still few and sparse.

Epidemiological studies showed that mild cognitive impairment of vascular origin is a frequent condition. In the population-based Canadian Study of Health and Aging, Rockwood and colleagues[4] have defined vascular cognitive impairment - no dementia as global cognitive performance lower than normal associated with clinical features suggestive of multi-infarct dementia (sudden onset, stepwise progression, and focal signs or symptoms). The authors found that these subjects amounted to 2.6% of the population over 65 years, i.e. about half of subjects with Alzheimer's disease, and that they had high rates of death and institutionalization.

However, cognitive deterioration due to a multi-infarct etiology (usually following occlusion of large arterial vessels) accounts for only part of the cases of cognitive impairment due to a vascular etiology. Some observations[5] indicate that the largest share of vascular cognitive impairment is due to small vessel disease affecting the subcortical white matter and basal ganglia (subcortical vascular dementia, SVD). Meyer and colleagues[6] in a 8-years longitudinal study of 291 cognitively normal subjects found that 27 developed vascular dementia. Of these patients, 15 (56%) developed vascular dementia from prodromal MCI stage and 12 (44%) directly from normal cognitive status, without passing through MCI stage. The comparison of clinical and neuroradiological characteristics between the two groups showed that the most part of vascular demented patients with prodromal MCI had SVD,

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while most of those without prodromal MCI had multi- or strategic-infarct dementia.

Recently, diagnostic criteria to recognize patients with mild cognitive impairment with subcortical cerebrovascular disease (svMCI) in clinical practice have been developed (table 1)[7]. These were clinical criteria of Erkinjuntti and colleagues for SVD modified to accomodate lower severity of cerebrovascular disease[8]. Core features required the evidence of relevant cerebrovascular disease by brain imaging, a cognitive profile of a dysexecutive "frontal" syndrome with relative sparing of memory, and neurological signs (gait disorders and extrapyramidal signs). Supporting features included urinary symptoms such as urgency and incontinence, and behavioural symptoms. We have found that when compared to aMCI patients, those with svMCI have poorer outcome: after a 3-year follow-up, 50% had died, while all aMCI were still alive. Moreover, of those alive, 68% of svMCI vs 17% of aMCI patients had reached one outcome among nursing home placement, functional loss, and severe cognitive deterioration[7].

We describe the case of a patient with a clinical pitcure typical of svMCI.

Case report

A 72-year old man was evaluated as part of a prospective project on the natural hystory of MCI ("Mild cognitive impairment and no dementia: preclinical condition of AD and vascular dementia.") for difficulties in problem solving reported by both the patient himself and the caregiver (his wife). The patient, presently retired, worked as a truck driver until some months before the observation. He had five years of education. No smoke and mild alcohol consumption was referred. Comorbility was absent: the patient himself pointed out that he had always been healthy and "never mixed with doctors".

Cognitive disturbances started one year ago, when the patient showed difficulties in some activities of daily living. For example, he started to drove the car less frequently because he was afraid he might not find a parking place. As soon as his usual routine showed some changes, the patient showed excessive anxiety and worries. When on holiday in a safe sea resort, he wanted his grandchild not to bathe because he could not swim and he would not be able to save her in case of danger. Moreover, he was inattentive: he often lost the thread of his actions and discourses. Since six months slowing in movements and thoughts were referred. The whole clinical picture progressively worsened until examination in the absence of functional impact: the patient was still independent in instrumental and basic activity of daily living.

On physical examination, high blood pressure values were found (160/90). Neurological examination evidenced extrapyramidal (mild to moderate upper arms rigidity, left more than right), focal (increased ROT in the left lower arm and left Babinski sign), and cortical dishinibition (snout, sucking, and glabellar) signs. The Mini Mental score was

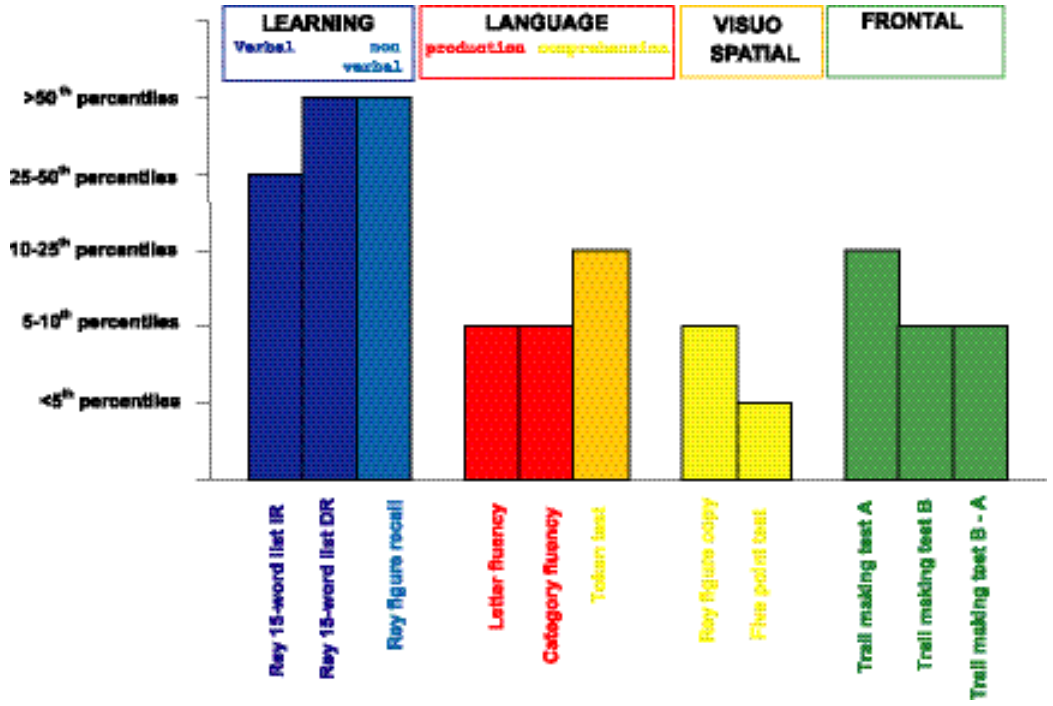

Figure 1. Neuropsychological features. Columns denote age-, gender-, and education-corrected test scores expressed as percentiles. IR: immediate recall; DR: delayed recall.

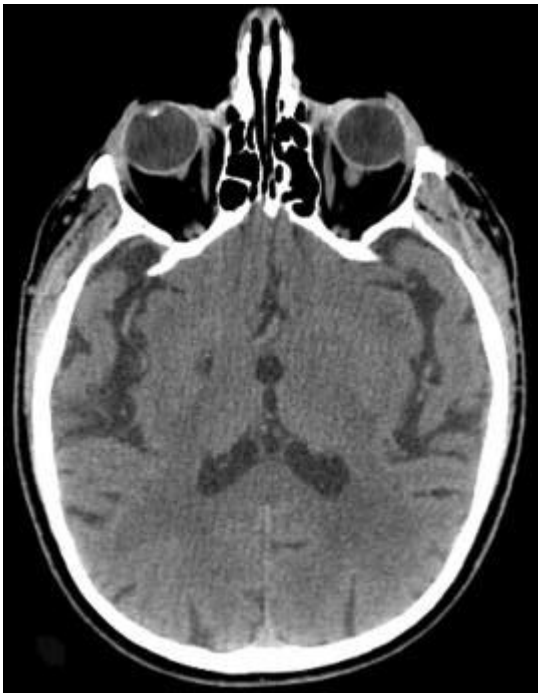

Figure 2.a. Neuroimaging features. CT shows a lacune in the striatum and diffuse leukoaraiosis with patchy lesions in frontal, parietal, and occipital regions. The subcortical vascular score based on the CT-based weighted rating scale for subcortical ischemic vascular disease[9] is 41.6 (severe disease).

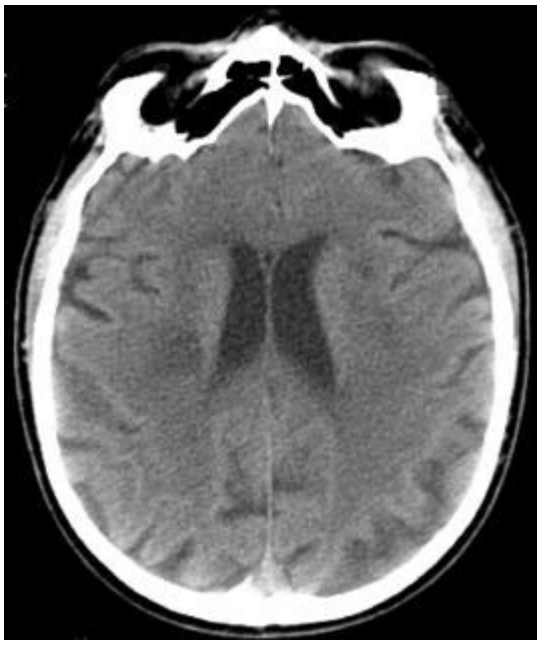

Figure 2.b. Neuroimaging features. T2 weighted MR imaging further defines the extent and severity of white matter disease: confluent white matter hyperintensities can be appreciated bilaterally in the paratrigonal areas and multiple punctate, early confluent, and confluent hyperintensities in the centrum semiovale. The score of Wahlund and colleagues' scale[10] for age-related white matter changes is 15/30 (severe changes).

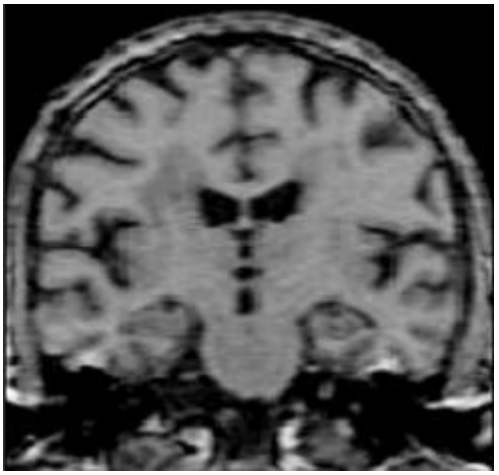

Figure 3. Medial temporal lobe assessment. T1 weighted MR coronal image through the hippocampus showing increased width of the choroid fissure, with normal width of the temporal horn, and normal height of the hippocampal formation. The visual medial temporal lobe atrophy rating is 1/4 (minimal atrophy) on Scheltens and colleagues's scale[11]

28/30: one point was lost in the attention and one point in the three words recall item. Neuropsychological evaluation evidenced borderline/low performance on language (category and letter fluency), visuospatial (Rey copy, five point test), and frontal (trial making test) functions, while memory functions were well preserved (figure 1). The EEG showed mild global slowing of electrical rhythms. The apoE genotype was 3/3.

Quantification of subcortical vascular load and medial temporal lobe atrophy was made on CT and MR films through validated scales. CT scan showed lacunes in basal ganglia and external capsule (right emisphere), with leukoaraiosis extending to the cortex and patchy hypodensities in frontal, parietal and occipital regions (right and left emispheres) (figure 2a). The subcortical vascular score based on a CT-based weighted rating scale for subcortical ischemic vascular disease[9] was 41.6, indicating severe subcortical vascular load. MR images confirmed severity of vascular lesions (figure 2b): the score of Wahlund and colleagues' scale[10] for age-related white matter changes was 15/30. On the contrary, medial temporal lobe atrophy was minimal (the subjective medial temporal lobe atrophy score based on the Scheltens and colleagues scale[11] was 1) (figure 3).

Discussion

We have described a patient with neuropsychological (dysexecutive "frontal" syndrome with relative sparing of memory), motor (gait slowing and extrapyramidal signs), behavioural (anxiety), and instrumental (evidence of

relevant cerebrovascular disease by brain imaging) features fulfilling diagnostic criteria for svMCI.

Although we cannot exclude that our svMCI patient would show AD on pathology, the clinical features are quite inconsistent with what is known of the pathophysiology and natural history of AD. Gait disturbances at the onset or very early in the course of the dementia is not typical for AD[12]. In a prospective community study of 422 non demented elderly persons followed for a median of 7 years, subjects with abnormal gait had greater risk of developing vascular dementia (hazard ratio 3.5) but not AD[13]. In a subgroup of 48 patients where autopsy was performed, vascular dementia, in particular microinfarcts, was the pathological condition more common in subjects with abnormal gait.

Although a distinctive profile of behavioral symptoms for different forms of dementia, such as SVD and AD, has not been yet defined, the prevalence of behavioral symptoms in patients with cognitive impairment is high. A recent population study of 362 patients with dementia and 320 with MCI showed that 80% of demented and up to 50% of MCI patients had developed at least one symptom, as assessed with the NeuroPsychiatric Inventory, since the

Table 1. Clinical criteria for mild cognitive impairment with subcortical vascular disease (svMCI)[7].

I. A and B must be both present:

A. COGNITIVE SYNDROME including all of the following:

A1. DYSEXECUTIVE SYNDROME: Impairment in goal formulation, initiation, planning, organizing, sequencing, executing, set-shifting and -maintenace, and abstracting

A2. MEMORY DEFICIT (may be mild): Impaired recall, relative intact recognition, less severe forgetting, benefit from cues

A3: PROGRESSION: deterioration of A1 and A2 from a previous higher level of functioning that are not per se interfering with complex (executive) occupational and social activities

B. CEREBROVASCULAR DISEASE including both B1 and B2.

B1. EVIDENCE OF RELEVANT CEREBROVASCULAR DISEASE BY BRAIN IMAGING defined as the presence of both:

- extensive periventricular and deep white matter lesions: patchy areas of low attenuation (intermediate density between that of normal white matter and that of intraventricular cerebro-spinal fluid) or diffuse symmetrical areas of low attenuation with ill defined margins extending to the centrum semiovale plus at least one lacunar infarct, and
- absence of cortical and/or cortico-subcortical non-lacunar territorial infarcts and watershed infarcts, haemorrhages indicating large vessel disease, signs of normal pressure hydrocephalus, and specific causes of white matter lesions (e.g. multiple sclerosis, sarcoidosis, brain irradiation).

B2. PRESENCE OR A HISTORY OF NEUROLOGIC SIGNS as evidence for cerebrovascular disease such as hemiparesis, lower facial weakness, Babinski sign, sensory deficit, dysarthria, gait disorder, extrapyramidal signs consistent with subcortical brain lesion(s).

II. Clinical features supporting the diagnosis include the following:

a. Episodes of mild upper motor neuron involvement such as drift, reflex asymmetry, incordination.
b. Early presence of a gait disturbance (small-step gait or marche a petits-pa magnetic, apraxic-ataxic or Parkinsonian gait).
c. History of unsteadiness and frequent, unprovoked falls.
d. Early urinary frequency, urgency, and other urinary symptoms not explained by urologic disease.
e. Dysarthria, dysphagia, extrapyramidal signs (hypokinesia, rigidity).
d. Behavioral and psychological symptoms such as depression, personality change, emotional incontinence, psychomotor retardation.

III. Features that make the diagnosis uncertain or unlikely include:

a. Early onset of memory deficit and progressive worsening of memory and other cognitive functions such as language (transcortical sensory aphasia), motor skills (apraxia), and perception (agnosia), in the absence of corresponding focal lesions on brain imaging.
b. Absence of relevant cerebrovascular lesions on brain CT or MRI.

onset of cognitive impairment[14]. The most frequent clinically significant symptoms in MCI were sleep disturbances and irritability. However, MCI patients were not further categorized into amnestic and vascular.

The neuropsychological profile we found in our svMCI patient is that of impairment of frontal functions, with sparing of memory. Several studies have found frontal executive dysfunction in SVD and have underlined its relevance in the differential diagnosis with AD[15,16]. Some also showed better recognition memory in SVD, while delayed recall was equally impaired, probably reflecting retrieval deficit[15]. However, the lack of pathological studies in SVD and svMCI patients who also had accurate neuropsychological testing prevents a direct confirmation of this view.

We conclude that svMCI is a condition with a definite clinical picture. The availability of diagnostic criteria allow to recognize these patients in the clinical routine. Further studies should devise appropriate strategies that may help slowing or halting the progression of the disease.

Acknowledgements

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References

1. Petersen R.C., Doody R., Kurz A., Mohs R.C., Morris J.C., Rabins P.V., Ritchie K., Rossor M., Thal L., Winblad B., Current concepts in mild cognitive impairment. *Arch Neurol* 2001; 58: 1985-92.
2. Petersen R.C., Smith G.E., Waring S.C., Ivnik R.J., Tangalos E.G., Kokmen E., Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 1999; 56: 303-8.
3. Morris J.C., Storandt M., Miller J.P., McKeel D.W., Price J.L., Rubin E.H., Berg L., Mild cognitive impairment represents early-stage Alzheimer disease. *Arch Neurol* 2001; 58: 397-405.
4. Rockwood K., Wentzel C., Hachinski V., Hogan D.B., MacKnight C., McDowell I., Prevalence and outcomes of vascular cognitive impairment. Vascular Cognitive Impairment Investigators of the Canadian Study of Health and Aging. *Neurology* 2000; 54: 447-51.
5. Esiri M.M., Wilcock G.K., Morris J.H., Neuropathological assessment of the lesions of significance in vascular dementia. *J Neurol Neurosurg Psychiatry* 1997; 63: 749-53.
6. Meyer J.S., Xu G., Thornby J., Chowdhury M.H., Quach M., Is mild cognitive impairment prodromal for vascular dementia like Alzheimer's disease? *Stroke* 2002; 33: 1981-5.
7. Frisoni G.B., Galluzzi S., Bresciani L., Zanetti O., Geroldi C., Mild cognitive impairment with subcortical vascular features Clinical characteristics and outcome. *J Neurol* 2002; 249: 1423-32.
8. Erkinjuntti T., Inzitari D., Pantoni L., Wallin A., Scheltens P., Rockwood K., Roman G.C., Chui H., Desmond D.W., Research criteria for subcortical vascular dementia in clinical trials. *J Neural Transm Suppl* 2000; 59: 23-30.
9. Geroldi C., Galluzzi S., Testa C., Zanetti O., Frisoni G.B., Validation study of a CT-based weighted rating scale for subcortical ischemic vascular disease in patients with mild cognitive deterioration. *Eur Neurol* 2003; 49: 193-209.
10. Wahlund L.O., Barkhof F., Fazekas F., Bronge L., Augustin M., Sjogren M., Wallin A., Ader H., Leys D., Pantoni L., Pasquier F., Erkinjuntti T., Scheltens P., European Task Force on Age-Related White Matter Changes, European Task Force on Age-Related White Matter Changes: A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke* 2001; 32: 1318-22.
11. Scheltens P., Leys D., Barkhof F., Huglo D., Weinstein H.C., Vermersch P., Kuiper M., Steinling M., Wolters E.C., Valk J., Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. *J Neurol Neurosurg Psychiatry* 1992; 55: 967-72.
12. McKhann G., Drachman D., Folstein M., Katzman R., Price D., Stadlan E.M., Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; 34: 939-44.
13. Verghese J., Lipton R.B., Hall C.B., Kuslansky G., Katz M.J., Buschke H., Abnormality of gait as a predictor of non-Alzheimer's dementia. *N Engl J Med* 2002; 347: 1761-8.
14. Lyketsos C.G., Lopez O., Jones B., Fitzpatrick A.L., Breitner J., DeKosky S., Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. *JAMA* 2002; 288: 1475-83.
15. Tierney M.C., Black S.E., Szalai J.P., Snow W.G., Fisher R.H., Nadon G., Chui HC., Recognition memory and verbal fluency differentiate probable Alzheimer disease from subcortical ischemic vascular dementia. *Arch Neurol* 2001; 58: 1654-9.
16. Traykov L., Baudic S., Thibaudet M.C., Rigaud A.S., Smagghe A., Boller F., Neuropsychological deficit in early subcortical vascular dementia: comparison to Alzheimer's disease. *Dement Geriatr Cogn Disord* 2002; 14 :26-32.

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Sunday, July 18, 2004

Epidemiology of Alzheimer's Disease:
Lessons from Cardiovascular Studies
Lenore Launer, PhD
National Institute on Aging, Bethesda, Maryland U.S.A.

Genetics of Alzheimer's Disease
Gerald Schellenberg, PhD
University of Washington, Seattle, Washington, U.S.A.

ApoE and Alyheimer's Disease: A 10-Year Update
Karl Weisgraber, PhD
University of California, San Francisco, California, U.S.A.

Monday, July 19, 2004

Oxidative Mechanisms, Inflammation,
and Alzheimer's Disease Pathogenesis
Flint Beal, MD
Cornell University, Ithaca, New Tork, U.S.A.

Biological Function of APP
Edward Koo, MD
University of California, La Jolla, California, U.S.A.

Tau and Tauopathies
Eva-Maria Mandelkow, MD, PhD
Max Planck Institute, Hamburg, Germany

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Alzheimer's Disease and Related Disorders

Tuesday, July 20, 2004

Behavioral and Psychological Symptoms of Dementia: Nature and Treatment
Constantine Lyketsos, MD
Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A.

Pharmacological and Non-Pharmacological Treatments in Alzheimer's Disease
Rachelle Doody, MD, PhD
Baylor College of Medicine, Houston, Texas, U.S.A.

Therapeutic Strategies for Alzheimer's Disease
Steve Paul, MD
Eli Lilly and Company, Indianapolis, India, U.S.A.

Wednesday, July 21, 2004

Cellular Models of Alzheimer's Disease
Nikolaos Robakis, Phd
Mount Sinai School of Medicine, New York, U.S.A.

Animal Models of Alzheimer's Disease
Karen Hsiao Ashe, PhD
University of Minnesota, Minneapolis, Minnesota, U.S.A.

Biology of Gamma Secretase
Takeshi Iwatsubo, MD
University of Tokyo, Tokyo,Japan

Thursday, July 22, 2004

Molecular Pathology/Histopathology of Alzheimer's Disease
John Q. Trojanowski, MD, PhD
University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A.

Imaging in Prediction and Longitudinal Assessment of Alzheimer's Disease
Clifford R. Jack, MD
Mayo Clinic, Rochester, Minnesota, U.S.A.

Biomarkers in Alzheimer's Diasease
Kaj Blennow, MD, PhD
University of Goteborg, Molndal, Sweden

Important Details

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Beginning in Septembrie 2003, information about submitting abstracts will be available on-line. Abstract will be solicited for both oral and poster presentation on all aspects of Alzheimer's disease and related disorders, including epidemiology, genetics, molecular and cellular biology, pathology, neuroimaging, experimental therapeutics and evidence-based patient management and social-behavioral research.

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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]
6. Benton A., Tranel D., Visuoperceptual, Visuospatial, and Visuoconstructive Disorders. In: Heilman K.M. and Valenstein E., eds., *Clinical Neuropsychology*. Oxford University Press, 1993: 195-212 [for edited books]
7. Luria A., *The Working Brain.* New York: Basic Books, 1973.[for monographs]

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