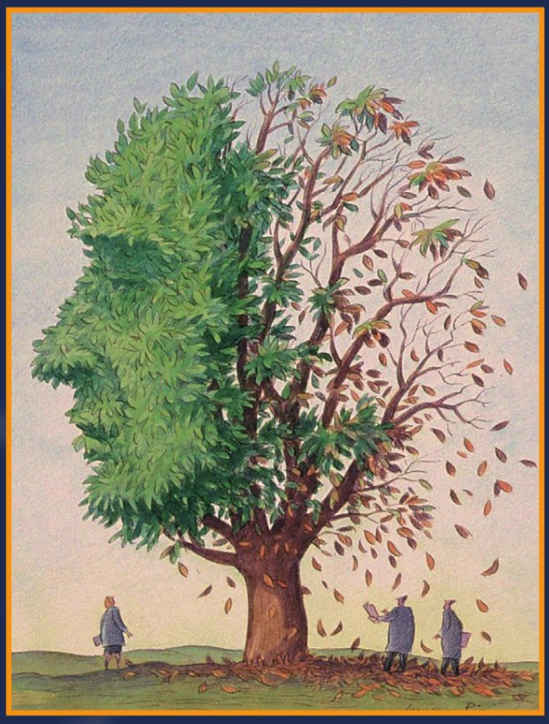
Research and Perspectives in Alzheimer's Disease

M. Jucker · K. Beyreuther · C. Haass
R. Nitsch · Y. Christen (Eds.)

Alzheimer: 100 Years and Beyond



FONDATION IPSEN
POUR LA RECHERCHE THERAPEUTIQUE

Springer

RESEARCH AND PERSPECTIVES IN ALZHEIMER'S DISEASE

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M. Jucker K. Beyreuther
C. Haass R.M. Nitsch Y. Christen (Eds.)

Alzheimer: 100 Years and Beyond

With 143 Figures

Springer

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One hundred years of Alzheimer research

Few medical or scientific addresses have so unmistakeably made history as the presentation delivered by Alois Alzheimer on November 4, 1906 in Tübingen. The one-hundred year anniversary of that event has been marked on several occasions in 2006, most notably at the very site of the original lecture, namely the Institute of Psychiatry of the University of Tübingen in Germany on November 2–5, 2006.

The celebratory event, "Alzheimer 100 Years and Beyond" organized on the initiation of the Alzheimer community in Germany and worldwide, in collaboration with the Fondation Ipsen, was the highlight of the Year of Alzheimer. However, beyond these few months of tributes, the centennial offers a unique opportunity to assess both the progress achieved and the uncertainties remaining. This volume, a collection comprised mainly of articles by the invited speakers and also of a few other prominent researchers, is meant to be a record of those events.

Over the last century of Alzheimer research (1906-2006), remarkable progress has been achieved in many areas:

- *Progress towards understanding the pathogenesis*: In this field progress has been very much dependent on developments in technology and other speciality areas. This was true from the very start: Alois Alzheimer's research would not have been possible without then-nascent microscopic staining techniques. It has proven true again over the last few decades, with molecular and cellular biology, genetics, and brain imaging all making contributions while benefiting from related fields. For instance, the discovery of the role of apolipoprotein E enabled Alzheimer scientists to apply research findings from the cardiovascular field. On the other hand, research into the pathogenesis of Alzheimer's disease revealed fundamental findings regarding protein aggregation, or regulated intramembrane proteolysis that has not only been applied to other neurodegenerative diseases but also paved the road to understanding completely unexpected signalling pathways.
- *Recognition of the disease and clinical treatment*. Although no cure has been found for Alzheimer's disease yet, the outlook is promising. Alzheimer's disease has ceased to be considered as an inescapable consequence of ageing.
- *Economic and social considerations*. With the aging of our society over the last 100 years, there has been a continuous increase in the number of Alzheimer patients and the burden for individual care-takers, as well as social and medical institutions. Demography has helped to make the public aware and prepared of the social and economic consequences of this devastating form of dementia.

Yet, none of the advances made have yet culminated in a fully satisfactory outcome. The pathogenic mechanisms of the disease remain inadequately understood and are at

the centre of serious controversy, such as determining whether the observed lesions in post mortem brains of patients with Alzheimer's disease are the cause or a consequence of the disease; in no country have the public authorities provided fully for patients; and treatment options remain largely insufficient, despite hope from various symptomatic treatments and ongoing promising clinical studies targeting the pathological mechanisms.

Despite these shortcomings, the last one hundred years have been full and active ones. The aim of the meeting held in November 2006 in Tübingen, like that of this volume, is not to lay out the final legacy of a scientific corpus that is, to the contrary, constantly-developing, nor to crown the contributions of a specific researcher, at the expense of his colleagues or competitors. Instead, it is to honor the work accomplished and provide material for the history of science. We asked the invited authors to present their pioneering research explaining the conditions under which they were conducted from their viewpoint, and thus intentionally leaving room for a certain degree of subjectivity. Their testimonials contain unavoidably some contradictions, in particular regarding their part in certain essential discoveries. The editors of this book did not, at any time, wish to take sides in the possible ownership squabbles, but only to provide readers with information from the very individuals who have made Alzheimer research what it is, over the past few decades. The only editing changes related to form alone - no article was changed in substance, none were censured and none were rejected. Some authors invited to present their research at the meeting in Tübingen were unable to hand in their contributions in time, however contributed significantly to the meeting, such as Monique Breteler, Nick Fox, Michael Hutton, Steven Paul, Gerard D. Schellenberg, Sangram S. Sisodia, Bengt Winblad, Bruce Yankner.

Lastly, several of the pioneers passed away too early to be able to attend the Tübingen meeting and contribute to this work: George Glenner, Henry Wisniewski, Tsunao Saitoh, Eva Braak, Jean-Louis Signoret, Yvon Lamour, Nelson Butler, Luigi Amaducci, and others. We dedicate this volume to them.

Mathias Jucker
Konrad Beyreuther
Christian Haass
Roger Nitsch
Yves Christen

Some “players” of the AD story (1987–2006)

01 Paris, 1988, Stanley Rapoport, Charles Epstein
02 Paris, 1988, Carleton Gajduzek, Henry Wisniewski
03 Paris, 1987, Dennis Selkoe
04 Angers, 1987, *front row*: Annick Pouplard-Bartheleix, Toshiharu Ishii, Mrs Ishii, Taihei Miyakawa – *back row*: Philippe Brachet, Colin Masters, Konrad Beyreuther, Hugh Fudenberg, Henry Wisniewski, Mrs Glenner, George Glenner, Jacqueline Mervaillie, Mrs Miyakawa, Dennis Selkoe, Jean Emile, Piet Eikelenboom, Jean Lapresle, Yves Christen, André Delacourte
05 Angers, 1987, Toshiharu Ishii, Yves Christen, George Glenner
06 Paris, 1988, Carleton Gajduzek, Yves Christen, Françoise Forette

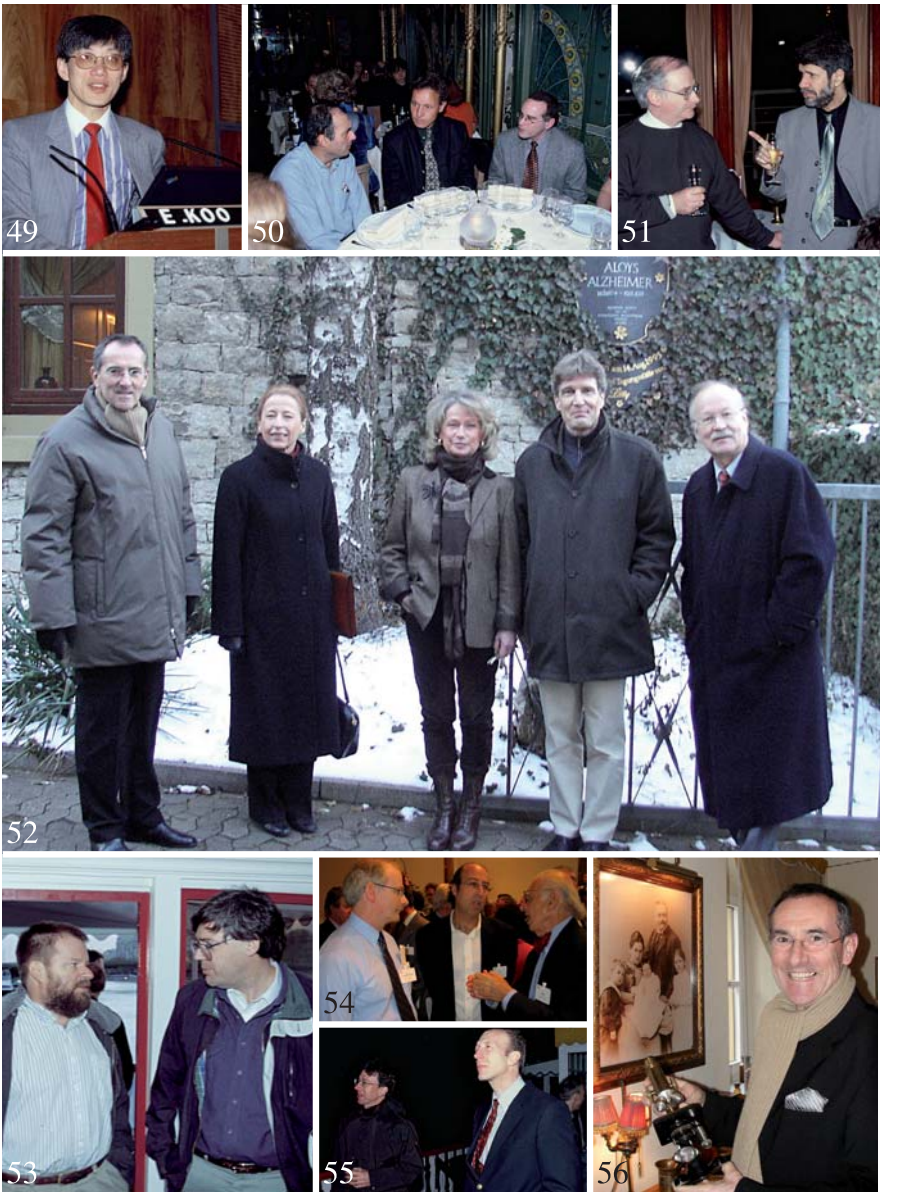
07 Montpellier, 1988, Allen Roses, Annie Saunders
08 Paris, 1989, Marshal Folstein, Stanley Rapoport
09 Toulouse, 1989, Tsunao Saitoh, Rudolph Tanzi
10 Toulouse, 1989, Jean-Jaques Hauw
11 Paris, 1988 and 2006 (Charles de Gaulle airport), Christine Van Broeckhoven
12 Toulouse, 1989, John Blass, Peter Davies
13 Toulouse, 1989, André Rascol, Jean-Louis Signoret
14 Toulouse, 1989, Rudolph Tanzi, Yves Agid
15 Toulouse, 1989, André Delacourte, Henry Wisniewski, Krystina Wisniewski, Tsunao Saitoh, Ushi Beyreuther, Konrad Beyreuther, Andrew Haynes, John Atack, Jay Pettergrew, François Boller, Jacqueline Mervaillie, Yves Christen, Kenneth Kosik

16 Marseille, 1992, Luigi Amaducci
17 Strasbourg, 1990, Dan Lindholm, Yoshihiro Arakawa, Robert Terry, William Mobley, Nikolaos Robakis, Kenneth Kosik
18 Paris, 1989, William Klunk
19 Marseille, 1992, Jean-François Foncin, Peter St. George-Hyslop
20 Toulouse, 1989, Henry Wisniewski, Konrad Beyreuther, André Delacourte
21 Strasbourg 1990, Robert Terry, Albert Aguayo
22 La Jolla, 1991, Jean-Pierre Changeux, Robert Katzman, Robert Terry
23 La Jolla, 1991, John Morrison, Francis Crick
24 Dijon, 1993, Steven Younkin, Marie-Christine Chartier-Harlin, Lydia Hendriks, Yves Christen, Jacqueline Mervaillie, Stanley Prusiner

25 Paris, 1997, John Hardy
26 Paris, 2003, Charles Duyckaerts
27 Paris, 1995, Blass Frangione, Allen Roses, Kazuhiko Ikeda
28 Aix-en-Provence, 1992, *first row*: André Nieoullon, Zaven Khachaturian, Helen Chui, Yves Christen, Jacqueline Mervaillie – *second row*: Sam Gandy, Allen Roses, Marcel Mesulam, Victor Bulyzenkov, Richard Mayeux – *above*: Michel Poncet, Thomas Bird, Henri Dehen, François Boller, François Chain, Bruno Dubois, Peter St. George-Hyslop, John Hardy, Charles Duyckaerts, Dennis Dickson
29 Paris, 1995, Yvon Lamour, Judes Poirier
30 Paris, 1988, Alison Goate
31 Dijon, 1993, David Small, Helen Price, Stanley Prusiner, Donald Price, Rudolph Tanzi

32 Lyon, 1993, Rudolph Tanzi, Ashey Bush, Steven Younkin, Colin Masters
33 Lyon, 1993, Sam Sisodia, Sam Gandy
34 Paris, 1996, Heiko Braak, Zaven Khachaturian
35 Paris, 1997, John Hardy, Michael Wolfe, Christian Haass
36 Paris, 1997, Roger Nitsch, Steven Younkin
37 Paris, 1996, David Felten, Jacqueline Mervaillie, Yves Christen, François Boller, Zaven Khachaturian, Donald Stein, Mark West, Charles Duyckaerts, Eliezer Masliah, Jennifer Altman, Paul Coleman, Dora Games, Bradley Hyman, Gary Van Hoesen, Pierre Tariot
38 Paris, 1997, Karen Duff
39 Paris, 1999, Eckhard Mandelkow, Eva-Maria Mandelkow
40 Paris, 1996, Dora Games, Yves Christen, Eliezer Masliah

41 Paris, 1999, Peter Lansbury, Virginia Lee, John Trojanowski
42 Paris, 2000, Mathias Jucker, Joachim Herz, Bart de Strooper
43 Paris, 1997, Dennis Selkoe, Christian Haass, Roger Nitsch
44 Paris, 1999, Michel Goedert, Luc Buée
45 Paris, 1997, Jean Mariani, Paul Fraser, Christan Haass, Karen Duff, John Hardy, Yves Christen, Steven Younkin, Rudolph Tanzi, Wilma Wasco, Roger Nitsch, Sam Sisodia, Jacqueline Mervaillie, Dennis Selkoe
46 Paris, 1998, Robert Katzman, Richard Mayeux
47 Paris, 2002, Dale Schenk
48 Paris, 1998, Albert Hofman, Jean-François Dartigues



49 Paris, 2001, Edward Koo
50 Paris, 2001, Alain Israel, Christian Haass, Michael Wolfe
51 Paris, 2001, Peter St. George-Hyslop, Frédéric Checler
52 Markbreit, 2005, Konrad Beyreuther, Jacqueline Mervaillie, Ulrike Maurer, Mathias Jucker, Konrad Maurer
53 Paris, 1999, Gerard Schellenberg, Kirk Wilhelmsen
54 Paris, 2003, Peter St. George-Hyslop, Kenneth Kosik, Eric Kandel
55 Paris, 2002, Dale Schenk, David Holtzman
56 Markbreit, 2005, Konrad Beyreuther (A. Alzheimer's microscope)

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Alois Alzheimer

Concerning a unique disease of the cerebral cortex

*Alois Alzheimer**

A. reports a case which he observed in the Mental Asylum in Frankfurt a.M. and whose central nervous system was given to him for study by the director Mr. Sioli.

Clinically, this case presented an unusual picture that did not fit any known disease, while anatomically it demonstrated a finding that was distinct from other known disease processes.

A 51 year old woman showed as her first obvious symptom jealousy concerning her husband. Soon a very rapidly progressive impairment of memory became noticeable. She could no longer find her way around her apartment, moved objects to and fro and hid them. At times she believed someone wanted to kill her and began to scream loudly.

In the Asylum, her behavior is characterized by a total helplessness. She is totally disoriented to place and time. Occasionally she complains that she is at a complete loss to understand all this. She greets the doctor like a visitor and asks to be excused for not being done with her housework, then screams loudly that the doctor wants to cut her, or she rejects him with intense indignation using phrases which indicate she is afraid that he wants to assault her sexually. Sometimes she is completely delirious, carrying around her bedclothes, calling for her husband and her daughter, and seeming to have auditory hallucinations. Often she screams with a horrible cry for many hours.

Because of her inability to understand the situation, she begins screaming loudly as soon as one starts to examine her. Only with frequent repetition was it finally possible to determine anything.

Her memory is greatly disturbed. If one shows her objects, she is able to name these correctly, but immediately afterward has forgotten everything. While reading, she skips from one line to another, reads phonetically or with a senseless intonation. While writing she repeats single syllables many times, skips others and quickly becomes distracted. While speaking, she often uses embarrassing phrases, single paraphasic expressions (milk-pourer instead of cup), sometimes becoming stuck and stopping. Some questions she seems not to hear. She no longer seems to know the use of certain objects. Her gait is undisturbed, she uses her hands equally well. Her patellar reflexes are present. The pupils react. Her radial artery is a bit hard, there is no enlargement of the heart impulse, and no proteins.

* This is the talk A. Alzheimer gave on November 4, 1906 in Tübingen. It is a case report on the first Alzheimer patient Auguste D.; it was written by Alois Alzheimer himself and published in 1907 (Alzheimer 1907a). A first English translation was written by Solomon C. Fuller and published in 1912. In 1977 another translation by Hochberg CN and Hochberg FH was published. The present translation was written by Clifford B. Saper (Harvard Medical School, Boston, USA) and Horst Herbert (Graduate School of Neural & Behavioural Sciences, University of Tübingen, Tübingen, Germany).

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In the course of the disease, these phenomena, interpreted as focal signs, waxed and waned but were always present to some extent. However, the general dementia progressed. After 4 1/2 years of the disease, death took her. At the end, the patient was completely apathetic, lying on her bed with retracted legs, incontinent and had decubitis ulcers despite good nursing care.

The autopsy showed a uniformly atrophic brain without any macroscopic focal abnormalities. The larger cerebral vessels showed arteriosclerotic changes.

In preparations processed using Bielschowsky's silver method, remarkable neurofibrillary changes were observed. In a normal appearing cell, first one or a few fibrils occur, which are prominent because of their thickness and their unusual impregnation. Later in the course, many fibers showing similar changes are seen running side-by-side. Then these coalesce into dense bundles and gradually progress to the surface of the cell. Finally the nucleus and the cell degenerate and only a tangled bundle of fibrils outlines the locus where the nerve cell was situated.

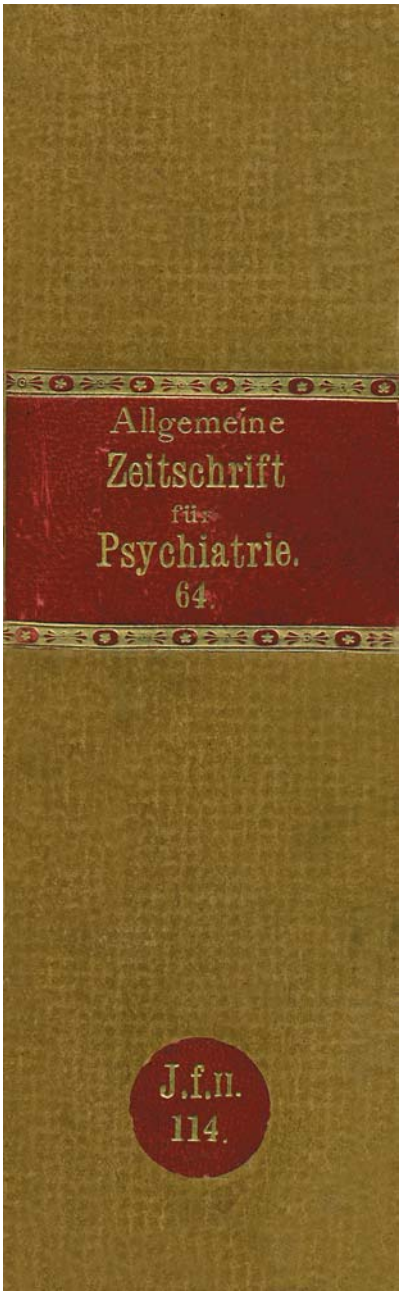
As these fibrils take up different stains than normal neurofibrils, a chemical change of the fibrillar substance must have occurred. This seems to be the reason that the fibrils persist beyond the destruction of the cell. The transformation of these fibrils seems to go hand in hand with the storage in the nerve cell of a yet-to-be-identified pathological metabolic product. About 1/4 to 1/3 of all neurons in the cerebral cortex show these changes. Numerous neurons, especially in the upper cell layers have entirely disappeared.

Distributed over the entire context, especially numerous in the upper layers, one finds miliary foci, which are due to the storage of a peculiar substance in the cortex. These are already visible, even without staining, but are very refractory to staining.

The glia have developed numerous fibers and other glial cells have large lipid droplets.

There is complete absence of vessel infiltration. On the other hand, there is endothelial proliferation and scattered formation of new blood vessels.

All in all, we are evidently confronted here by a unique disease process. Such unusual disease processes have in recent years been established in greater numbers. This observation makes it clear that we should not be satisfied, even after expending great effort, with placing any clinically unclear disease in a known disease group. There are doubtless more psychic diseases than are listed in our textbooks. In some such cases, later histological study will then establish their uniqueness. Then we will gradually come to the point where we are able to clinically distinguish single diseases from the large disease groups in our textbooks, and to outline them more precisely.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Psychiatrie.
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ALLGEMEINE ZEITSCHRIFT
FÜR
PSYCHIATRIE
UND
PSYCHISCH-GERICHTLICHE MEDIZIN

HERAUSGEGEBEN VON

DEUTSCHLANDS IRRENÄRZTEN

UNTER DER MITREDAKTION VON

BONHOEFFER (BRESLAU) CRAMER (GÖTTINGEN) v. GRASHEY (MÜNCHEN) KREUSER (WINNENTAL) PELMAN (BONN) SCHÜLE (ILLENAU)

DURCH

HANS LAEHR
SCHWEIZERHOF

VIERUNDSECHZIGSTER BAND

NEBST EINEM BERICHT
ÜBER DIE PSYCHIATRISCHE LITERATUR IM JAHRE 1906

REDIGIERT VON

E. SCHULTZE und **O. SNELL**
GREIFSWALD LÜNEBURG

BERLIN

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1907

Inhalt.

Erstes Heft.

Originalien.

Verhandlungen psychiatrischer Vereine.

Verhandlungen psychiatrischer Vereine.

37. Versammlung Südwestdeutscher Irrenärzte in Tübingen am 3. und 4. November 1906.

Geschäftsführer: *Kreuser*-Winnental, *Wollenberg*-Straßburg.
Schriftführer: *Buder*-Winnental, *Finckh*-Tübingen.

Präsenzliste: (88 Teilnehmer und Gäste) *Alzheimer*-München, *Aumüller*-Hördt, *Bezzola*-Schloß Hardt, *Brauchli*-Münsterlingen, *Bumke*-Freiburg, *Buder*-Winnental, *Busch*-München, *Basler*-Tübingen, *Binswanger*-Konstanz, *Barbo*-Pforzheim, *Böss*-Illenau, *Becker*-Baden-Baden, *Baisch*-Tübingen, *Camerer*-Stuttgart, *Curschmann*-Tübingen, *Dreyfus*-Heidelberg, *Daiber*-Weinsberg, *Dietz*-Goddelau-Philippshospital, *Dannenberger*-Ahrweiler, *Damköhler*-Klingenmünster, *Döderlein*-Tübingen, *Ewald*-Frankfurt a. M., *Eisenbach*-Winnental, *Finckh*-Tübingen, *Frank*-Zürich, *Feldmann*-Stuttgart, *Fleischer*-Tübingen, *Frank*-Tübingen, *v. Grützner*-Tübingen, *Gaupp*-Tübingen, *Glatzel*-Göppingen, *Geelvink*-Frankfurt a. M., *Groß*-Tübingen, *Hoche*-Freiburg, *Hackländer*-Giessen, *Heiligenthal*-Baden-Baden, *Hegar*-Wiesloch, *Habermaas*-Stetten i. Remsthal, *Herbert*-Tübingen, *Hoppe*-Pfullingen, *Jung*-Burghölzli-Zürich, *Isserlin*-Heidelberg, *Kreuser*-Winnental, *Krimmel*-Zwiefalten, *Klüpfel*-Urach, *Köstlin*-Stuttgart, *Klewe*-Emmendingen, *Krauß*-Kennenburg, *Kurz*-Pfullingen, *Laquer*-Frankfurt a. M., *Landerer*-Freiburg, *Levi*-Stuttgart, *Merzbacher*-Tübingen, *Nadler*-Emmendingen, *Neumann*-Karlsruhe, *Nitsche*-München, *Nissl*-Heidelberg, *Pfersdorff*-Straßburg i. E., *Ransohoff*-Stephansfeld, *Romberg*-Tübingen, *Römer*-Stuttgart, *Römer*-Hirsau, *Rosenfeld*-Straßburg i. E., *Reis*-Neckargemünd, *v. Rümelin*-Tübingen, *Staiger*-Hohenasperg, *Sayler*-Weinsberg, *Stengel*-Bruchsal, *Sick*-Tübingen, *Sauberschwarz*-Elisabethenberg bei Lorch, *Schultes*-Illenau, *Schott*-Weinsberg, *Stade*-Hub, *Schneider*-Goddelau-Philippshospital, *Schnorr v. Carolsfeld*-Obersendling-München, *Stein*-Budapest, *Schleich*-Tübingen, *Thomsen*-Bonn, *Urstein*-Heidelberg, *Wollenberg*-Straßburg i. E., *Wiehl*-Schussenried, *Max Weil*-Stuttgart, *Weinland*-Weißenau, *Wiedenmann*-Rottenmünster, *Weiler*-München, *Wolff*-Katzenelnbogen, *Wilmanns*-Heidelberg, *Zahn*-Stuttgart.

1. Sitzung am 3. November 1906, 2¾ bis 6 Uhr. Vorsitzender *Hoche*-Freiburg. Begrüßung der Versammlung durch den Einführenden *Wollenberg*-

Straßburg. Redner gedenkt in warmen und anerkennenden Worten des verstorbenen Psychiaters Prof. Dr. *Karl Fürstner* und gibt einen Überblick über das Leben und die wissenschaftlichen Verdienste und Arbeiten des Verstorbenen. Die Versammelten erheben sich zum ehrenden Andenken *Fürstners* von ihren Sitzen. *Kreuser*-Winnental berichtet über die an Geh. Rat *Ludwig*-Heppenheim zur Feier seines 80. Geburtstages eingereichte Glückwunschadresse und die Danksagung des Jubilars.

Vorträge.

1. *Bürker*-Tübingen: Zur Thermodynamik des Muskels.

Die dynamischen und elektrischen Verhältnisse der Muskelmaschine sind Gegenstand vielfältiger Untersuchungen gewesen. Zur genaueren Analyse der Wirkungsweise einer Maschine genügt aber nicht die Kenntnis ihres dynamischen Effektes, noch weniger die des nebenher auftretenden elektrischen, es muß hierzu vielmehr ermittelt werden: wieviel Brennmaterial wendet die Muskelmaschine auf und wieviel nutzbringende Arbeit leistet sie dabei? mit andern Worten: es muß bekannt sein der thermische Wirkungsgrad, die indizierte und die effektive Leistung.

Solche Untersuchungen ermöglicht wenigstens an Kaltblütermuskeln die thermodynamische Methodik. Mit ihrer Hülfe wurde ermittelt, daß die Muskelmaschine unter den verschiedenen äußeren und inneren Einflüssen, wie sie die verschiedene Jahreszeit mit sich bringt, über gesetzmäßig verschiedene Mengen von Brennmaterial verfügt und dieses auch in den einzelnen Jahreszeiten in verschiedener Weise verwertet, daß die weiblichen Froschmuskeln in der Laichzeit reich an Brennmaterial und daher sehr leistungsfähig sind, daß Krötenmuskeln unter sonst gleichen Bedingungen zur Ermöglichung einer maximalen Zuckung nur halb so viel Energie aufwenden und Arbeit leisten als Froschmuskeln, daß das Adduktorenpräparat mit halb so viel Brennmaterial doppelt soviel Arbeit zu leisten vermag als das Gastrocnemiuspräparat, was außerordentlich auffallend erscheint, daß es eine Heizung des Muskels auf Nervenreiz hin, ohne daß es zu einer Kontraktion kommt, nicht gibt, daß es bezüglich des Energieaufwandes gleichgültig ist, ob direkt oder indirekt gereizt wird, falls nur die Arbeitsleistung gleich groß ausfällt, daß bei einer Muskelzuckung der Zug des angehängten Gewichtes nicht nur im Stadium der steigenden Energie, sondern auch in dem der sinkenden Energie exothermische Prozesse, wenn auch in geringerem Maße, auslöst.

(Eigenbericht).

Keine Diskussion.

2. *Alzheimer*-München: Über eine eigenartige Erkrankung der Hirnrinde.

A. berichtet über einen Krankheitsfall, der in der Irrenanstalt in Frankfurt a. M. beobachtet und dessen Centralnervensystem ihm von Herrn Direktor Sioli zur Untersuchung überlassen wurde.

Er bot schon klinisch ein so abweichendes Bild, daß er sich unter keiner der bekannten Krankheiten einreihen ließ, anatomisch ergab er einen von allen bisher bekannten Krankheitsprozessen abweichenden Befund.

Eine Frau von 51 Jahren zeigte als erste auffällige Krankheitserscheinung Eifersuchtsideen gegen den Mann. Bald machte sich eine rasch zunehmende Gedächtnisschwäche bemerkbar, sie fand sich in ihrer Wohnung nicht mehr zurecht, schleppte die Gegenstände hin und her, versteckte sie, zuweilen glaubte sie, man wolle sie umbringen und begann laut zu schreien.

In der Anstalt trug ihr ganzes Gebaren den Stempel völliger Ratlosigkeit. Sie ist zeitlich und örtlich gänzlich desorientiert. Gelegentlich macht sie Äußerungen, daß sie alles nicht verstehe, sich nicht auskenne. Den Arzt begrüßt sie bald wie einen Besuch und entschuldigt sich, daß sie mit ihrer Arbeit nicht fertig sei, bald schreit sie laut, er wolle sie schneiden, oder sie weist ihn voller Entrüstung mit Redensarten weg, welche andeuten, daß sie von ihm etwas gegen ihre Frauenehre befürchtet. Zeitweilig ist sie völlig delirant, schleppt ihre Bettstücke umher, ruft ihren Mann und ihre Tochter und scheint Gehörshalluzinationen zu haben. Oft schreit sie viele Stunden lang mit gräßlicher Stimme.

Bei der Unfähigkeit, eine Situation zu begreifen, gerät sie jedesmal in lautes Schreien, sobald man eine Untersuchung an ihr vornehmen will. Nur durch immer wiederholtes Bemühen gelang es schließlich, einiges festzustellen.

Ihre Merkfähigkeit ist aufs schwerste gestört. Zeigt man ihr Gegenstände, so benennt sie dieselben meist richtig, gleich darauf aber hat sie alles wieder vergessen. Beim Lesen kommt sie von einer Zeile in die andere, liest buchstabierend oder mit sinnloser Betonung; beim Schreiben wiederholt sie einzelne Silben vielmals, läßt andere aus und versandet überhaupt sehr rasch. Beim Sprechen gebraucht sie häufig Verlegenheitsphrasen, einzelne paraphasische Ausdrücke (Milchgießer statt Tasse), manchmal beobachtet man ein Klebenbleiben. Manche Fragen faßt sie offenbar nicht auf. Den Gebrauch einzelner Gegenstände scheint sie nicht mehr zu wissen. Der Gang ist ungestört, sie gebraucht ihre Hände gleich gut. Die Patellarreflexe sind vorhanden. Die Pupillen reagieren. Etwas rigide Radialarterien, keine Vergrößerung der Herzdämpfung, kein Eiweiß.

Im weiteren Verlaufe treten die als Herdsymptome zu deutenden Erscheinungen bald stärker, bald schwächer hervor. Immer sind sie nur leicht. Dagegen macht die allgemeine Verblödung Fortschritte. Nach 4½ jähriger Krankheitsdauer tritt der Tod ein. Die Kranke war schließlich völlig stumpf, mit angezogenen Beinen zu Bett gelegen, hatte unter sich gehen lassen und trotz aller Pflege Decubitus bekommen.

Die Sektion ergab ein gleichmäßig atrophisches Gehirn ohne makroskopische Herde. Die größeren Hirngefäße sind arteriosklerotisch verändert.

An Präparaten, die mit der Bielschowskyschen Silbermethode angefertigt sind, zeigen sich sehr merkwürdige Veränderungen der Neurofibrillen. Im Innern einer im übrigen noch normal erscheinenden Zelle treten zunächst

eine oder einige Fibrillen durch ihre besondere Dicke und besondere Imprägnierbarkeit stark hervor. Im weiteren Verlauf zeigen sich dann viele nebeneinander verlaufende Fibrillen in der gleichen Weise verändert. Dann legen sie sich zu dichten Bündeln zusammen und treten allmählich an die Oberfläche der Zelle. Schließlich zerfällt der Kern und die Zelle, und nur ein aufgeknäueltes Bündel von Fibrillen zeigt den Ort, an dem früher eine Ganglienzelle gelegen hat.

Da sich diese Fibrillen mit anderen Farbstoffen färben lassen als normale Neurofibrillen, muß eine chemische Umwandlung der Fibrillensubstanz stattgefunden haben. Diese dürfte wohl die Ursache sein, daß die Fibrillen den Untergang der Zelle überdauern. Die Umwandlung der Fibrillen scheint Hand in Hand zu gehen mit der Einlagerung eines noch nicht näher erforschten pathologischen Stoffwechselproduktes in die Ganglienzelle. Etwa 1/4 bis 1/3 aller Ganglienzellen der Hirnrinde zeigt solche Veränderungen. Zahlreiche Ganglienzellen, besonders in den oberen Zellschichten, sind ganz verschwunden.

Über die ganze Rinde zerstreut, besonders zahlreich in den oberen Schichten, findet man miliare Herdchen, welche durch Einlagerung eines eigenartigen Stoffes in die Hirnrinde bedingt sind. Er läßt sich schon ohne Färbung erkennen, ist aber Färbungen gegenüber sehr refractär.

Die Glia hat reichlich Fasern gebildet, daneben zeigen viele Gliazellen große Fettsäcke.

Eine Infiltration der Gefäße fehlt völlig. Dagegen sieht man an den Endothelien Wucherungserscheinungen, stellenweise auch eine Gefäßneubildung.

Alles in allem genommen haben wir hier offenbar einen eigenartigen Krankheitsprozeß vor uns. Solche eigenartigen Krankheitsprozesse haben sich in den letzten Jahren in größerer Anzahl feststellen lassen. Diese Beobachtung wird uns nahe legen müssen, daß wir uns nicht damit zufrieden geben sollen, irgend einen klinisch unklaren Krankheitsfall in eine der uns bekannten Krankheitsgruppen unter Aufwendung von allerlei Mühe unterzubringen. Es gibt ganz zweifellos viel mehr psychische Krankheiten, als sie unsere Lehrbücher aufführen. In manchen solchen Fällen wird dann eine spätere histologische Untersuchung die Besonderheit des Falles feststellen lassen. Dann werden wir aber auch allmählich dazu kommen, von den großen Krankheitsgruppen unserer Lehrbücher einzelne Krankeiten klinisch abzuscheiden und jene selbst klinisch schärfer zu umgrenzen.

(Eigenbericht.)

Keine Diskussion.

3. *Frank*-Zürich und *Bezzola*-Schloß Hard: Über die Analyse psychotraumatischer Symptome. — *Frank*-Zürich:

Ref. verweist auf seine und Dr. *Bezzolas* Ausführungen in der gleichen Versammlung vor vier Jahren in Stuttgart. Ihre heutigen Berichte über weitere Erfahrungen auf dem Gebiete der Psychoanalyse sind veranlaßt durch

Konrad Maurer

The history of Alois Alzheimer's first case Auguste D.

How did the eponym "Alzheimer's Disease" came into being?*

Konrad Maurer[1]

The year 2006 marks the centenary of Alois Alzheimer's remarkable presentation on "A Characteristic Disease of the Cerebral Cortex". Alzheimer read this paper during an afternoon session of the 37th Assembly of Southwest German Psychiatrists in Tübingen. Eighty-eight physicians and researchers were present including Binswanger, Curschmann, Döderlein, Levi, Merzbacher, Nissl and Romberg. Besides Alzheimer Binswanger and Levi were to become well know eponymists. Carl Gustav Jung from the Burghölzli Hospital in Zürich was also present. He later developed analytic psychology. The paper read by Alzheimer was the 11th contribution and published in the same year 1906 as abstract in the "Neurologische Centralblatt" with the title "Über einen eigenartigen schweren Erkrankungsprozess der Hirnrinde" (Neurologisches Centralblatt 1906; 23: 1129–36).

One year later, in 1907, the "Allgemeine Zeitschrift für Psychiatrie und Psychisch-Gerichtliche Medizin" (General Journal of Psychiatry and Psycho-Forensic Medicine) printed the lecture in full under the rubric "Proceedings of Psychiatric Associations" as the second contribution under the title "A Peculiar Disease of the Cerebral Cortex". This two-page article, and subsequent publications by Bonfiglio (1908), Perusini (1909), and again Alzheimer in 1911, led to the eponym *Alzheimer's Disease* first used by Emil Kraepelin in his 1910 textbook of psychiatry. In his 1906 and 1907 papers, Alzheimer described Auguste D., a 51-year-old woman from Frankfurt who had exhibited progressive cognitive impairment, focal symptoms, hallucinations, delusions, and psychosocial incompetence. At postmortem she exhibited arteriosclerotic changes, senile plaques, and neuro-fibrillary tangles. Although die eponym *Alzheimer* was originally used to describe "presenile" dementia, it was later also applied to dementing processes of old age.

This chapter describes the discovery and the contents of the long-lost file of Auguste D. and provides some biographical data on Alois Alzheimer and information on the derivation of the eponym. The type of Auguste D.'s dementia will also be reviewed in this context.

Alois Alzheimer's Life

Alois Alzheimer was born on June 14, 1864, in Marktbreit, a small town in lower Franconia on the Main river in Bavaria, southern Germany. His father was a Royal Bavarian

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* From: Concepts of Alzheimer Diseasae, Biological, Clinical and Cultural Perspectives; Eds. Peter J. Whithehouse, Konrad Maurer, Jesse Ballenger (2000) The John Hopkins University Press Baltimore and London. pp 5–29

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Fig. 1. Alois Alzheimer 1884 as a member of the Franconia fraternity in Würzburg

Notary. When he graduated from high school in the district capital of Aschaffenburg, Alzheimer's teachers certified that he was "excellent in the sciences." Science was also his hobby. Alzheimer studied medicine in Berlin, Würzburg, and Tübingen. He returned to Würzburg, where he graduated in 1888 after writing a doctoral dissertation, "On the Ceruminal Glands of the Ear." His doctoral adviser was the famous swiss-born physiologist Albert Koelliker. Alzheimer completed his state medical exams in the same year (Fig. 1).

After graduation Alzheimer worked for a short period in Koelliker's histologic laboratory in Würzburg. The young Alzheimer quite likely acquainted himself with the topical problems of the microscopic construction of the nervous system and was involved in the neurohistologic discussions oft that time.

In 1888 Alzheimer went to Frankfurt to work in the Municipality Asylum for the Mentally Sick and Epileptics, directed by Dr. Emil Sioli, an open-minded, liberal psychiatrist (Fig. 2). The young Alzheimer, at this time assistant house-officer, continued to be very fond of working with the microscope, a fascination that remained with him all his life. He was especially interested in researching the cortex of the human brain.

At the turn of the century, the number of mentally ill patients was increasing rapidly in Germany as elsewhere. Sexually transmitted diseases were widespread, and the number of patients with neuropsychiatric complications of progressive paralysis was increasing. In this atmosphere Alzheimer gained abundant practice as a psychiatrist. He was in close contact with his patients and "wanted to help psychiatry with the microscope" (Kraepelin 1924).

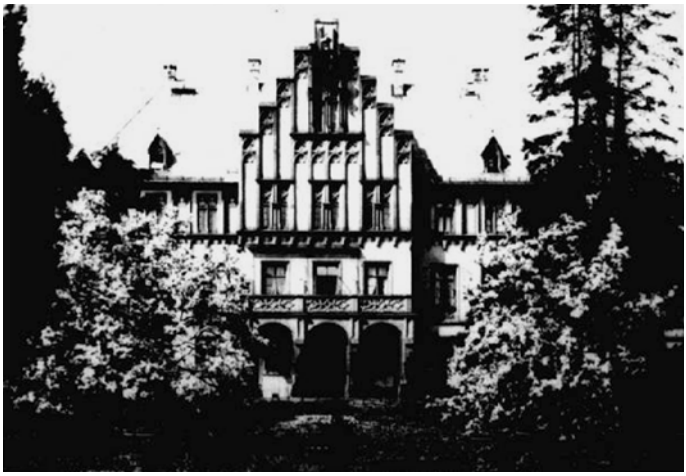


Fig. 2. Municipality Asylum for the mentally sick and epileptics

Dr. Franz Nissl, Alzheimer's superior, who had arrived in Frankfurt in April 1889, discovered better tissue-staining techniques. Nissl and Alzheimer became friends and close colleagues. During the day they worked together in the hospital, and in the evening they sat side by side in the laboratory doing research and discussing their results. Alzheimer believed that clinical practice and laboratory research complemented each other. "Why should not the physician improve his competence by enlarging scientific knowledge of psychiatry besides doing his daily clinical practice?" he once wrote (Maurer and Maurer 1998).

In 1894 Alzheimer married Cecilie Geisenheimer, nee Wallerstein, a wealthy jewish widow. They had three children: Gertrud (who married the psychiatrist Georg Stertz), Hans, and Maria (Fig. 3). When his young wife died in 1901, Alzheimer's younger sister, Maria, came to take care of the three children. As a result of his marriage, Alzheimer had gained considerable financial independence.

While in Frankfurt, Alzheimer expressed the desire to have a position in which he could combine research and clinical practice. In 1895 Alzheimer's friend Nissl moved to Heidelberg, where Emil Kraepelin held the chair of psychiatry. Kraepelin heard of Alzheimer's application for the post of managing director of a mental asylum. Being exclusively a research scientist, Kraepelin did not think much of this idea. Instead, Kraepelin invited Alzheimer to come to Heidelberg to write his "Habilitationsschrift." Alzheimer accepted and completed his research project under Kraepelin's supervision. In 1903 Alzheimer followed Kraepelin to Munich, where Kraepelin had recendy been appointed director of the Nervenklinik.

In Munich Alzheimer was appointed head of the neuroanatomic laboratory, which became an important center for brain research. He was joined by a number of renowned psychiatrists and neuropathologists, including Gaetano Perusini, Francesco Bonfiglio, Ugo Cerletti, Alfons Jakob, Hans Gerhard Creutzfeldt, Nicolas Achucarro, Karl Kleist, and Smith Ely Jeliffe. Alzheimer and his co-workers completed thousands of microscopic preparations (Fig. 4).

Fig. 3. Photograph of Mr. and Mrs. Alzheimer and the Children Hans, Maria and Gertrud

Alzheimer's research interests were wide-ranging. During his years in Frankfurt and Munich, he published about seventy papers. He finished his inaugural dissertation, "Histological Studies on the Differential Diagnosis of Progressive Paralysis," in Munich in 1904. This work was based on 320 postmortem cases he had collected in Frankfurt. In addition to dementia of vascular and degenerative origins, Alzheimer was interested in areas such as forensic psychiatry, delirium, mental deficiency, indications for induced abortion in mentally ill women, and histopathology of psychoses.

The Friedrich-Wilhelm University of Breslau in Silesia appointed Alzheimer chair of the Department of Psychiatry and director of the University Psychiatric Clinic on July 16, 1912. He viewed the post as the fulfillment of his scientific and academic aims (Fig. 5). On his way to Breslau, which was then in East Germany (though today it is Wroclaw, in Poland), Alzheimer caught a severe and persistent cold, which developed

Fig. 4. Scientific co-workers of Alzheimer
Last row from left to right: Lotmar (1), Rosenthal (3), Allers (4), Alzheimer (6), Achucarro (7), Lewy (8) Sitting: Grombach (1), Cerletti (2), Binfiglio (4), Perusini (5)

into subacute bacterial endocarditis. He never recovered completely. On December 19, 1915, during the second winter of World War I, Alois Alzheimer died in a uremic coma. He had not reached his fifty-second birthday. His body was transferred to Frankfurt and was laid to rest at the principal cemetery next to his wife, who had been buried there on February 28, 1901 (Fig. 6).

Auguste D. and her File

Until 1989 the whereabouts of Alzheimer's birthplace were largely unknown. That year, on the occasion of a symposium to celebrate Alzheimer's 125th birthday, the house, located in Marktbreit was discovered by Dr. and Mrs. Mony de Leon from New York on a side trip from the Würzburg Imaging in Psychiatry conference organized by Dr. Maurer, verified as correct by Dr. Konrad Maurer, subsequently fitted by the Maurer couple with a memorial plaque, and after purchase by Eli Lilly, converted into a museum and conference center that ist managed by Mrs. Maurer (Fig. 7).

Before this event, the author had done an intensive search for the file of Auguste D., which had been lost since Alzheimer's and Perusini's descriptions of the case in 1907 and 1909. The author located the file in the archives of his department in Frankfurt on December 21, 1995 (Maurer et al. 1997).

After 90 years the blue cardboard file was still in pristine condition. It contained 32 pages, including the patient's admission report, several medical and administrative certificates, and three versions of the case history—one in Latin script and two in the now-outdated Germanic script "Sütterlin."

Fig. 5. Portrait of Alois Alzheimer, photographed in Berlin Before he went to Breslau

The case history begins with an interview of the patient's husband, followed by clinical findings and the details of the course of her disease. A report on her death includes an anatomopathologic diagnosis. A small sheet of paper with the handwriting of Auguste D., dated by Alois Alzheimer, shows "anamnestic writing disorder". Alzheimer's handwritten notes, also in German script, document in detail the patient's symptoms from the first five days of her hospitalization on. In between Alzheimer's signatures are two handwritten notes by Auguste D., samples of Auguste's attempts to write her name. The file also contains four photographs of the patient (Fig. 8). The course of the disease is documented beginning in February 1902 until the day of her death, *April* 8, 1906. The file also includes a one-page case report from the Royal Psychi-

Fig. 6. Tomb of Cecilie and Alois Alzheimer at the principal cemetery in Frankfurt am Main

atrie Department in Munich, in which Alzheimer summarizes the history and course of August D.'s disease.

Auguste D.'s case history

Auguste D. was admitted to the clinic in Frankfurt on November 25, 1901. The case history in the file reads as follows: D., Auguste, wife of an office clerk, aged 51-and-a-half years. The patient's mother suffered convulsive attacks after menopause; it seems that she did not lose consciousness and did not drop objects that she was holding in her hands. Her mother died at the age of 64 of pneumonia; her father died at the age of 45. Three healthy brothers. No alcoholism or mental illness in the family history.

Previously, the patient had never been sick. She had been happily married since 1873, had borne a healthy daughter, and had had no abortions. Very diligent and tidy, slightly anxious and fearful, but polite. There seems to have been no syphilitic infection in either the patient or in her husband. Until March 1901, nothing outstanding occurred. Around March 18, 1901, the patient suddenly asserted, without any reason, that her husband had gone for a walk with a neighbour. From then on she remained very cool toward him and the lady. Soon afterward, she started to have difficulty in remembering things. Two months later, she started making mistakes in preparing meals, paced nervously and without reason in the apartment, and was not careful with the household money. She progressively became worse. She asserted that a wagon driver who often came to her home might do something to her, and she assumed

Fig. 7. Birthplace of Alois Alzheimer of Marktbreit

that all conversations of the people around her were about her. She had no language disturbances and no paralysis. Later she often had a fear of dying and nervous anxiety during which she started to tremble. She would ring all the bells of the neighbors and knock on their doors. She could not find certain objects that she had put away.

Alzheimer's case report

A full transcription of Alzheimer's questions and Auguste D.'s answers appears in previous publications as well as in a biography of Alzheimer (Maurer and Maurer 1998), and will not be printed here in their entirety. Alzheimer's notes in the file begin on November 26, 1901. He asked very simple questions and wrote down the dialogues systematically. His questioning continues on four handwritten pages, dated through November 30, 1901 (Fig. 9).

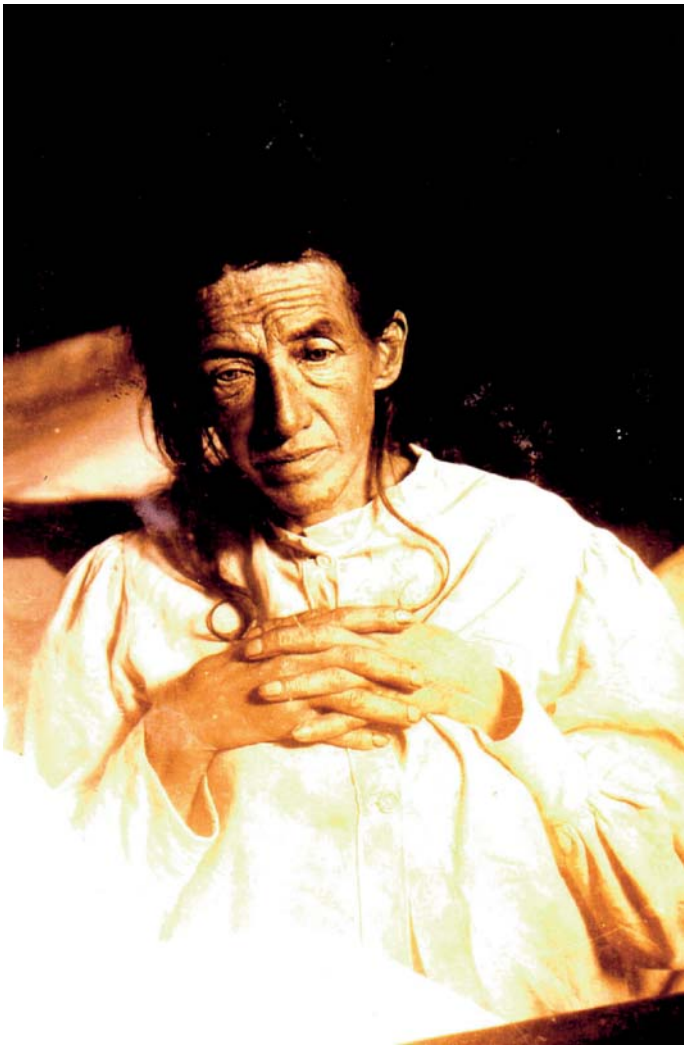


Fig. 8. Portrait of Auguste D. aged 51

November 26, 1901

ALZHEIMER'S NOTE: She sat on her bed with helpless expression.

Alzheimer: What is your name?
Auguste D.: Auguste.
Alzheimer: Last name?
Auguste D.: Auguste.
Alzheimer: What is your husband's name?
Auguste D.: Auguste, I think.

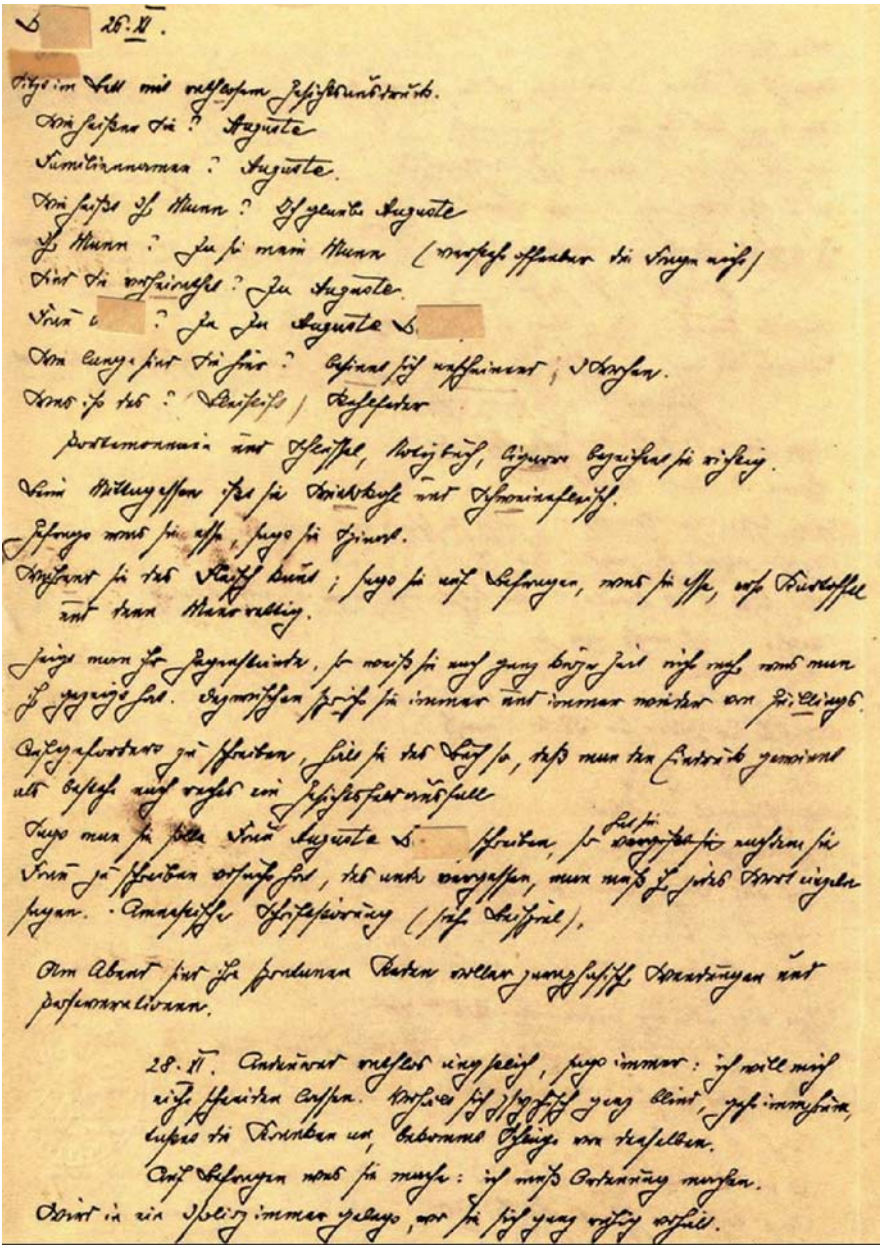


Fig. 9. Findings on admission of November 26, 1901

Alzheimer: Your husband?
Auguste D.: Ah, my husband? *[Sbe looks as if she doesn't understand tbe question.]*
Alzheimer: Are you married?
Auguste D.: To Auguste.
Alzheimer: Mrs. D.?
Auguste D.: Yes, Auguste D.
Alzheimer: How long have you been here? *[She seems to be trying to remember.]*
Auguste D.: Three weeks.
Alzheimer: What ist his? *[I showed her a pencil.]*
Auguste D.: A pen.

ALZHEIMER'S NOTE: A purse, a key, a diary, a cigar are named correctly. At lunch she eats cauliflower and pork. Asked what she was eating, she answers "spinach." As she was chewing the meat and was asked what she was eating, she answered "potatoes" and then "horseradish." When objects were shown to her, after a short time she did not remember what objects had been shown. In between she always speaks about "twins." When she is asked to write, she holds the book in such a way that one has the impression that she has a reduction of the right visual field.

Asked to write "Mrs. Auguste D.," she tries to write "Mrs." and forgets the rest. It is necessary to repeat every word. Amnestie writing disorder ("Amnestische Schriftstörung"). In the evening her spontaneous speech is full of paraphrasic derailments and perseverations.

November 28

She continuously looks helpless, anxious, and says, "I do not want to be cut." She behaves as if blind, touching other patients on their faces while they fight her. When asked what she is doing, she replies: "I must tidy up." She had been brought into an "isolation room," where she behaved very quietly.

November 29

ALZHEIMER'S NOTE: Helpless, refuses everything.

Alzheimer: … What is your name?
Auguste D.: Mrs. D., Auguste.
Alzheimer: When were you born?
Auguste D.: Eighteen hundred and …
Alzheimer: Your birthday?
Auguste D.: This year, a past year.
Alzheimer: When born?
Auguste D.: Eighteen-hundred, I don't know.
Alzheimer: What did l ask you?
Auguste D.: Ah, D., Auguste.
Alzheimer: Do you have children?
Auguste D.: One daughter.
Alzheimer: What is her name?
Auguste D.: Thekla.
Alzheimer: How old is she?

Auguste D.: She is married in Berlin, Mrs. S.
Alzheimer: Where does she live?
Auguste D.: We live in Kassel.
Alzheimer: Where does your daughter live?
Auguste D.: Waldemarstreet, no different.
Alzheimer: What is the name of your husband?
Auguste D.: I do not know how I came to this. I cannot go on this way.
ALZHEIMER'S NOTE: She seems not to understand the question.
Alzheimer: What is the name of your husband?
Auguste D.: I don't know.
Alzheimer: What is your husband's name?
Auguste D.: My husband is not here at this time.
Alzheimer: What is the name of your husband?

ALZHEIMER'S NOTE: She suddenly and quickly answered, "August Wilhelm Carl. I don't know if I can say that."

Alzheimer: What is your husband?
Auguste D.: Office clerk. I am so wrong, so wrong. I cannot …
Alzheimer: How long have you been here?
Auguste D.: Rather long.
Alzheimer: Where are you now?
Auguste D.: But this is Wilhelmshöhe.
Alzheimer: Where is your flat?
Auguste D.: In Frankfurt am Main.
Alzheimer: Which street?
Auguste D.: Not the Waldemarstreet but another one. … Just wait, I am very, very …
Alzheimer: Are you ill?
Auguste D.: Well, more the spine.
Alzheimer: Do you know me?
Auguste D.: I think you have seen me two times. Please excuse me. … I cannot … in this way.
Alzheimer: What is the current year?
Auguste D.: 1800.
Alzheimer: Are you ill?
Auguste D.: Second month.
Alzheimer: What are the names of the patients? *[She answers quickly and correctly.]*
Alzheimer: Which month is it now?
Auguste D.: The eleventh.
Alzheimer: What is the name of the eleventh month?
Auguste D.: The last one, if not the last one.
Alzheimer: Which one?
Auguste D.: I don't know.
Alzheimer: What color is the snow?
Auguste D.: White.
Alzheimer: The soot?
Auguste D.: Black.
Alzheimer: The sky?

Auguste D.: Blue.
Alzheimer: The meadows?
Auguste D.: Green.
Alzheimer: How many fingers do you have?
Auguste D.: Five.
Alzheimer: Eyes?
Auguste D.: Two.
Alzheimer: Legs?
Auguste D.: Two.
Alzheimer: How many dimes are in a mark?
Auguste D.: 100.
Alzheimer: How many marks are in one thaler?
Auguste D.: One mark, yes, one mark.
Alzheimer: How much does an egg cost?
Auguste D.: Six or eight.
Alzheimer: Six or eight, what?
Auguste D.: Yes.
Alzheimer: Six or eight marks?
Auguste D.: Yes, mark.
Alzheimer: What does a pound of meat cost?
Auguste D.: Twenty.
Alzheimer: Twenty, what?
Auguste D.: I don't know.
Alzheimer: One roll?
Auguste D.: Three dimes.
Alzheimer: If you buy six eggs for seven dimes, how much does it cost?
Auguste D.: Differently.
Alzheimer: On what street do you live?
Auguste D.: I can tell you. I must wait a little bit.
Alzheimer: What did I ask you?
Auguste D.: Well, this is Frankfurt am Main.
Alzheimer: On which street do you live?
Auguste D.: Waldemarstreet … not … no …
Alzheimer: When did you get married?
Auguste D.: I don't know at present. The woman lives on the same floor.
Alzheimer: Which woman?
Auguste D.: The woman where we are living. *[The patient calls]* Mrs. G., Mrs. G., here a step deeper. She lives …

ALZHEIMER'S NOTE: I show her a key, a pencil, and a book and she names them correctly.

Alzheimer: What did I show you?
Auguste D.: I don't know, I don't know.
Alzheimer: It is difficult, isn't it?
Auguste D.: So anxious, so anxious.
Alzheimer: How many fingers?
Auguste D.: Three.
Alzheimer: Are you still anxious?

Auguste D.: Yes.
Alzheimer: How many fingers did I show you?
Auguste D.: Well, this is Frankfurt am Main.

ALZHEIMER'S NOTE: The patient was asked to recognize objects by touch, closing her eyes. A toothbrush, a sponge, bread, a roll, a spoon, a brush, a glass, a knife, a fork, a plate, a purse, a mark, a cigar, a key.

She recognizes them quickly and correctly.

By touch, she calls a brass cup "a milk jug" and "a teaspoon," but when she opens her eyes she immediately says, "a cup." She writes as we have already described. When she has to write, "Mrs. Auguste D.," she writes "Mrs.," and we must repeat the other words because she forgets them. The patient is not able to progress in writing, and repeats, "I have lost myself."

Reading, she passes from one line to the other and repeats the same line three times. But she correctly reads the letters. She seems not to understand what she reads. She accents the words in an unusual way. Suddenly she says, "Twins." "I know Mr. Twin." She repeats the word twin during the whole interview.

The pupils accommodate to light without delay. The tongue has normal mobility and is dry, yellow-red-brown. No disturbance in speech articulation. She frequently interrupts herself about the pronunciation of words during the interview as if she would not know if she said something correctly. She carries a denture. No facial nerve differences. Muscular strength on the left side is considerably reduced in comparison with the right side.

Patellar reflex is normal. Radial reflex is a bit (but not relevantly; rigid. Cardiac ictus is not felt. Cardiac obtusity is not enlarged. The second pulmonary and aortic tones are not accentuated.

During the physical examination she cooperates and does not show anx-iety. She suddenly says, "Just now a child called. Is he there?" She hears her calling …; she knows Mrs. Twin. When she was brought from the isolation room to the bed, she became agitated, screamed, and was noncooperative. She shows great fear and repeats, "I don't want to be cut." "I do not cut myself."

November 30

She frequently stays in the living room, touches the faces of other patients, and hits them. It is difficult to figure out what she wants. Therefore, she must be isolated. When we try to speak with her, she says, "I do not have either the will or the time. I don't want …"

Alzheimer: How are you?
Auguste D.: During the last days I was very good.
Alzheimer: Where are you?
Auguste D.: Here and everywhere. Here and now. You don't mind.
Alzheimer: Why are you here?
Auguste D.: We are going to live there.
Alzheimer: Where is your bed?
Auguste D.: Where should it be?

Alzheimer: How did you sleep?
Auguste D.: Very good.
Alzheimer: Where is your husband?
Auguste D.: In the clerk's office.
Alzheimer: How old are you?
Auguste D.: Fifty-seven years.
Alzheimer: Where are you living?
Auguste D.: Waldemarstreet.
Alzheimer: Have you already eaten today?
Auguste D.: Soup and other things.
Alzheimer: What are you doing?
Auguste D.: To clean and something like that.
Alzheimer: Why didn't you put on your clothes?
Auguste D.: I had something to do.
Alzheimer: How long have you been here?
Auguste D.: You did write it, fifty-seven?
Alzheimer: Fifty-seven what?
Auguste D.: With the years.

ALZHEIMER'S NOTE: The behavior of the patient indicates that she is suspicious. She says to the doctor, "You do not have anything to do here."

After that she greets him in a friendly way. "Please have a seat. I did not have time." She wants to live, screams terribly, like a small child. She shows signs of occupational delirium. She takes some bedspreads and folds them up or puts them under the bed. "I am making order." Sometimes she sweats profusely and calls, "Karl" or "Thekla" (the names of her husband and daughter). If she is asked to name her husband, she normally says, "Auguste." When asked where she is, she says, "at home" and after that, "at the hospital." When asked to knit, she pulls out the needles from the work and begins to pick up the single loops. When asked what a bedside table is, she answers, "This is a bedside chair, and needs a cover."

Alzheimer's hand-written report ends November 30, 1901. The other two copies, written in German old script and Latin, continue to document the course of the patient's disease from January 1902. The Latin copy contains a registration from 1902 to the beginning of 1906.

Shortly before Auguste D.'s death, the file states: "Tendency to decubitus since the beginning of 1906. Development of a sacral and left trochanteric ulcer. Very weak, high fever up to 40 °C within the last days. Pneumonia of both inferior lobes."

The last documentation is dated April 8, 1906: "Within the morning, exitus letalis. Cause of death: Septicemia due to decubitus. Anatomical diagnosis: Moderate external and internal hydrocephalus. Cerebral atrophy. Arteriosclerosis of the small cerebral vessels. Pneumonia of both inferior lobes. Nephritis."

How did the eponym Alzheimer's disease come into being?

In the autumn of 1903, Alois Alzheimer left Frankfurt. Following a short stay in Heidelberg, he moved to Munich to continue his scientific and medical activities at

the Royal Psychiatric Clinic under director Emil Kraepelin. After Auguste D. died on April 8, 1906, Alzheimer asked that the record and the brain be sent to Munich. He immediately did a report on the admission formulas used in Munich at this time and wrote a full-page epicrisis. After this he made an entry to the autopsy book of the clinic under the number 181, dated 28 April 1906, Frankfurt, followed by the last name "D." and the source of the tissue as Frankfurt (Graeber et al. 1998). This proves that the brain had been analyzed in his famous neuropathologic laboratory. Within six months, **on** November 3, 1906, he presented his findings at the thirty-seventh meeting of the Southwest German Psychiatrists in Tübingen. In 1907 the lecture was published in *Allgemeine Zeitschrift für Psychiatrie und Psychisch-Gerichtliche Medizin* under the title, "A Characteristic Serious Disease of the Cerebral Cortex."

In this paper, Alzheimer described Auguste D.'s disease as follows:

The patient showed early clinical symptoms that deviated from the common ones and could not be classified under any well-known clinical patterns. The anatomical findings were also different from those of the usual disease processes. This disease started with a strong feeling of jealousy toward her husband. Very soon she showed rapidly increasing memory impairments. … She was disoriented as to time and place. Within half a year, Auguste developed symptoms typical for presenile dementia, later called Alzheimer's disease. Her neurological status was normal. There were no motoric disturbances in her gait or use of her hands. Her pupils reacted normally. … After four-and-a-half years of illness, the patient died. She was completely apathetic in the end and was confined to bed in a fetal position (with legs drawn up), was incontinent, and in spite of all the care and attention given to her, she suffered from decubitus. The autopsy showed an evenly affected atrophic brain without macroscopic foci. The larger cerebral vessels showed arteriosclerotic changes.

Concerning histopathology, Alzheimer wrote:

The Bielschowsky silver preparation showed very characteristic changes in the neurofibrils. However, inside an apparently normal-looking cell, one or more single fibers could be observed that became prominent through their striking thickness and specific impregnability. At a more advanced stage, many fibrils, arranged parallel, showed the same changes. Then they accumulated, forming dense bundles, and gradually advanced to the surface of the cell. Eventually the nucleus and cytoplasm disappeared, and only a tangled bundle of fibrils indicated the site where once the neuron had been located. As these fibrils can be stained with dyes different from the normal neurofibrils, a chemical transformation of the fibril substance must have taken place. This might be the reason why the fibrils survived the destruction of the cell. It seems that the transformation of the fibrils goes hand in hand with the storage of an as yet not closely examined pathological product of the metabolism in the neuron. About one-quarter to one-third of all the neurons of the cerebral cortex showed such alterations. Numerous neurons, especially in the upper cell layers, had totally disappeared.

Dispersed over the entire cortex, and in large numbers, especially in the upper layers, miliary foci could be found, which represented the sites of deposition of a peculiar substance in the cerebral cortex. It was even possible to recognize these without staining, but they were much more evident once stained.

The glia had abundant formed fibers; in addition, many glia cells showed large deposits. There was no infiltration of the vessels. Against this, focal lesions in the endothelium could be observed, and in some sites new vessel formation could also be seen (Fig. 10).

Alzheimer concluded:

On the whole, it is evident that we are dealing with a peculiar, little-known disease process. In recent years these particular disease processes have been detected in great numbers. This fact should stimulate us to further study and analysis of this particular disease. We must not be satisfied to force it into the existing group of well-known disease patterns. It is clear that there exist many more mental diseases than our textbooks indicate. In many such cases, a further histological examination must be done to determine the characteristics of each single case. We must reach the stage in which the vast, well-known disease groups must be subdivided into many smaller groups, each one with its own clinical and anatomical characteristics.

We learn more about Alzheimer's 51-year-old female patient in a 1909 article written by E. Perusini, "On Histological and Clinical Findings of Some Psychiatrie Diseases of Older People." On the Suggestion of Alzheimer, Perusini "examined four cases all characterized by clinical and especially anatomopathological signs." In this publication, Alzheimer's patient (case 1) was investigated again concerning her symptoms and histopathology. The initials of the surname, the complete Christian name, and the profession of her husband were mentioned for the first time ("D. Auguste, wife of an office clerk, aged 51 1/2 years"). Perusini and Alzheimer thanked Dr. Sioli in Frankfurt for the use of the case history and the brain for microscopic research. These facts prove that Perusini's case l was identical to the case described by Alzheimer in 1907.

Concerning Auguste D.'s plaques, Perusini stated, "In preparations for myelin sheath, clear yellow-grey or yellow spots of different sizes are seen between the darkly colored fibers. It is difficult to count the number of those plaques. Many are seen in the preparations that show plaques." In another part of the paper, Bielschowsky's method is mentioned and described as "especially favorable for showing such tonnation; by this method the plaques are seen impregnated more or less intensely with silver nitrate". Concerning neurofibrils, Perusini stated that "some cells are recognized only by their fibrillar skeletons: between the single fibrils that are clearly present there exists a particular myelin substance; this is colored metachromatically with toluidine blue".

Perusini concluded that "the pathological process recalls the main features of senile dementia. However, the alterations in the cases described are more far-reaching, although some of them represent presenile diseases. Regarding the clinical symptoms, those cases are peculiar as well. Apart from varying affective anomalies and varying psychotic symptoms, serious amnesia and rapid weakness of intelligence are present very early in the course of this disease."

Besides the two essential publications of Alzheimer (1907a) and Perusini (1909), Kraepelin must have been familiär with the publications of Bonfiglio and Sarteschi. In 1908 Bonfiglio reported in Italian on the initiative of Alzheimer. Bonfiglio's 60-year-old patient exhibited similar symptoms and histopathologic findings. Sarteschi also published cases in Italian.

In the eighth edition of his *Handbook of Psychiatry* (1910), Kraepelin completely reorganized the chapter on senile dementia (Fig. 11). Kraepelin mentioned Alzheimer dis-

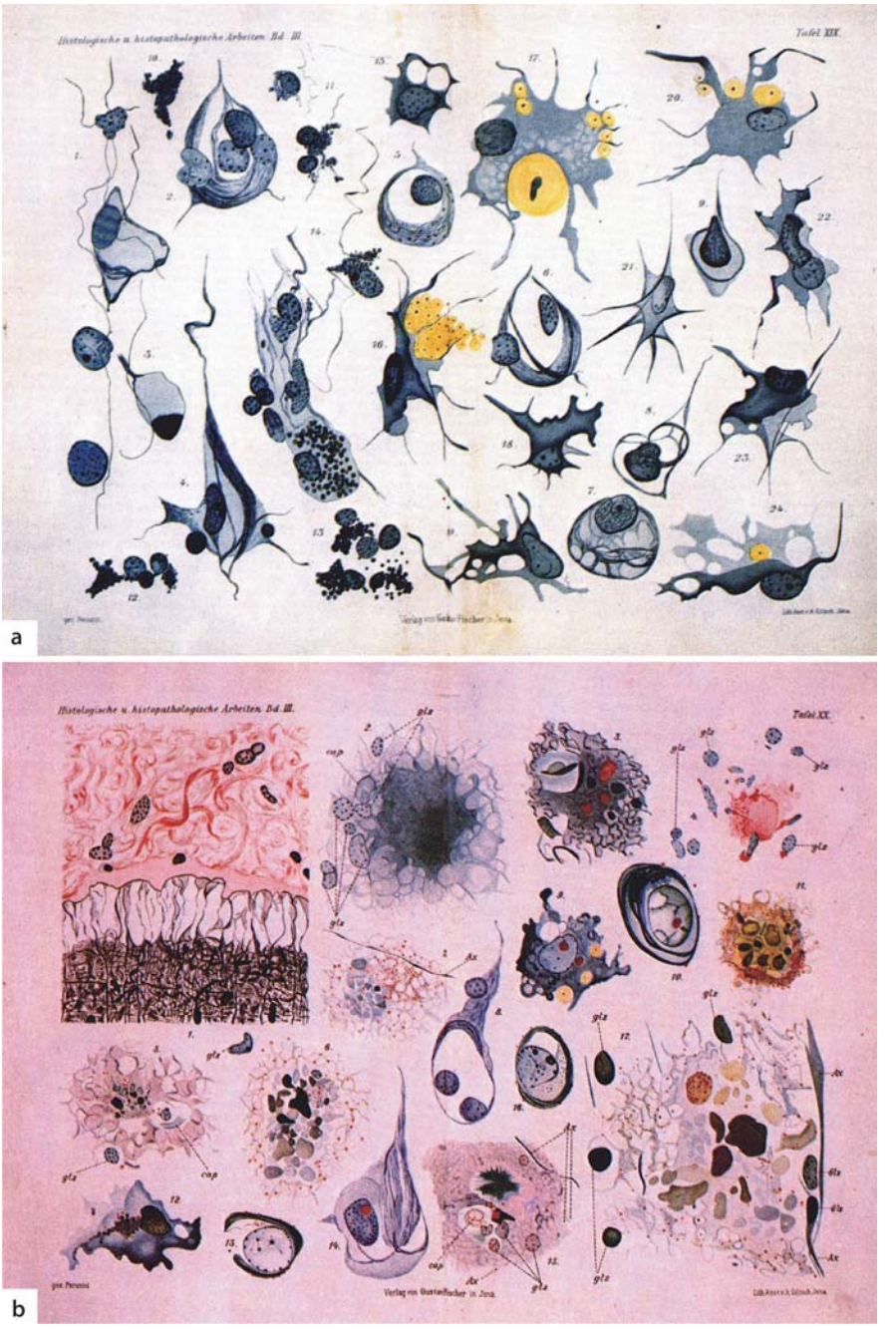
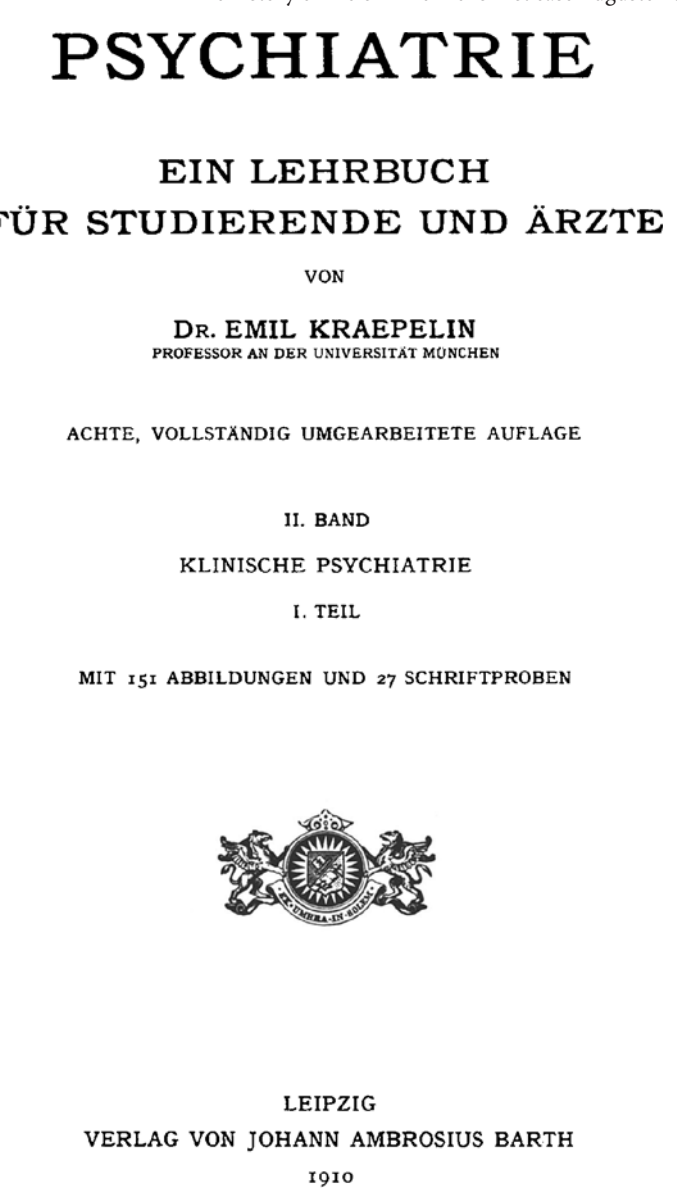


Fig. 10. (**a**) Neurofibrils of Auguste D. (**b**) Pictures drawn with Abbe's camara lucida. Under 2 and 3 plaques of Auguste D.



PSYCHIATRIE

EIN LEHRBUCH
FÜR STUDIERENDE UND ÄRZTE

VON

DR. EMIL KRAEPELIN
PROFESSOR AN DER UNIVERSITÄT MÜNCHEN

ACHTE, VOLLSTÄNDIG UMGEARBEITETE AUFLAGE

II. BAND

KLINISCHE PSYCHIATRIE

I. TEIL

MIT 151 ABBILDUNGEN UND 27 SCHRIFTPROBEN

LEIPZIG
VERLAG VON JOHANN AMBROSIUS BARTH
1910

Fig. 11. Front page of Kraepelin's textbook

ease for the first time in the following text: The clinical interpretation of this Alzheimer's disease is still confusing. While the anatomical findings suggest that we are dealing with a particularly serious form of senile dementia, the fact is that this disease sometimes starts as early as the late 40s. In such cases we should at least assume a "senium praecox," if not rather a more or less age-independent peculiar disease process. The

clinical picture involving an extraordinarily serious dementia, serious speech disorder, spastic signs, and seizures differs distinctively from "presbyophrenia," because pure senile cortical changes accompany this disease. Perhaps relations with one or more presenile diseases exist. (Kraepelin 1910)

In introducing the eponym *Alzheimer's disease*, Kraepelin likely knew only the few cases in table 1 (Fig. 12). This was confirmed by Alzheimer himself in his 1911 paper, "Über eigenartige Krankheitsfälle des späteren Alters," pointing only to his own 1907 publication and those of Bonfiglio (1908) and Perusini (1909). One case from Sarteschi (1909), describing a 67-year-old female, does not fit into the scheme.

It is of some interest that Alzheimer's case of Auguste D. is, in fact, Perusini's case 1. Some features have been changed (i.e., the postmortem results no longer showed arteriosclerosis). Besides that, the Perusini paper described Auguste D.'s histopathologic peculiarities in detail, with numerous plaques showing pictures drawn with Abbe's camera lucida or photographs with Zeiss plana. Likewise, Perusini's fourth case (Leonhard Sch.) was the same as that described by Bonfiglio (1908).

Thus, Kraepelin had knowledge of only four cases (Auguste D., Leonhard Seh., R.M. male, and BA female) and knew their histopathologic findings.

The second case published by Alzheimer in 1911 involved Johann F., who had been admitted to the hospital on November 12, 1907, and died on October 3, 1910. Certainly Kraepelin knew him from his ward rounds and was familiar with his history and clinical signs. However, it is unlikely that he knew the histopathologic findings of Johann F. when he was writing the eighth edition of his textbook and defining the term "Alzheimer's disease."

Why did Kraepelin, who introduced the eponym *Alzheimer disease,* use Alzheimer's name and not that of Perusini or Bonfiglio? Alzheimer was the editor of the Journal in which Perusini published his 1909 paper, which starts as follows: "On the Suggestion of Dr. Alzheimer, I examined the following four cases characterized by clinical and especially anatomo-pathological signs in common." Of interest is the fact that most of the papers in the series, *Histologicel und histopathologische Arbeiten über die Großhirnrinde* (Histological and Histopathological Studies on the Cerebral Cortex), edited by Nissl and Alzheimer, had single authors. Thus, according to modern convention, Alzheimer was the senior author of the publication in which Perusini described the four cases. It was also common at this time for the editor of such an important Journal to stay in the background.

Case	Author / Year of Publication	Age (yr.)
Auguste D.	Alzheimer, 1907	51
Leonard Sch.	Bonfiglio, 1908	60
Auguste D.	Perusini, 1909	51
R.M.	Perusini, 1909	45
B.A.	Perusini, 1909	65
Leonard Sch.	Perusini, 1909	60

Fig. 12. Table 1: Published cases of AD available to Kraepelin in 1910

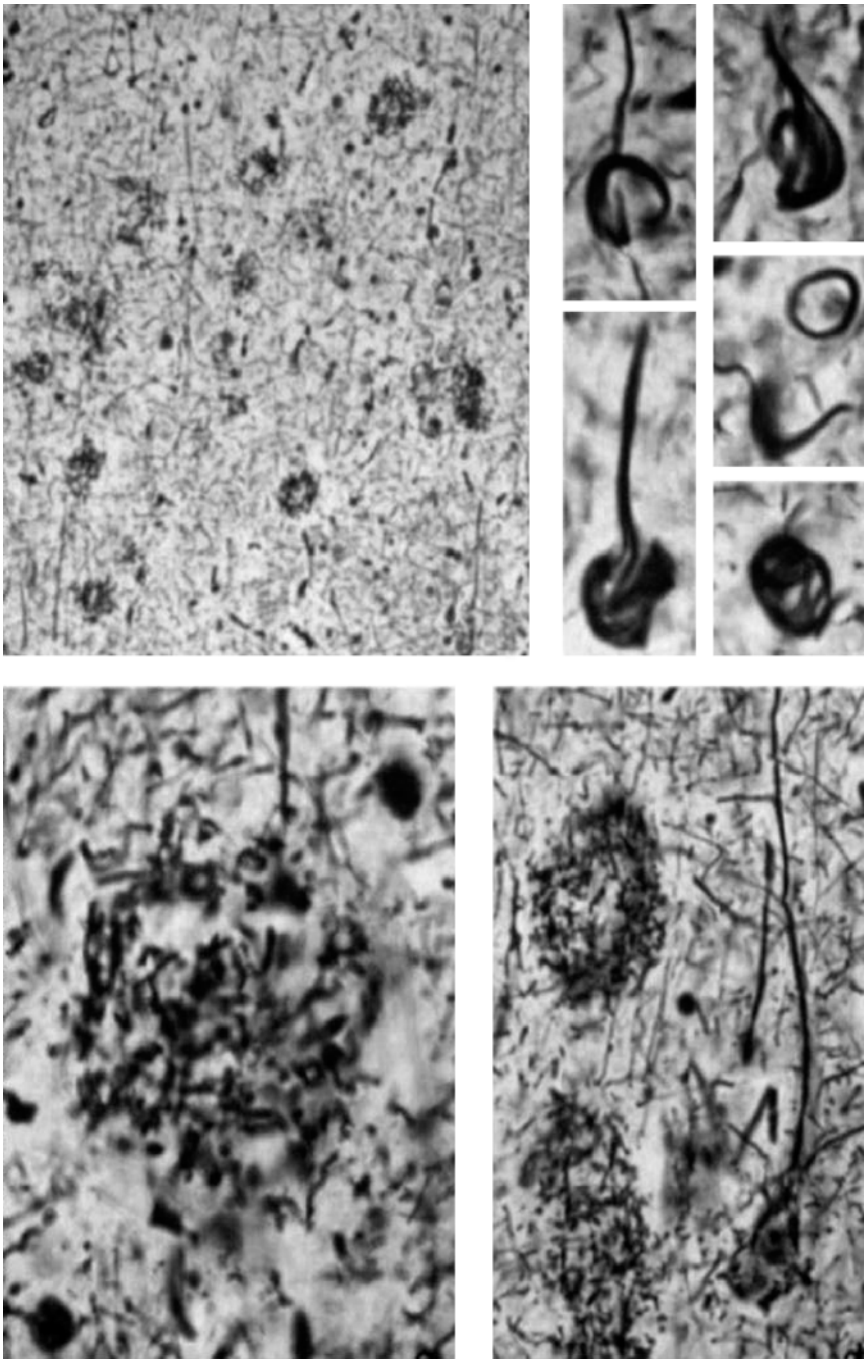


Fig. 13. Histological examination of Auguste D.'s brain, showing neurofibrillary tangles and amyloid plaques

Of the four cases (Auguste D., Leonhard Seh., R.M., and B.A.) that had been verified histopathologically at the time the eponym was created, that of Auguste D. was the most prominent. Since that early start in 1907, the Auguste D. case has been cited numerous times and was used for introductions to publications and articles covering the field of AD. We are convinced that the eponym *Alzheimer disease* was based mainly on Alzheimer's 1907 report of Auguste D. and the few cases published by Perusini.

Several hypotheses have been suggested to account for the haste with which Kraepelin created the eponym. Beach (1987) says that Kraepelin did so for scientific reasons (i.e., because he believed that Alzheimer had discovered a new disease). Another reason might have been the existing rivalry between Kraepelin's department and that of Pick in Prague and Kraepelin's desire for prestige for his Munich laboratory. Also plausible is Kraepelin's wish to show the superiority of his school over psychoanalytical theories and to show (vis-a-vis Freud) that some mental disorders were organically based. The most likely explanation, however, is the close collaboration that existed between Kraepelin and Alzheimer, and Kraepelin's awareness of Alzheimer's clinical and scientific work on presenile cases.

Auguste D/s dementia

In addition to Alzheimer disease, other hypothetical diagnoses of Auguste D.'s disease have been put forward, especially arteriosclerosis and, astonishingly, metachromatic leukodystrophy. Many postmortem diagnoses listed arteriosclerosis at this time. In Auguste D.'s file, Alzheimer himself noted "Arteriosklerotische Gehirnatrophie." The question mark is interesting and also appeared in the autopsy report: "Arteriosklerose der kleinen Hirngefäße." However, the histopathologic details in the 1907 and 1909 publications always pointed to vessels without arteriosclerosis: Perusini found "that the large vessels, the arterial circle of Willis, and the Sylvian arteries showed no significant sign of arteriosclerosis"; only "some regressive alterations of the arterial walls" were described. In both papers the presence of the neuritic plaques and neurofibrillary tangles was confirmed.

There are a number of convincing arguments against the assumption of a metachromatic leukodystrophy in Auguste D.'s case, as suggested by Amaducci (Amaducci et al. 1991). The clinical picture of Auguste D. bears only a limited resemblance to the symptoms of metachromatic leukodystrophy. In particular, key symptoms caused by involvement of the peripheral nervous system are lacking. Alzheimer was an experienced neuropsychiatrist, and it is unlikely that he would have missed clear symptoms concerning the disease.

All discussions about Auguste D.'s illness should end now that the tissue sections from Auguste D., discovered by Graeber and co-workers in 1998, have been examined (Fig. 13) "There were numerous neurofibrillary tangles and many amyloid plaques, especially in the upper cortical layer of this patient. Yet, there was no microscopic evidence for vascular—i.e., arteriosclerotic lesions (Graeber et al., 1998). Thus, considering the publications and the file of Auguste D., it becomes more and more evident that she is the initial case of Alzheimer's disease."

Ralf Dahm

Alois Alzheimer and the beginnings of research into Alzheimer's disease

Ralf Dahm[1]

A century ago – on November 3, 1906 – a young doctor delivered a talk at the annual meeting of the South-West German psychiatrists. In this talk, he described the psychiatric symptoms and changed brain histology of his late patient, Auguste D. This moment marked the beginning of research into what was to become one of the most infamous afflictions, the disease that today bears his name: Alzheimer's disease.

Alzheimer's early years and medical studies

Aloysius (Alois) Alzheimer (Fig. 1) was born on June 14, 1864, to the royal notary Eduard Alzheimer and his second wife, Therese, in the small Bavarian town of Marktbreit.[2] Early on in school, he showed a vivid interest in the natural sciences. His school leaving certificate remarked on his academic achievements: "This student shows outstanding knowledge of the Natural Sciences, which he has studied with particular predilection throughout his time at high school." In the autumn of 1883, after having left school, Alzheimer followed this interest and began to study medicine at the University of Berlin, a hub for the medical and biological sciences at the time.

The late 19th century was an exciting time for neuroscience. Many of the fundamental concepts of our understanding of the brain were emerging. By the 1870s, the study of lesions or targeted stimulations of specific brain regions had led to the concept of cortical localization, and ever more mental faculties were localized to specific regions of the brain. In the 1880s, neurons were increasingly advocated as the elementary units of the nervous system (neuron doctrine) and their morphology was being elucidated. Dendrites and axons were named, and synapses were postulated to be points of contact between neurons (reviewed in Finger 1994; Shepherd 1991).

In Berlin, Alzheimer was introduced to novel approaches regarding the study of brain pathology as an important tool in psychiatric investigations. Scientists increasingly tried to find anatomical causes, particularly damage to the brain, to explain mental disorders. But despite the great opportunities Berlin offered to the young student, Alzheimer was deeply rooted in his South German homeland. After just one

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[2] Detailed accounts of the life and achievements of Alois Alzheimer can be found in the biography by Konrad and Ulrike Maurer (translated by Neil Levi; Maurer et al. 2003) and a recent article by Manuel B. Graeber (Graeber 2006). Further information can be obtained from the obituary by Franz Nissl (Nissl 1916), Walther Spielmeyer's posthumous appraisal of Alzheimer's life and work (Spielmeyer 1916) and Emil Kraepelin's memoirs (Kraepelin 1987).

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Alzheimer: 100 Years and Beyond

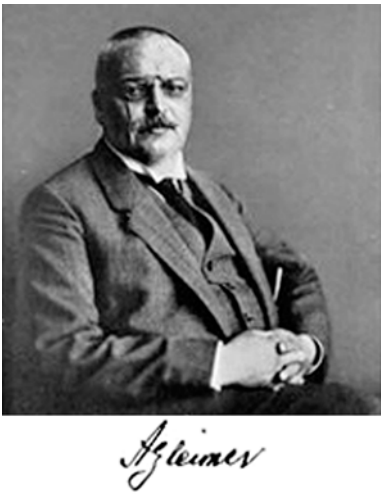

Fig. 1. Alois Alzheimer, the person after whom Alzheimer's disease was named. Below the portrait is his signature

semester in Berlin, he joined his elder brother Karl, who studied in the Franconian city of Würzburg. Alzheimer continued his medical studies and – with the exception of the winter semester 1886/7 spent studying at the University of Tübingen – finished them in Würzburg. During his time in Würzburg, he encountered Albert von Kölliker. von Kölliker, a renowned histologist and pioneer of microscopic anatomy, introduced Alzheimer to microscopy. The solid foundations in histology acquired in Kölliker's laboratory, together with the new concepts on the basis of mental disorders he had learned about in Berlin, would prove a crucial combination for his future investigations. In 1887, Alzheimer also performed his doctoral work on the microscopic anatomy of the ceruminal glands of the ear with von Kölliker (amongst others) and in 1888, received the "approbation" as a medical doctor.

The Frankfurt Institution of the Mentally Ill and Epileptics

After his studies, Alzheimer spent five months as a personal physician to a wealthy mentally ill lady. This assignment stimulated his interest in psychiatry. And thus, in 1888, he applied for a position at the Municipal Institution for the Mentally Ill and Epileptics in Frankfurt (am Main), Germany. Despite not being one of the hubs of neuroscience research at the time, Frankfurt's psychiatric institution was an innovative work place. Its founder, Heinrich Hoffmann – today better remembered for his children's book, Struwwelpeter (Shock-Headed Peter) – believed in an organic origin of mental disorders. Unlike many of his contemporaries, he firmly believed in a psychiatry based on scientific investigation and followed the credo that diseases of the mind are diseases of the brain. Through countless autopsies of the brains of deceased patients suffering from psychiatric or neurological disorders, he tried to uncover the anatomical causes of these disorders. Moreover, Hoffmann tried to implement more humane treatments for his patients. It was in this spirit that, in 1864. he established a new institution for the mentally ill in Frankfurt (Fig. 2).

Fig. 2. The former psychiatric clinic in Frankfurt. The Institution for the Mentally Ill and Epileptics was an imposing edifice built in the German neo-gothic style. It was erected in 1864 under the auspices of the notable German psychiatrist, Heinrich Hoffmann. It had several gardens, courts and a ballroom. Due to its grand appearance, the citizens of Frankfurt deridingly referred to it as the "palace of the mad." It was this institution Auguste D. was admitted to and where she was examined by Alois Alzheimer

In late 1888, at the age of 79 and after having headed Frankfurt's psychiatric institutions for over three decades, Hoffmann retired and Emil Sioli was appointed as his successor. Like Hoffmann, Sioli believed in non-restraint and that the brain holds the key to understanding mental disorders. To continue the tradition of the Frankfurt Institution, he sought to recruit assistants who were both dedicated medical practitioners with an interest in psychiatric disorders and scientists with a passion for studying brain histology. When Alzheimer applied for the position, Sioli immediately accepted him. On December 19, 1888, Alzheimer took up his new post and, shortly thereafter, Sioli appointed Franz Nissl, one of the greatest neuropathologists of his day and still famous today for the development of the Nissl histological staining technique, as assistant medical director.

The three men harmonized exceptionally well and continued the tradition of the clinic as a progressive psychiatric institution. They introduced innovative treatments for psychiatric disorders and established interviews with their patients as important sources of information to understand their mental disorders. Alzheimer's close interaction with Nissl was crucial for their scientific studies. Alzheimer's capabilities as a psychiatrist were essential in evaluating the clinical symptoms of their patients and Nissl's protocols for staining neurons became an important tool in their histological examinations. Together the two men substantially advanced the understanding of the histopathology of the cortex.

The close intertwining between their clinical work and their microscopic analyses was crucial to correlating psychiatric symptoms with histological results obtained from autopsies of the brains of their deceased patients. In other areas of medicine, histopathology had already been widely successful in correlating disease symptoms with organic causes. Alzheimer's main aim was to use microscopic analyses to achieve the same for psychiatry: to understand the physical bases of mental afflictions and,

based on this understanding, to develop criteria for their classification. He was convinced that only if the different mental diseases could be clearly defined would it be possible to understand and possibly treat them (Nissl 1916). The combination of being a compassionate psychiatrist and an astute scientist allowed Alzheimer to make important contributions in various areas, including, for example, the various forms of dementia, cerebral atherosclerosis, progressive paralysis, damage caused by acute syphilis infections or chronic alcohol abuse, epilepsy, psychoses and forensic psychiatry (Graeber 2006). But his fame today rests mainly on his case study of a woman in her early fifties who had been admitted to the Frankfurt psychiatric institution on November 25, 1901: Auguste D.

Auguste D. – the first patient diagnosed with Alzheimer's disease

On the November 26, 1901, Alzheimer examined Auguste D. for the first time. The entry into her case file revealed that, eight months earlier, Auguste D. had developed increasing signs of a change in her personality (Maurer et al. 1997, 2000, 2003). Her memory failed her. More and more, she neglected her household chores and began hiding objects. When preparing food, she made mistakes and spoiled the dishes. She became agitated and aimlessly paced around her apartment. Frequently, she was lost in familiar situations and developed a fear of people well acquainted to her. She became paranoid and unduly jealous of her husband. At times she even believed that someone wanted to kill her and began to shout wildly. At the time, Auguste D. was 51 years of age. She had never been afflicted by any serious illnesses and, apart from being underweight, her physique was normal. When examined upon admission at the Frankfurt clinic by the institution's personnel, Auguste D. was diagnosed as being spatially and temporally disoriented, generally confused, anxious and reluctant to cooperate with the institution's personnel (Alzheimer 1907a).

To be able to better diagnose her affliction, Alzheimer interviewed Auguste D. She spoke clearly and articulated well. Alzheimer noted, however, that she often stopped in the middle of a sentence or even a word, as if she was at a loss or indecisive as to whether she was saying the right thing. When reading, she often pronounced words in a meaningless fashion or spelled them out letter by letter. When writing, she sometimes repeated syllables multiple times, omitted letters or entire syllables and generally abruptly ended her speech mid-statements. For example, when asked to write her name ("Frau Auguste D."), she stopped after the word "Frau" (Fig. 3). Only when she was told to write every word individually, one after the other, could she note them down correctly (a symptom termed "amnesic writing disorder" by Alzheimer). When failing to be able to write down something that was said to her, Auguste D. herself remarked, "I have, so to say, lost myself" – an apt summary of the tragic changes in mental capabilities and personality that many patients suffering from Alzheimer's disease experience.

Alzheimer had never encountered a patient with such symptoms. He was fascinated by Auguste D.'s case and decided to examine her more closely. He systematically

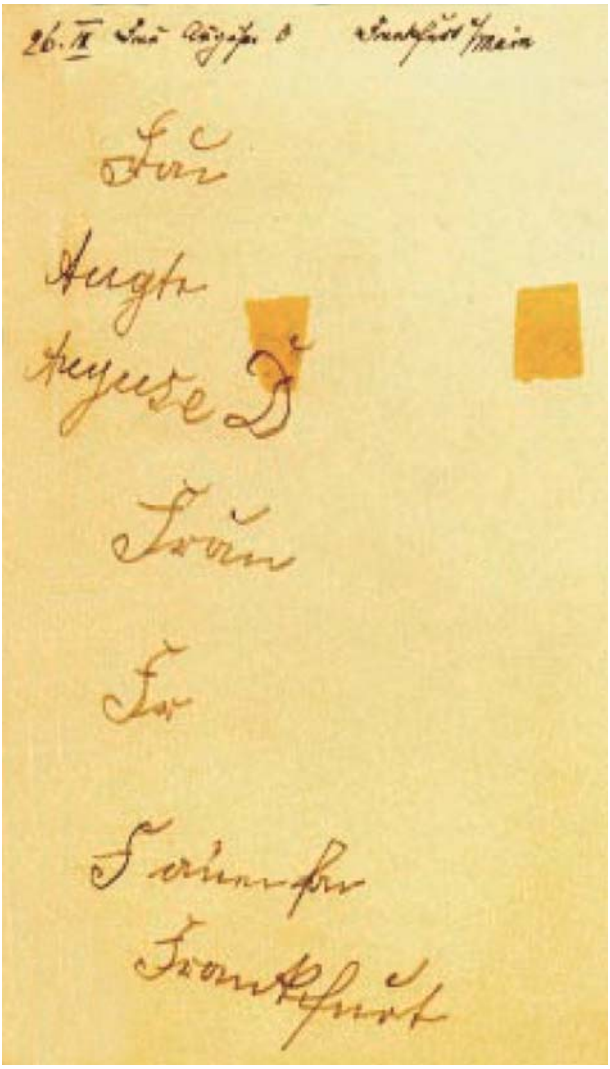

Fig. 3. Handwriting of Auguste D. Sheet of paper showing attempts by Auguste D. to write her name ("Frau Auguste D.") and the name of the city she lived in ("Frankfurt"). At the top, the sheet was annotated by Alzheimer with the date, name of the patient and place

interviewed her and recorded her answers to his questions in detailed protocols.[3] When he addressed her with specific questions, her replies often did not relate to his questions. When shown objects, she generally named them correctly. However, some

[3] A selection of Alzheimer's transcripts of these interviews, together with additional information on the clinical symptoms of Auguste D., is given in the chapter by Konrad Maurer in this volume.

she evidently did not recognize anymore and did not know how to use them. When talking spontaneously, she frequently evaded questions, used paraphasic expressions and inappropriate words (e.g., milk pourer instead of cup) or words in wrong and senseless combinations (Alzheimer 1907a).

Over time, Auguste D.'s symptoms generally worsened. Occasionally, she tended to occupy herself with senseless tasks in a delirious manner, carried bedding about and engaged in aimless activities. Increasingly, she began to hallucinate, for example, insinuating that the physicians wanted to injure or abuse her, getting very angry and demanding them to leave the house. On other occasions, she greeted her doctors like visitors and apologized for not being ready to receive them yet. She frequently shouted nonsensically, often for hours on end. At times, she was in a state of intense fear and vigorously resisted all attempts to examine or wash her. At other times, she was completely apathetic (Alzheimer 1907a). Alzheimer's last entry into Auguste D.'s case file dates from November 30, 1901. After that time, Alzheimer's colleague Paul Nitsche continued the file, but Alzheimer kept following the case with great interest.

Alzheimer joins Kraepelin

In 1903, after 14 years in Frankfurt, Alzheimer left the Frankfurt institution. Emil Kraepelin (Fig. 4), one of the most influential psychiatrists of his time, had offered him a position as scientific assistant at his clinic in Heidelberg. Alzheimer's close friend, Franz Nissl, who had previously also moved to Heidelberg, persuaded Alzheimer to join them. Alzheimer's stay in Heidelberg, however, was to be short. Only six months after he had taken up his new position, in the autumn of 1903, Kraepelin moved to Munich to head the Royal Psychiatric Clinic (Nissl 1916). Alzheimer went with Kraepelin to Munich and took over the clinic's large anatomical laboratory. Under Alzheimer, the laboratory quickly filled with students and guest scientists from various countries

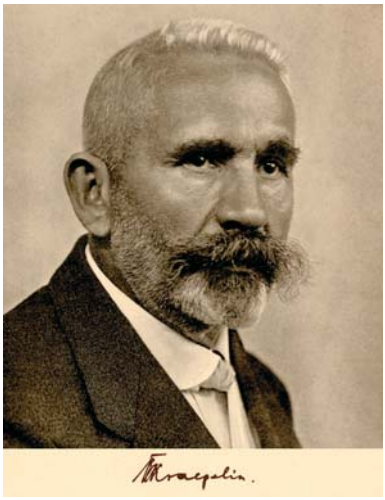

Fig. 4. Emil Kraepelin. This picture is the frontispiece from Kraepelin's 9th edition of his volume on clinical psychiatry (Leipzig 1927)

(Fig. 5). These included Friedrich H. Lewy, famous today for the Lewy bodies named after him, as well as Hans-Gerhard Creutzfeld and Alfons Maria Jakob, who in the early 1920s would be the first to describe the disease that now bears their names, Creutzfeld-Jakob disease. Also Alzheimer's extensive teaching duties increasingly impinged on his time to do research. Yet despite these new tasks, he never lost interest in the presenile dementias, including his key case of Auguste D.

Sioli had regretted losing Alzheimer, but he promised to keep him up to date on the development of Auguste D. Her physical and psychological state progressively worsened. She became more and more distant, shouting and hitting when being examined. Her speech became completely unintelligible. Later she stopped talking altogether, only humming or shouting wildly, often for hours and without apparent triggers. She ate irregularly, had to be fed and continuously soiled herself. In her final year, she became completely apathetic and spent most of her time hunched up in her bed. In early 1906, she developed pneumonia and on April 8, 1906, five weeks short of her 56th birthday, Auguste D. died. In the file describing her case, her cause of death is given as septicaemia due to a decubitus (Maurer et al. 1997).

The case of Auguste D., as recorded by Alzheimer, accurately describes the clinical course of many patients suffering from Alzheimer's disease: her increasingly failing memory, notably her early problems to establish and maintain memories for recent events; her impaired comprehension, unpredictable behavior and psychosocial ineptitude; as well as her disorientation and progressively developing aphasia. Overall, the

Fig. 5. Alois Alzheimer and guest scientists in his anatomical laboratory at the Royal Psychiatric Clinic in Munich. Top row, left to right: F. Lotmar (Switzerland), unknown, St. Rosental (Poland), Allers (?), unknown, Alois Alzheimer, Nicolás Achúcarro (Spain), Friedrich H. Lewy (Germany). Bottom row, left to right: Adele Grombach (Alzheimer's technician), Ugo Cerletti (Italy), unknown, Francesco Bonfiglio (Italy), Gaetano Perusini (Italy)

clinical symptoms displayed by Auguste D. fit well into the range of symptoms associated with Alzheimer's disease today. Furthermore, the examination of her brain was to result in the discovery of the characteristic changes associated with the disease.

Shortly after her death, Sioli sent Auguste D.'s brain to Alzheimer for detailed morphological examination. From this examination, Alzheimer hoped to uncover the histopathological changes responsible for the symptoms he had observed and thus to understand this new and "peculiar" disease. The first anatomical and histological studies confirmed Alzheimer's suspicion that this was an exceptional case and well worth pursuing further. On a gross anatomical level, the brain showed a widespread atrophy. Together with two visiting Italian physicians, Gaetano Perusini and Francesco Bonfiglio, Alzheimer meticulously examined the histological sections of Auguste D.'s brain. The sections again revealed the massive loss of cells that had occurred in various brain regions. But in addition to the atrophy, Alzheimer and his colleagues observed peculiar thick and strongly staining fibrils in the remaining neurons, a discovery made possible in large part by the silver stain recently developed by Max Bielschowsky. They also discovered deposits of an unidentified substance in the form of plaques throughout the cerebral cortex.

The brain of Auguste D. thus displayed what are considered today hallmarks of the brains of patients suffering from Alzheimer's disease: a loss of neurons as well as the accumulation of amyloid plaques and neurofibrillar tangles. For Alzheimer and his colleagues, though, the histological findings in Auguste D.'s brain represented a novel and as yet uncharacterized pathology. To some extent, they were reminiscent of changes in senile dementia, a pathology observed in elderly patients (for a discussion, see Alzheimer 1911 and references therein). What was peculiar about Auguste D.'s case, however, was that the changes occurred in a woman who was only 55 years old when she died and that they were much more profound than those in patients suffering from senile dementia in their 70s or 80s.

First presentation of "Alzheimer's disease"

On the occasion of the 37th meeting of South-West German psychiatrists in Tübingen on November 3, 1906, Alzheimer presented his results on the case of Auguste D. for the first time. His talk was entitled "Über eine eigenartige Erkrankung der Hirnrinde" (On a peculiar disease of the cerebral cortex). Alzheimer began by remarking that the clinical manifestations of the disease were so different from any other described so far that it was impossible to assign it to any of the known disorders. He proceeded by recounting Auguste D.'s clinical symptoms and the results from his physical examinations.

Alzheimer then turned to describing the findings of his histopathological analysis of her brain, relating that the necropsy showed a uniformly atrophic brain lacking macroscopic foci, with the major vessels of the brain showing some arteriosclerotic changes. He next summarized his analyses of the histological sections of Auguste D.'s brain stained with Bielschowsky's silver stain. These revealed "very strange changes in the neurofibrils" (Alzheimer 1907a), which clumped into tangles that eventually replaced the perished cells. Alzheimer speculated on the process giving rise to these neurofibrillary tangles: "Inside an otherwise still normal appearing cell, one or more fibrils become very prominent due to their unusual thickness and particular staining

properties. Later, several of these fibrils running alongside each other display the same changes. They then cluster into larger bundles and progressively move towards the surface of the cell. Ultimately, the nucleus and the cell disintegrate, such that only a bundle of tangled fibrils indicates the location of a former nerve cell" (Alzheimer 1907a).

Alzheimer continued to report that these fibrils displayed staining properties different from those of normal neurofibrils, indicating that they had undergone a chemical transformation. He conjectured that this transformation was also the cause of their surviving the extinction of the cell. He elaborated that one-quarter to one-third of all "ganglion cells" (neurons) in the cerebral cortex displayed such changes and that numerous cells, particularly in the uppermost cell layers, had disappeared entirely. Alzheimer then went on to describe the abundant "miliary foci" (amyloid plaques) he had seen in sections of Auguste D.'s brain. He reported that they could be found throughout the entire cerebral cortex, with the highest density in the uppermost layers, and had likely been formed by the "deposition of a peculiar substance." This deposition could be discerned even without any staining and was indeed very refractory towards staining.

Alzheimer concluded by reiterating that the pathology observed in Auguste D. did not fit into any of the categories of psychiatric disorders in use at the time. He even speculated that numerous psychiatric disorders with clinical symptoms deviating from the classical pathologies would turn out to be discrete pathologies upon histopathological analysis. He advanced the view that histological analyses like the one he had just presented would lead to a division of the large groups of disease patterns commonly used for classification at the time into discrete pathologies with clearly defined clinical symptoms and histopathological characteristics.

Much to Alzheimer's disappointment, there were no questions or discussion following his presentation. Similarly, the organizers of the meeting considered his talk unsuitable for publication in the meeting proceedings. Only the local newspaper – the Tübinger Chronik – which covered the meeting in its November 5 issue, mentioned his talk in a single sentence: "Dr. Alzheimer from Munich reported of a peculiar, severe disease process which in a period of 4 1/2 years causes a substantial loss of neurons." In 1907, however, the meeting organizers changed their minds and a summary of Alzheimer's talk was published as a short report in the Allgemeine Zeitschrift für Psychiatrie und Psychiatrisch-gerichtliche Medizin (Journal of Psychiatry and Psychiatric-forensic Medicine; Alzheimer 1907a).[4] In just two pages, Alzheimer summarised his findings of the clinical and neurohistopathological symptoms of Auguste D. No illustrations were included in this report.

Initially, Alzheimer's findings did not receive widespread interest. Alzheimer himself, however, remained fascinated by the peculiar disease and decided to examine more cases of presenile dementia he had since encountered. Between 1907 and 1908, three additional patients of his (Mrs. B. A., Mr. Sch. L. and Mr. R. M.) died after having displayed symptoms very similar to the ones of Auguste D. Together with Perusini, Alzheimer studied the brains of these new cases and compared them to the changes

[4] An English translation of the short summary of Alzheimer's talk held at the 37th meeting of South-West German psychiatrists, which was printed in the Allgemeine Zeitschrift für Psychiatrie und Psychiatrisch-gerichtliche Medizin in 1907, has been published (Bick 1987).

observed in Auguste D.'s brain. They discovered that the four cases shared key characteristics, including the neurofibrillary tangles and the formation of plaques throughout the cerebral cortex. Their results, including the first pictures of the histopathological changes of Auguste D.'s brain, were published in 1909 by Perusini in a journal edited by Nissl and Alzheimer himself (Perusini 1909). In this paper, Alzheimer's earlier statement that the major vessels of the brain showed some arteriosclerotic changes (Alzheimer 1907a) was retracted following the more detailed microscopic examination of the sections by Alzheimer and Perusini (Perusini 1909). This finding finally ruled out the possibility that Auguste D. had suffered from vascular dementia.

Introduction of the term "Alzheimer's disease"

Between 1908 and 1910, Kraepelin worked on the eighth edition of his renowned textbook on psychiatry (Kraepelin 1910). In the chapter on "Das senile und präsenile Irresein" (Senile and Presenile Dementias), Kraepelin first introduced the terms, "Alzheimers Krankheit" and "Alzheimersche Krankheit" (both translate as Alzheimer's disease). Kraepelin was one of the most influential psychiatrists of the early 20th century and his textbook was standard reading for psychiatry students as well as for his colleagues worldwide. It was thus that the world first encountered the eponym Alzheimer's disease. He wrote, "a peculiar group of cases with severe cellular changes has been described by Alzheimer" and went on to expertly describe the clinical symptoms and histological abnormalities of this new disease. "According to Alzheimer, the necropsy shows changes that represent the most severe form of senile dementia. The plaques were extraordinarily numerous and nearly a third of the cortical cells appeared to have died. In their places were strangely tangled, strongly staining fibrillary bundles, apparently the last remains of the perished cell bodies" (Kraepelin 1910). Kraepelin also included three illustrations of these fibrillary tangles characteristic of Alzheimer's disease.

He concluded his discussion of Alzheimer's disease with a speculation on its integration into the spectrum of dementias known at the time. "The clinical interpretation of this Alzheimer's disease is currently unclear. While the anatomical findings suggest that we are dealing with a particularly severe form of senile dementia, the aspect that the disease occasionally already begins in the [patient's] late 40s seems to somewhat contradict this. One would have to presume a "Senium praecox" [premature ageing], if it is maybe not indeed a peculiar disease process, which is more or less independent of age …" (Kraepelin 1910). With these speculations, Kraepelin seemed to anticipate that, next to advanced age, there can be other factors causing an early onset of Alzheimer's disease (genetic factors, for example, as we know today). Kraepelin also included the case of another of Alzheimer's patients who displayed clinical symptoms similar to those of Auguste D., Johann F. Johann F. had been admitted to the Munich hospital in November 1907 at the age of 56. At the time when Kraepelin was finishing his book, he was still alive and thus Kraepelin could not include information on the histopathology of his brain.

Alzheimer himself published the first comprehensive account on Auguste D.'s cases only in 1911 (Alzheimer 1911).[5] In this paper, he also included a detailed description

[5] An English translation of Alzheimer's paper printed in Zeitschrift für die Gesamte Neurologie und Psychiatrie in 1911 has been published (Alzheimer et al. 1991).

of the clinical history and histopathology of Johann F., including numerous figures illustrating the different histological changes observed by Alzheimer in sections of the brains of Auguste D. and Johann F.

Even though Alzheimer, Kraepelin and their colleagues in Munich meticulously described the clinical symptoms and histopathological manifestations of the disease, their ability to alleviate them was very limited. They carefully supervised and cared for the often frail and decrepit patients, ensured adequate nutrition, tried to alleviate their fears by administering small doses of opium and their insomnia by means of bathing the patients or the occasional application of barbiturate drugs, such as paraldehyde or Veronal.

Re-analysis of the first described cases of Alzheimer's disease

The diagnosis of Auguste D. has since been confirmed by a re-examination of Alzheimer's original histological slides (Graeber et al. 1998; Graeber and Mehraein 1999). These analyses verified the loss of neurons in various areas of the cortex, the accompanying gliosis, as well as the presence of large numbers of typical neurofibrillary tangles and amyloid plaques in her cerebral cortex, precisely as had been described and depicted by Alzheimer. Moreover, the studies proved the absence of any significant changes to the brain's vessels or any changes suggestive of a metachromatic leukodystrophy (both pathologies had variously been suggested as the real causes of Auguste D.'s affliction (see Graeber et al. 1998 and references therein). Combined with Auguste D.'s clinical symptoms (Alzheimer 1907a; Maurer et al. 1997), these data confirm this first case of Alzheimer's disease as being a typical example of the disease now bearing Alzheimer's name.

Isolation of DNA has recently also been accomplished from Alzheimer's original histological sections of Auguste D.'s brain. Genotyping of this DNA revealed that Auguste D. was homozygous for apolipoprotein E (APOE) allele epsilon3 (ε3/ε3) (Graeber et al. 1998) – an allele not associated with an increased risk for developing Alzheimer's disease. Due to the limited availability of tissue for DNA extraction, the mutational analysis of other loci associated with the development of Alzheimer's disease, such as those for amyloid precursor protein (APP), presenilin-1 and presenilin-2, has been deferred until the emergence of novel, more sensitive techniques for the detection of mutations.

Similarly, a recent re-analysis of Alzheimer's original sections of Johann F.'s brain confirmed Alzheimer's findings on this patient. His brain shows classical amyloid plaques (with cores) in the cerebral cortex; the pyramidal neurons and neurites within the plaques, however, display no signs of neurofibrillary changes (Graeber et al. 1997; Moller and Graeber 1998). With today's knowledge, Johann F. would be classified as having suffered from a less common form of Alzheimer's disease, which is referred to as "plaque-only" Alzheimer's disease.

A recent analysis of Johann F.'s family history shows a strong familial predisposition to developing presenile dementia (Klunemann et al. 2002). Other members of his family afflicted by dementia include Johann F.'s mother, his maternal grandfather, great-aunt and great-grandfather as well as three of his eight brothers and sisters. In addition, five offspring of two of his affected siblings are known to have developed dementia.

The inheritance of the disorder appears to follow an autosomal dominant pattern with variable penetrance and an age of onset ranging from the thirties to the late sixties. Given the "plaque only" phenotype of Johann F.'s brain, it would be interesting to identify the genetic alterations underlying his pathology. Similar to Auguste D.'s case, it has recently been possible to isolate DNA from original histological sections of Johann F.'s brain and spinal cord. Genotyping of this DNA revealed that Johann F., like Auguste D., was homozygous for APOE allele epsilon3 and no mutations could be detected at codons 692, 693, 713 and 717 of the APP gene (Graeber et al. 1997). As with the DNA isolated from Auguste D.'s tissue, the screening of Johann F.'s DNA for mutations in other genes associated with Alzheimer's disease has been postponed.

Intriguingly, with two patients published under his name, Alzheimer already provided an early illustration of the range of pathological manifestations of Alzheimer's disease known today. In his second article on the disease, Alzheimer accepted this fact. And he even made the first steps towards including not only early-onset (presenile) cases of Alzheimer's disease in the disease spectrum but also cases of senile dementia showing very similar histological changes that had been observed by Alzheimer himself and others (see, e.g., Fischer 1907, for a discussion; see (Alzheimer 1911) and references therein).

Alzheimer's final years

Alzheimer was a passionate scientist. He worked very long hours and rarely took time off for a holiday. In Munich, he worked without a salary for years and even paid a large part of the cost incurred by his research from his private funds. In 1909, his commitment was honored and he was appointed as extraordinarius (assistant professor) at the University of Munich. His scientific merits are reflected by his appointment as editor of a newly established psychiatric journal in 1910, at a time when the eponym Alzheimer's disease was becoming increasingly recognized by psychiatrists worldwide. Over time, however, his hard work began to exhaust him.

Nonetheless, when in 1912 he received an offer to become a full professor and director of the Psychiatric and Neurological Clinic at the Silesian Friedrich-Wilhelm-University in Breslau, he rapidly accepted. The clinic was a prestigious institution at the time. Alzheimer succeeded scientists such as Heinrich Neumann, Carl Wernicke and most recently Karl Bonhoeffer, who had been appointed to the Charité Hospital in Berlin. However, during his move to Breslau, Alzheimer fell seriously ill from an infection, from which he never fully recovered. Yet Alzheimer tried to fulfil his duties as best he could. In addition to running the clinic, he devoted a lot of his time to teaching. He continued to publish articles on his research and, in October 1913, even hosted the annual meeting of the society of German psychiatrists in Breslau.

After the outbreak of World War I, the psychiatric institutions in Europe faced a wave of new admissions. The increased workload was a heavy burden on the weakened Alzheimer. He tried to tackle the problems as well as was possible under the circumstances, even publishing an article on the effects of war on the psyche, but the exertions of the last years began to take their toll. Alzheimer progressively weakened and, in October 1915, he became bedridden. On the December 19, 1915, Alzheimer

died at the age of only 51. The day before Christmas Eve 1915, he was interred in the principal cemetery in Frankfurt am Main.

In his obituary, Alzheimer's close friend and collaborator Nissl stressed that Alzheimer was more than an outstanding scientist who contributed greatly to our knowledge of the histopathology of the brain. He was "first and foremost a psychiatrist who strove to advance psychiatry by using a microscope" (Nissl 1916). Besides being an astute scientist, always bound by the highest standards, Alzheimer was also a very considerate physician. Contrary to the growing movement that regarded disabled people as inferior, Alzheimer treated his patients with great compassion. His treatise on the indications for an abortion testifies to this considerate and humane approach (Alzheimer 1907b). In it, Alzheimer argues that most cases of psychiatric disorder in the mother do not warrant an abortion. He makes the point that the understanding of the bases of mental disability at the time would not allow for any judgment to be made on the worth of a human life and hence on whether to terminate a pregnancy on the basis of the mother being afflicted by a mental disorder. Today, Alzheimer's name is associated with one of the cruellest diseases and the mere mention of his name conjures up associations of inexorable mental decline. However, it was his genuine interest in the troubles of his patients and his realization of the pathological basis of the disease named after him that paved the way to a better understanding of the pathological process that might ultimately lead to ways of treating or preventing it.

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Yves Christen

Alois Alzheimer and the myth of the pioneer

Yves Christen[1]

From a historical point of view, what can be referred to as the myth of the pioneer finds expression in a two-way contradictory process. On the one hand, there is a tendency, from a hagiographical viewpoint, to give the creator full credit for the discovery. Meanwhile, others, taking an anti-establishment standpoint, endeavour to demonstrate how small the creator's contribution was, since it is in fact always possible to find forerunners of the greatest of pioneers. Alois Alzheimer is no exception. What, therefore, is his actual contribution?

Was there a discovery?

Alzheimer, of course, is not the father of the concept of dementia, or even of dementia in the elderly. Some have been aware of this concept for at least 2,500 years (Hoyer 1987; Berchtold and Cotman 1998), and if it were to be accredited to somebody, that person would be the French psychiatrist Jean-Étienne-Dominique Esquirol (1772–1840), who worked with Philippe Pinel – the man who on September 1793 freed the mentally disabled of the Bicêtre in Paris by removing their chains – and coined the term senile dementia (Esquirol 1830). Finally, the idea that neuropsychiatric disorders result from negative changes in the brain can, of course, be traced back to Hippocrates who, unlike Aristotle, believed that the brain, and not the heart, was the seat of the mind (but omitted to include senile dementia, or even dementia, in his list of mental disorders). More recent points of reference include Wilhelm Griesinger (1817–1868) and Theodor Meynert (1833–1892), who were convinced that neuropsychiatric disorders were caused by the diseased brain.

Officially, Alois Alzheimer's "discovery" dates back to the meeting of Southwest German psychiatrists that took place on November 3 and 4, 1906, in Tübingen. Here, under the title "Uber eine eigenartige Erkrantung der Hirnrinde," he presented the case of a patient, who later became known as Auguste D., whose "peculiar disease of the cerebral cortex" (Maurer et al. 1997; Maurer and Maurer 1998) allowed Alzheimer to describe neurofibrillary degeneration (Alzheimer 1907a). In 1911, Alzheimer reported the case of another patient, aged 56, in whom he detected the other characteristic of "his" illness: senile plaques (Alzheimer 1911).

What is Alzheimer's contribution? It is well known that senile plaques were originally described in epileptics in 1892 by P. Blocq and G. Marinesco, were identified in senile atrophy by E. Redlich in 1898 as "miliary sclerosis" (Miliare Herdchen; Redlich

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1898), were examined by André Leri in 1906 (Petit and Leys 1988), were considered as a marker for senile dementia by Oskar Fischer in his important 1907 article (Fischer 1907), and were christened "senile plaques" in 1911 by T. Simchowicz (1911). Therefore, Alzheimer's contribution is generally considered to be limited to describing neurofibrillary degeneration. However, this is not accurate either, as Berrios very ably explained (1990). Others had spoken earlier about the existence of neurofibrillary degeneration, notably Fragnito (1904), Bianchi (1906) and above all Salomon Fuller (1907) who, six months before the Tübingen meeting, had talked of "neurofibrillar bundles in senile dementia." Still others, including Alzheimer's Italian colleagues Perusini (1909) and Bonfiglio (1908), can also be looked on as pioneers.

The concept of Alzheimer's disease was new because it was distinguished from forms of senile dementia. However, the term presenile dementia was introduced by Binswanger in 1898 and most notably used by Kraepelin in 1899. Indeed, the credit for this way of seeing things – if there is credit to be attributed – is not actually due to Alzheimer, but to Emil Kraepelin. Kraepelin endorsed Alois Alzheimer's achievement by proposing, on page 627 of the 8th edition of his famous book, *Psychiatrie*, that the form of presenile dementia described in 1906 be named Alzheimer's disease (Kraepelin 1910). The concept of presenile dementia fitted with his own viewpoint, since it is cited in his *Compendium der Psychiatrie* from 1899. The initiative taken by Kraepelin, however, did not have unanimous support, as Berrios underlines (1990). Fuller (1912), Hakkebousch and Geier (1912) in Russia, and Lambert (1916) and Lugaro (1916) rejected the radicalness of this distinction, and it does not appear that Alzheimer himself considered it essential (Amaducci et al. 1986; Berrios 1990).

It is reasonable to question Kraepelin's motives. In them, logically, can be seen a real conviction, since Kraepelin believed in the distinction between senile dementia and presenile dementia (Beach 1987). Similarly, the emphasis on this discovery was in line with Kraepelin's organicist view and, therefore, counter to that of Freud and his supporters. From this viewpoint, both Alzheimer and Kraepelin deserve to be considered as neuroscientists (Devi and Quitschke 1999), in opposition to the interpretations of non-biological psychiatry. Mention can also be made of the academic competition with Pick's school, with Kraepelin in a way using Alzheimer's discovery by attributing his name to a disease that could just as well have been named "Fischer's disease" (Fischer worked with Pick). It could also be conjectured, in the spirit of the competition in modern science, that it was justification for the spending of the Munich laboratory (Thomas and Isaac 1987).

Was there a rediscovery?

In terms of the history of science, Alzheimer's case presents an unusual problem, as it is a discovery in the process of expansion. Admittedly, there are large numbers of concepts or theories that are at first undervalued and whose full significance only becomes evident rather belatedly. But, Alzheimer's disease is not a theory or an idea. It is a disease that, since Alois Alzheimer's report in 1906, has never stopped affecting humans and which, of course, affected them before this time. How could it be possible, then, for this disease to have been considered of great importance only for the past 30 years? Or, to ask the same question but to personalize it more, how could it be possible

for Alzheimer to emerge as a central figure in medicine only relatively recently? To an observer, it would seem that this disease was rediscovered in the 1960s, thanks to Roth, Terry, Kidd, Blessed and a few others.

But was it really rediscovered? The history of science often teaches us that the thinkers who we seem to have lost sight of have not, in fact, been forgotten. The most famous example has to be Gregor Mendel, the father of genetics and author of a first printed edition published in 1866 and rediscovered in 1900 by Hugo de Vries, Carl Correns and Erich Tschermak (which equates to an eclipse of around half a century, comparable to the time that separates the death of Alzheimer from the first modern papers by Roth, Terry, Blessed, etc.). Unlike Mendel, while he was alive, Alzheimer received full recognition as a renowned academic, and his co-workers were among the most distinguished of his time (while Mendel, in contrast, was isolated in his monastery).

Acknowledged in his lifetime, Alzheimer and "his" disease were not forgotten with the death of the pioneer. Proof of this lies in the work of E. Grunthal (1926) on the relationship between neurofibrillary degeneration and senile plaques, P. Divry's studies (1927) on the amyloidal nature of senile plaques, the observations of J. Lhermite and Nicolas (1923) in France, and the theses by J. Cuel (1924) and H.M. Dubruille (1924).

Contrary to what we may have been led to believe, Alzheimer has never been rediscovered, for the simple reason that he has never been forgotten. What is new, however, is the change in the status of Alzheimer's disease from what was originally considered a relatively rare form of presenile dementia to what has now been established to be an extremely common illness. It is this conceptual shift that is the source of the development of modern "Alzheimerology," which began in the 1960s. The work published by J.A.N. Corsellis and P.H. Evans in 1965, showing that, as a rule, arteriosclerosis is no more prevalent in elderly subjects with dementia than in healthy subjects, seems to have played an essential role (Corsellis and Evans 1965). A short time later, B.F. Tomlinson, G. Blessed and M. Roth confirmed that the dementia from which the majority of elderly subjects suffered was indeed that described by Alzheimer (Tomlison et al. 1968, 1970). The medical literature therefore brought together Alzheimer's disease and senile dementia under the name Senile Dementia of the Alzheimer's Type (SDAT), before abandoning this label in favor of Alzheimer's disease, which was no longer seen as a form of presenile dementia but as the major cause of dementia in the elderly population (Katzman 1976). This condition gradually established itself as a blight on society that was "sanctioned" by the media.

Both a posthumous achievement and a retraction

Since the work of Thomas Kuhn, the history of science cannot be approached without an attempt to tease out the paradigm shifts that mark out the major stages in the development of a scientific discipline. How can the evolution of Alzheimerology be analyzed from this standpoint? It seems clear that a major change took place around the middle of this century. Those who contributed to the development of medicine at the turn of the 19th century were, in some respects, inclined towards taxonomy. They sought out the rare in the same way that zoologists went in search of mysterious and as yet undiscovered animals. This resulted in the impressive list of diseases and syndromes

that we know today: Pick's disease, Alzheimer's disease, Creutzfeldt–Jakob disease, etc. (Beighton and Beighton 1986). From this point of view, it is not without reason that Emil Kraepelin was described as "the Linnaeus of psychiatry;" his disposition towards classification made his work the basis for the future, successive editions of the DSM.

Towards the 1950s, this way of looking at things completely changed in connection with at least two separate events. The first was the increasing interest in theories and general ideas. The development of molecular biology was in line with this trend, leading to the formation of notions as general as the concept of the genetic code. Further evidence of this process of generalization was the fact that the social significance of issues was increasingly taken into account in the fields of science and medicine. From this perspective, a rare form of Alzheimer's disease was of less interest than a common form of Alzheimer's disease. This approach, probably rarely practised in Alzheimer's time, goes hand in hand with the obligations governing the search for sources of funding. It is now common practice for research reports, budget applications and scientific papers to have an introduction intended to underline the quantitative importance of the disease under study, with the aim of showing that public funds will be spent on a cause of social importance.

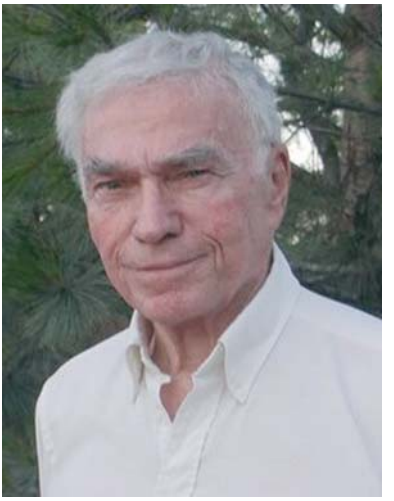
How did this change in how we look at things come about? How can context influence how science views its subject? A Darwinian evolutionist model could be applied to the history of science, where the setting – i.e., intellectual trends, or dominant ideologies – acts as a selective environment contributing to the selection of certain ideas over others. I think that this schema applies to the issue of the status of Alzheimer's disease. Indeed, it is indisputable that some authors had, well before the 1960s, considered the possibility that there was a link between presenile dementia and senile dementia. In his thesis, for example, Dubruille (1924) wrote: "We note that a person of a doctrinal mind would be sure to do away with the concept of senile dementia and break it up into arteriosclerotic dementia and argyrophilic grain disease. Senile dementia would be classed as a special case of Alzheimer's disease." Other authors, such as E. Grunthal (1927), D. Rotschild (Rotschild and Kasanin 1936), W.H. McMenemey (1940) and later R.D. Newton (1948) and M.A. Neumann and R. Cohn (1953), published studies consistent with the idea that there is a natural similarity between senile dementia and presenile dementia. It would, therefore, be totally inaccurate to say that this hypothesis was not considered at all before the 1960s; rather, the selective environment in which it was put forward did not allow it to be retained. Conversely, it would be wrong to view the homogenization of Alzheimer's disease as absolutely and incontestably obvious in the light of modern research. If only at the clinical level, a certain form of heterogeneity is by no means excluded (and for sure, there is a genetic heterogeneity). This also confirms that the socio-scientific context of the time plays the role of a selective environment contributing to the highlighting of one schema rather than another. And this context – a preference for general theories and a requirement to be concerned with socially significant situations – is currently working in the direction of homogenization.

Posthumously, Alzheimer has benefited from this move towards the "socially significant" aspect of a common disease. However, this situation represents a paradox that is significant in terms of the history of science, as this achievement comes at the expense of the very idea that prevailed when "Alzheimer's disease" was given its name, because if the condition described at Tübingen was simply a variant of all the forms of senile dementia, there is nothing to justify the label chosen by Kraepelin. The paradox

here is that the very reasons for the success of Alzheimer in modern times could be used as arguments by those who would prefer to see this pathology called "Fischer's disease," or simply senile dementia. There are therefore elements of retraction in the current success enjoyed by Alzheimer (Christen 1997).

That said, the fact remains that the study of the early forms of senile dementia described by Alzheimer paved the way for current research. It led to the identification of the first genes linked to this disease. It has also been possible to identify some of these mutations in the case described in 1911 (Graeber et al. 1997). From Alzheimer to John Hardy, with Jean-François Foncin in between, there is a real logic to the road travelled. That is what matters the most, since it is a well-known fact that it is less important to know what we owe to individual pioneers than to embark upon fruitful research.

The beginning of modern research



Robert D. Terry

Alzheimer's Disease at mid-Century (1927–1977) and a little more

Robert D. Terry, M.D.[1]

In 1959, when Saul Korey and I began to think about planning a study of Alzheimer's disease (AD), the situation seemed quite simple and straightforward. It was widely accepted that AD was exclusively a rare pre-senile disorder, that it was entirely different from senile dementia, that it had a simple dominant genetic inheritance, and that there had been little or no work related to the problem for several decades. All of these ideas soon showed themselves to be at least partially wrong. Nevertheless, we did have a few advantages: Saul was the head of the Department of Neurology at the Albert Einstein College of Medicine and was also a well-trained (for 1959) neurochemist, and I, a neuropathologist, was asssigned a new Siemens electron microscope, much different from my previous RCA. The combination of chemist plus electron microscopist was a promising, but untried collaboration. A world-class neurosurgeon, Leo Davidoff, had agreed to do the brain biopsies. It now seems remarkable how primitive the relevant clinical science was less than 50 years ago – no imaging, very little psychology.

Of course, it is quite typical for one entering a new field to believe that its understanding began pretty close to that day. But, perhaps somewhat reluctantly, we did look at the recognizably beautiful silver impregnations prepared by Alzheimer himself and by his Italian associates, Bonfiglio and Perusini (Bick et al. 1987). The rivalry between Kraepelin, for whom Alzheimer was working in Frankfort, and Freud in Vienna was surely important to Alzheimer. Kraepelin expected to find organic causes of psychiatric problems, while Freud insisted on the opposite. So Kraepelin was delighted with Alzheimer's finding and promoted it, while Freud probably ignored it (Torack 1978).

Apparently only a little was added to the literature after Alzheimer's death in 1915, but there were a couple of major new points The core of the plaque had been called "amorphous material" until Divry applied the Congo Red stain and determined that the core was amyloid (Divry 1927). But the tangles also stained with Congo Red, so they were also thought to be amyloid until 1959, when Margolis demonstrated significant staining differences (Margolis 1959).

Pre-senile AD was apparently accepted as an entity, at best very rare, but most observers, such as Rothschild (Rothschild and Kasanin 1936) and Alvarez (1948), held that senile dementia was entirely different. The former had seen plaques in specimens from cognitively normal patients, so he would not accept those lesions as significant, while Alvarez, a gastro-enterologist, insisted that senile dementia was the result of ischemic brain disease related to multiple occlusions of small cortical vessels. The

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pathologist Newton got it right: senile dementia did indeed usually have to do with plaques and tangles and was very relevant to AD (Newton 1948).

At the time we began our study of AD, it was exclusively a problem for neuropathologists, and we seemed to be the only Americans working in the field. Therefore, many fine co-workers joined us; Nick Gonatas, Kinuko Suzuki, Mike Shelanski, Henry Wisniewski, Dino Ghetti, Khalid Iqbal, Cedric Raine, Dick Horoupian, Jim Goldman, Dennis Dickson, and others. Several have continued to work in related areas.

In 1961, our EM readily recognized tangles with no difficulty, but we found no histologic precedent for the twisted appearance of the intracellular fibers. Some cross sections looked circular, so they were interpreted as twisted tubules (Terry 1963). Working at about the same time in McMenemy's neuropathology lab at Maida Vale in London, Michael Kidd more correctly recognized them to be paired helical filaments – PHF (Kidd 1963). It also seemed to us that microtubules were abnormally sparse in the neuronal cytoplasm, and that suggested a problem with axoplasmic transport (Suzuki and Terry 1967).

Plaques were much more complex in these electron images, but they were quite entirely deciphered within about a year: amyloid fibrillar core, surrounded by unmyelinated dystrophic axons and dendrites containing filaments, dense bodies (lysosomes; Suzuki and Terry 1967) and PHF (Terry et al. 1964). Fibrous astrocytes lay on the periphery, whereas microglia infiltrated the lesion in close contact with amyloid. Sometimes an abnormal synaptic complex was included (Gonatas et al. 1967). The quantitative studies of plaques published by Blessed, Tomlinson and Roth had major influences on subsequent studies, since the numbers seemed to correlate with cognition, implying a major role for amyloid (Blessed et al. 1968). Our own cases never provided the statistical strength shown by their work because we were concerned only with AD cases whereas the British had considered many instances of plaques without dementia and dementia without plaques as well as AD specimens. These findings gave rise to the beginning of my own skepticism regarding the role of amyloid as it is classically defined: fibrillar, extra-cellular and congophilic. The Aβ oligomers so prominent in our current pathogenic thinking do not fulfill that admittedly narrow classical definition of amyloid.

Tangles also came into some reconsideration, since we found numbers of typical AD cases without neocortical tangles among patients over 70 years of age (Terry et al. 1987b). On re-examination in 1997, Alzheimer's second case (1911) was found to be of this type (Graeber et al. 1997). One might ask whether there might be a factor in the aged brain that blocks the formation of PHF or whether these specimens have less phosphorylated Tau.

Neurons were counted in the AD neocortex by image analysis (with results not significantly different from those more recently done by stereology) and were found to correlate with various cognitive tests (Terry et al. 1981). But the statistical strength is not as great as that provided by measures of synaptic population density (Terry et al. 1991).This latter finding was particularly satisfying in that the correlation is very strong and the physiologic rationale is so convincing. Our techniques utilized antisynaptophysin, and thus recognized pre-synaptic terminals. M. and A. Scheibel saw loss of dendritic spines – the pre-synaptic side – with their Golgi impregnations of AD specimens (Scheibel 1976).

There can be no doubt about a relationship between AD and the process of normal aging, but very little is said of it. The Sjogren and Sourander genetic study included familial instances on both sides of the 65 year line (Sjogren an Sourander 1962). Reports prior to the 1960s all made much of a normal loss of cortical neurons (Brody 1955). Our own cell counts by image analysis of normally aged specimens were startlingly different in that they did not demonstrate normal loss of neurons but rather a significant shrinkage of the large pyramids with maintenance of the total (Terry et al. 1987a). One might well predict synaptic loss in particular areas, and that cognitive change might well accompany it. Neocortical pre-synaptic loss has been demonstrated in normal aging, but the correlation with cognition is still lacking (Masliah et al. 1993).

During these early years, there had been really very little interest on the part of clinical neurologists. That changed quite smartly in 1976 with the publication of a letter from Robert Katzman to the editor of the Archives of Neurology, in which the frequency and mortality of AD were pointed out (Katzman 1976). Epidemiologists, clinical neurologists, radiologists, psychiatrists, and psychologists quickly became active developing better diagnostic methods.

At about the same time, there was growing interest in the cholinergic transmitter system as related to AD. Drachman found that scopolamine, an anti-cholinergic drug, created in normal patients a picture of memory loss and confusion similar to that seen in AD patients (Drachman and Leavitt 1974). Davies and Maloney, in Scotland, reported a significant deficiency of choline acetyl transferase (ChAT), the enzyme that catalyzes the formation of acetylcholine, in AD cortex (Davies and Moloney 1976). Two other British groups showed similar results within months (Perry et al. 1977; Bowen et al. 1976). Now the field became interesting to neuropharmacologists and neurochemists: geneticists, for the most part, came along a little later

Biomedical research is expensive, and beginning in the 1960s, our major support came from the National Institutes of Health. Private support began with the formation of the Alzheimer's Association and its first president, Jerome Stone. He had brought his wife, who had AD, to my office in the 1960s, and we talked about the need for an educational and funding organization. He said that if we started one, he would then help. About 15 years later he did indeed join in, and he played a major role as a skilled executive and a powerful fundraiser.

The years have passed at astonishing speed, and research on AD has become a worldwide industry. I've (reluctantly) retired from lab work. But I'm still watching!

Zaven S. Khachaturian

A chapter in the development on Alzheimer's disease research

A Case Study of Public Policies on the Development & Funding of Research Program

Zaven S. Khachaturian[1]

Summary. Although Alzheimer's disease, as a clinical-neuropathologic entity, was described one hundred years ago by Alois Alzheimer, the preponderance of knowledge on the disease was accumulated since the 1970s. The dramatic research advances during the last three decades propelled the disease from near obscurity to the forefront of modern biomedical science. The remarkable transformation of this field of study is reflected by the exponential increase in the numbers investigators, publications and funded projects. The current preeminence of dementia research is largely due to the increasing numbers and quality of significant breakthroughs in understanding the molecular neurobiology of the disease. Multiple promising leads now have created an atmosphere of optimism about the prospects of discovering effective interventions to delay the progression of the disease. Some of the key factors that influenced the pace of progress and helped to change the 'status' of dementia research were: a) increases in research funding, b) recruitment of new scientific talent; convergence of know-how and technologies, c) several crucial discoveries in molecular neurobiology and d) National Institute on Aging (NIA) initiatives to promote interdisciplinary research programs by creating nationwide network of collaborating investigators and the establishment research infrastructure/resource. This is an account how the evolution of Alzheimer research occurred; a story about critical findings, people and public policies that influenced the amazing progress in understanding the underlying molecular mechanisms of the disease and setting the stage for the discovery of a cure.

Preface

Although the phenomena of aging, cognitive impairments and dementia in various forms have always been part of the human experience, the precise beginning of systematic attempts to characterize the behavioral sequelae of brain aging [a.k.a. 'senility'] is not known. The prolonged history of scientific efforts to characterize better the clinical features of dementia perhaps can be described in the context of six arbitrarily defined epochs. Among these the first is the era of 'Phenomenology' or 'Descriptive Knowledge', which does not have a clear beginning but roughly covers a period until 1906. The second epoch is the 'Dawn of Systematic Studies' [1906–1960]. The third epoch, arguably represents the dawn of 'Modern Era of Neurobiology of Dementia' [1960–1980]. The fourth epoch is the era of 'Building Research Capabilities'; during this period interdisciplinary teams were formed, research resources/infrastructures were established and advance were made in – diagnosis, genetics, molecular biology, neurochemistry, & clinical trials [1980–1990]. The fifth epoch is the 'Emerging Treatments & Hope' [1990–2000]; this period is characterized by breakthroughs in: genetics,

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molecular neurobiology & disease modifying drugs. The sixth epoch is the period of 'Struggle Toward Primary Prevention' [2000–2010?].

There are several excellent published reviews that have described the earlier history of dementia (Berchtold and Cotman 1998; Beach TG. 1987; Bick KL et al. 1987). This paper will not dwell on the early part of the story other than to note that the pre-World War II (WWII) history of dementia is marked by relatively sluggish progress in understanding the disease. Some of the factors that account for the comparatively slow pace of advances were: a) the low incidence rates of the disease b) scarcity of fundamental knowledge and scientific tools and, c) the lack of recognition as a medical problem. Shortly after WWII the first crescendo of studies began to appear in the UK (Europe). In the US also interest in dementia research gradually started, in the 1960s, to gain momentum. The critical events that propelled dementia to the forefront of biomedical research during the last three decades were: a) increase in the average life span; impact of demographic shifts in the age distributions of people in industrialized countries, b) increasing prevalence of dementia with age, c) public heath imperatives; costs of care, d) technological/scientific advances in many unrelated areas and, e) emergence of National Institutes of Health (NIH) as major patron of research in academia.

The primary focus of this essay is on the events that led to the development of a national initiative on Alzheimer disease in the Untied States (US), starting in 1978. The story of "*how* and *why*" the extramural research programs on ***Neurobiology of Aging*** and ***Dementia*** were developed by the National Institute on Aging (NIA), at the National Institutes of Health (NIH), is a unique case study in formulating-implementing public policies to address a major public health problem. This chronicle reviews not only the evolution of knowledge but also the role of people, policies and organizations that influenced progress in building the foundation(s) for some of the current promising leads for therapies.

This review of dementia research is admittedly from a narrow vantage point of NIA/NIH; it covers only a small part of a larger story with many more actors.

The 'state of knowledge' of a field, at any moment in history, rarely if ever reflects the singular contribution of a country; this is especially true for dementia research. Present knowledge about the disease grew as a river with the accumulation of observations, insights and findings from many widely dispersed origins. The breathtaking advances in understanding the neurobiology of dementia since the pioneering studies of the 'Founders' resulted from the convergence of ideas, people, resources, programs and public policies.

Early History – Pre WWII Era

The current approach of combining clinical and biological studies of dementia started to take shape at the beginning of the 20th century in parallel with advances in histology (chemistry of tissue stains), light microscopy and neuroanatomy (Beach 1987; Bick et al. 1987). The early pioneers of the field were able to make some groundbreaking observations about dementia because of access to a wealth of new technologies for studying the brain and the emergence of vibrant academic environments that fostered interactions and cross-fertilization among psychiatrist, neurologist and neuropathologist. Reports by Blocq and Marinesco [1892] and later by Redlich [1898] began to

describe the relationships between neocortical senile plaques and senile dementia. Oskar Fisher [1907] was one of the first to suggest that severity of dementia and memory loss might be associated with senile plaques. Alois Alzheimer, a psychiatrist with an abiding interest to "help psychiatry through the microscope" was among the first to exploit the newly emerging tools for histological study of the human brain. Alzheimer was able to make his contribution to the understanding of dementia because he had access to a wealth of new technologies for tissue staining and close interactions with the vibrant neuropathology community of Munich (Beach 1987; Braak and Braak 2000; Maurer et al. 2000). The 1907 paper by Alois Alzheimer became the index case for "Alzheimer's Disease", however the term did not receive broad endorsement until the eighth edition of Emil Kraepelin's "Textbook of Psychiatry" published in 1910. The study by Alzheimer was one of the crucial milestones in the annals of dementia research; a prototype for correlating meticulous clinical observations with the systematic neuropathological analysis of brain lesions. Alzheimer's approach of relating clinical observation with brain lesions set a precedent for subsequent interdisciplinary efforts (Maurer et al. 2000)

Nearly a century later, Alzheimer's original report remains a crucial milestone in the annals of dementia research. The study approach, by Alzheimer, of combining meticulous clinical observations with systematic neuropathological analysis of brain lesions become the template for NIA's strategic plan in 1978 to start building a national program of research. The unique center piece of NIA's program development efforts was to promote the *integration of basic and clinical studies* and *interdisciplinary research* on the causal relationships between the clinical-pathological phenotypes of dementia.

Mileposts in Characterizing Behavioral – Clinical Phenotypes

In the era between Alzheimer's initial report in 1907 and 1960s, the primary focus of scholarship on dementia was the *epistemology* of the disease and the struggle for consensus on the *clinical definitions* (Dillmann 2000) Progress in understanding the relationships between the behavioral expression and pathological phenotypes of dementia was relatively slow during this period due to two impediments: first, the lack of objective clinical assessment tools, and second, uncertainty in the definition of the clinical phenomenon.

In the 1960s, arguably the start of the modern era of dementia research, clinical studies on dementia faced several challenges concerning the precise clinical characterization, definition and objective measures of the phenomena. Among these the most critical questions was '*whether Alzheimer changes were simply an accentuation of normal senescence*'. The landmark investigations by the Blessed, Tomlinson and Roth in the mid-1960s (Blessed et al. 1968) began to address the challenge of distinguishing brain changes due to pathology from those alterations due to healthy aging were started in a number of. The studies by this group correlated *quantitative measures of dementia* (cognitive and functional impairments) with estimates of the *number of the lesions* (plaques), and the *volume* of brain destroyed by infarcts. The efforts to quantify the relationships between the clinical and biological indices of the disease established the foundation for later longitudinal studies (Blessed et al. 1968; Katzman 1976; Katzman,

et al. 1978) However, the dispute could not be settled without comparisons of the clinical/biological/neuropathological phenotypes of the disease. Such comparisons became possible in the early 1960s with, the introduction of the electron microscope (EM) as a research tool, and the development of quantitative measures of dementia. [The answer to the long-standing problem of whether Alzheimer is an extension of aging was provided only recently by the findings of Jean-Jacques Haw and Charles Duyckaerts (Salpêtrière) *that not all centenarians get AD*].

In 1963, Terry (in the US) and Kidd (in the UK) independently reported the findings of EM studies showing the ultrastructure of a single neurofibrillary tangle to contain masses of microscopic fibers with periodic structure: paired helical filaments (PHF). These ground breaking studies enabled the field to: 1) develop quantitative assessments of the hallmark lesions, 2) clearly delineate the ultrastructure of the amyloid core (neuritic plaque), 3) develop methods of isolating plaques, neurofibrillary tangles and preparation of enriched PHFs, and 4) set the stage for the discovery of more sophisticated molecular and immunological probes to further characterize the abnormal proteins associated with the disease. Thus, these early ultrastructural studies by Terry, Kidd and colleagues opened the door for more detailed molecular characterization of the two fibrous proteins and set the stage for the remarkable advances of the last few years in understanding the molecular neurobiology of AD.

The second crucial hurdle that impeded progress in clinical studies in this period was the need for objective/quantitative tools to assess mental status for functional measures of severity. This problem was surmounted in 1968, with the publication of the Blessed, Tomlinson and Roth *Dementia Scale (Information-Memory-Concentration Test)*. The instrument was an informant-based scale of memory function, orientation, information, concentration, activities of daily living, etc. The landmark prospective studies of this group for the first time correlated *quantitative measures of dementia* (cognitive and functional impairments) with estimates of the *number of the lesions* (plaques), and the *volume* of brain destroyed by infarcts. Although subsequently the validity of correlations has been questioned by Terry and others by arguing that loss of synapse is the more valid index of severity. Nevertheless, these early efforts to quantify the relationships between the clinical and biological indices of the disease established the foundation for subsequent program initiatives and several collaborative multi-site longitudinal studies launched by NIA.

In the mid-1960 to mid-1980 period, four categories of objective clinical measurement tools were developed and validated, some in longitudinal studies with autopsy confirmations. These include: *Mental Status Exams* (e.g., Dementia Scale or ICM Test-1968, Mini-Mental Status Exam -1975 (Folstein et al. 1975), Short Blessed Test -1983), *Global Measures of Dementia Severity* (e.g., Clinical Dementia Rating – 1993, Global Deterioration Scale – 1982, CAMDEX – 1986), *Behavioral Scales* (Geriatric Depression Scale – 1988, Agitation Inventory – 1986, CERAD Behavioral Rating Scale for Dementia – 1995, Clinical Impression of Global Change or CIBIC) and *Cognitive Assessment Batteries* (e.g., Alzheimer Disease Assessment Scale or ADAS-cog) (Rosen et al. 1984). The efforts to construct quantitative measures of cognition and the validation of instruments for objective evaluation of symptoms were critical to the refinements in the characterization of the disease. These advances in assessment of the severity of the disease became the foundation for much of the current "routine clinical-workup" and set the "standard" for clinical staging methods an essential element of clinical research.

The third clinical controversy, in the 1950s–1970s, revolved around the issue whether '*presenile*' and '*senile*' dementias were the same disorder. The lack of consensus on a clear "clinical" definition of the disease was an important hurdle for progress in clinical studies. The uncertainty about the true identity of Alzheimer's diseases lingered until the 1976 editorial by Katzman (Katzman 1976). This landmark paper was an important step towards the recognition a common cause for late-onset and pre-senile dementia, an earlier thesis suggested by Newton [1948], and by Neuman and Cohn [1953]. This editorial for the first time framed Alzheimer's disease as a medical and public health issue. However, it did not speak to need for specific *diagnostic criteria* because in this period the DSM-III (DSM-IV) criteria for diagnosis of dementia seemed to be adequate most clinical work (Diagnostic and Statistical Manual of Mental Disorders. 1987). The needs for more rigorous clinical and neuropathological diagnostic criteria for research were addressed in the 1980s. The publication of the NINCDS-ADRDA diagnostic criteria in 1984 further specified three levels of confidence; *probable*, *possible* and *definite* with *definite* requiring histopathological confirmation (McKhann et al. 1984).

Once the challenges of developing diagnostic criteria and objective assessment instruments were overcome, the next major hurdle for clinical research was the effort to 1) *validate* the diagnostic criteria with histopathological confirmations; thus the need for neuopathological criteria, 2) *standardize* (reliability, sensitivity, specificity) various clinical assessment instruments and, 3) *construct* new measurements for changes in behaviors, symptoms or various domains of cognition. The availability of standardized, well-validated quantitative assessment instruments were indispensable prerequisites for NIA's subsequent initiatives (Khachaturian 1985; Mirra et al. 1991; Braak and Braak 1991; Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. 1997. Consensus Report of the Work Group, 1998)

In order to expand research on diagnosis and treatments, in 1978 NIA began to promote the construction and validation of assessment tools specifically designed for cognitive changes in several different domains; i.e., the Alzheimer's Disease Assessment Scale (ADAS) in 1984. These early efforts were significantly facilitated by the establishment of three related clinical programs that provided necessary research infrastructure: [a) Alzheimer's Disease Centers (1984 b) Consortium to Establish Registries for Alzheimer's disease (CERAD) (1987)and, c) Alzheimer's Disease Cooperative Study (ADCS) (1991). Thus 1984 was a watershed year:

- NINCDS-ADRDA criteria provided a systematic clinical diagnostic system supporting comparisons across centers;
- Alzheimer Disease Research Centers were established;
- Glenner and Wong identified amyloid;
- Standardized cognitive assessment instruments and global measured of dementia severity were introduced (e.g., ADAS, CDR, CIBIC) and enabled multi-site collaborative clinical studies.

During the following two decades the improvements in the accuracy of the clinical diagnosis were remarkable. The procedures for clinical assessment steadily advanced towards well-validated algorithms for identification of positive clinical phenotypes of the diseases. Twenty years ago what would have been considered "mild" dementia now more likely would be staged as "moderate" dementia. Early diagnosis has become

one of the most the important research and clinical accomplishments with profound implications for: establishing the prevalence of AD, initiating treatment when it may have optimal benefit, and understanding the pathobiology of the disease. For example, the original cholinergic hypothesis was based on neuropathologic material from end-stage AD patients. Now that AD is diagnosed earlier, some investigators [e.g., Ken Davis and Steve DeKosky] have suggested that simple cholinergic hypofunction may not be a feature of the initial stages.

The introduction of *mild cognitive impairment (MCI)* as a potential precursor or prodrome of the disease (Petersen 2000) was another significant accomplishment. Several groups (e.g., Barry Reisberg, Steve Ferris and the NYU group, Thomas Crook formerly at NIMH, Ron Petersen and the Mayo Clinic group, Marilyn Albert and the MGH group, and John Morris and the Washington University group) contributed to the efforts to improve the definitions and algorithms for distinguishing the early stages from non-demented aging and in characterizing border zone conditions.

This work sets the stage for the exploration of biomarkers. Advances in molecular neurobiology and emerging imaging technologies promise to provide early markers of the asymptomatic stages. The classification of degenerative dementias is moving rapidly, not just toward diagnostic and prognostic biomarkers, but toward antecedent biomarkers; a system of categorization based on combined behavioral and protein abnormalities (e.g., amyloidopathy, tauopathies, synucleinopathies and prion protein disorders) (Cummings et al. 2003). The potential value of an amyloid imaging compounds [Pittsburgh Compound] for early diagnosis was recently demonstrated by Bill Klunk (Pittsburg), Henry Engler (Stockholm) and collaborators from Uppsala and Boston with their success in imaging Aβ containing lesions in the living human brain with AD.

The prospects are promising that validated molecular and biochemical markers may soon complement clinical approaches in making early and valid diagnoses. However, prior to use as a routine clinical tool any potential biomarker must detect a fundamental biological feature of the disease and needs to be validated in neuropathologic confirmed cases. Presently none of the many [proposed] putative bio-markers have been validated in adequately powered investigations. Recent advances in neuroimaging technologies [e.g., Pittsburgh Compound with PET] offer the potential to detect and follow longitudinally the clinical course of the disease. In the future, it might be possible for neuroimaging technologies, perhaps MRI, to allow more direct monitoring of some biological phenotypes of the disease (e.g., brain metabolic changes, Aβ, Tau, synapse loss or cell death via PET and other structural changes). In contrast to neuropsychological measurements, imaging measurements, when validated, could allow following the more proximal brain changes associated with disease progression.

Epidemiological studies of prevalence, incidence, selective risk factors and the interactions of genetic and epigenetic factors were critical to understanding the full clinical aspects of the disease. Epidemiological studies have provided some of the most important hypotheses concerning etiology and novel avenues for potential therapeutic strategies. One of the earliest contributions of epidemiological research was Ernest Gruenberg's 1961 study showing the important relationship between age and prevalence of dementia; later confirmed by the East Boston study led by Dennis Evans indicating the exponential increase in the prevalence of dementia with age. Robert Katzman, a neurologist, shifted the focus of his research by becoming a neuro-epidemiologist

to study the relationship between incidence of dementia and education. The possibility of a positive relationship between education, challenging occupations and dementia were confirmed by Richard Mayeux/Barry Gurland. An array of putative risk factors has been reported [e.g., culture-ethnicity (Hugh Hendie); ApoE-head trauma (Richard Mayeux); gender-ethnicity-ApoE (Linsday Farrer); Rotterdam study on vascular factors-lifestyle-diabetes-dietary effects of antioxidants (Albert Hoffman, Monique Breteler); NSAID (Brietner); PAQUID study in Bordeaux reporting beneficial effect of wine (Jean-François Dartigues and Orgogozzo); description of an "Artic mutation", which leads to the production of lower level of Aβ in plasma but enhanced protofibrill formation (Lannfeld)]. These findings have provided provocative and possibly useful new avenues for therapy development; however, none of these putative risk factors have been confirmed through prospective clinical trials; some which currently are underway.

In summary, these early struggles to *define the disease* made significant contributions to current clinical knowledge by laying the foundation for more recent efforts to: a) refine the clinical description of the phenomenon/symptoms, b) establish clinical – pathological correlations, c) develop objective measures of behavior – psychometric assessment instrument, d) establish diagnostic criteria, e) standardize diagnostic procedures, f) refine clinical assessment algorithms, g) develop validated screening instruments and/or biological markers for early detection of mild cognitive changes and h) establish infrastructures for longitudinal clinical-pathological studies. Perhaps the most significant factor for the subsequent successes of the field was the integration and parallel developments in defining both the behavioral and biological phenotypes of the disease. The cross-talk and interdependent advances in basic and clinical studies was a unique and important part of the story.

Mileposts in Discovering Biological Phenotypes

In the post WWII era, the factors that account for the accelerated pace of progress in Alzheimer's research were advances in biochemistry, neuroscience and methods to study the: a) detailed structural and functional features of single neurons, b) mechanisms of intra- and intercellular communication, c) protein chemistry–synthesis, degradation aggregation, turnover and folding, d) genomics-proteomics, and e) mechanisms of regeneration and apoptosis.

In 1961, the first NIH grant on Alzheimer's disease was awarded to Robert Terry. The award represented the conceptual-scientific link between the earlier studies in Europe and a new beginning in the US. Robert Terry, a neuropathologist, along with his longtime collaborator Robert Katzman, a clinical neurologist, began to develop modern interdisciplinary research in neuroscience, which had begun earlier in the UK. By the 1970's under their leadership, the Albert Einstein College of Medicine became one of the most important centers for research. The overall impact of the Terry-Katzman team is enormous because of their multiple roles as investigators, teachers, and patient advocates. Their legendary contributions set the scientific compass for subsequent explorations in the field.

The current compendium of what is known about the molecular underpinnings of the disease can be traced to the early studies fostered by the Terry-Katzman team

or their scientific progeny. They created an environment for the convergence of several threads of investigation that included studies of the a) clinical definition of the disease/diagnosis (Katzman), and detailed ultrastructural characterization of brain lesions (Terry), b) protein chemistry/immunochemistry of brain lesions, c) neurochemistry of cell signaling (Davies), d) genetics/molecular biology neurodegeneration and e) clinical research and therapy development (Thal). Terry pioneered several lines of research. The most significant were in quantitative/experimental neuropathology and EM studies of amyloid fibrils in extracellular plaques and the helical structure of the intracellular neurofibrillary tangles (NFTs). The early EM studies by Kidd, Terry and his collaborators (e.g., Wisniewski, Gonatas, Gambetti, Shelanski, and Selkoe) set the stage for purifying and determining the detailed characterization of the biochemical/molecular structure of the NFTs. Between 1979 and 1982, one of the major disputes was between Iqbal and Selkoe and concerned the molecular structure of NFT. Research resulting from this controversy led to determining that isolated tangles are virtually insoluble in all common reagents; eventually these studied evolved into the current research on tau pathology. The mid-1980s mark the period vibrant growth of several parallel of research that proved to be significant for later advances and the recruitment of new blood into the field. These were:

a) Neurochemistry – receptor identification/characterization, mechanisms of synthesis and release of neurotransmitters
b) Protein chemistry cytoskeletal abnormalities – NFTs and amyloid plaque
c) Molecular biology/genetics of abnormal proteins- sequencing of the amyloid protein (by Glenner in 1984).

Cholinergic Hypothesis The discovery of a link between the clinical symptoms of the disease (memory loss) and specific cholinergic deficits in the brains of people with AD, by Peter Davies in 1976, was a landmark because it opened the door for modern neurochemistry (Davies and Maloney 1976). In the late 1970s the field desperately needed s *scientific hook* to elevate the quality of the science and shift the directions of studies away from descriptions toward the search for mechanisms. The concept of the "*Cholinergic Hypothesis*" provided investigators the first *promising lead*, which then attracted new investigators from neurochemistry. Although the concept of the cholinergic hypothesis was introduced by Elaine Perry, there was an army of scientists making significant contributions to the dominant scientific orthodoxy of the 1970's [e.g., David Drachman; David Bowen; Peter Davies; Elaine Perry, Joseph Coyle; Don Price, Leon Thal, Kenneth Davis, Ezio Giacobini, Larry Butcher, Ray Bartus; Richard Wurtman and M. Marsel Mesulam].

The discovery of lower levels of choline acetyltransferase (ChAT) in people with AD was followed with studies showing reduced levels of ChAT in the cortex of animals with basal forebrain lesions (the Johns Hopkins group, Coyle, Whitehouse, Price, Struble, Delong and colleagues in 1983). The combined neurochemical and anatomical studies [e.g., Mesulam studies] led to the development of an animal model for the cholinergic deficits. These early studies, which showed links between cognitive impairment, cholinergic deficits, loss of central cholinergic neurons of the basal forebrain and altered processing of the amyloid precursor protein (APP), eventually led to larger clinical studies that attempted to modulate the cholinergic system, increasing the signal strength. These efforts culminated in the FDA approval of four acetyl cholinesterase inhibitors

(AChEI) for symptomatic treatment of mild to moderate patients. [Cognex® – Warner-Lambert/Pfizer; Aricept® – Pfizer/Easai; Exelon® – Novartis; Reminyl® – Janssen; and more recently glumatergic compound (NMDA antagonist) for treatment of moderate to severe symptoms: Memantine – Namenda® – Forest Labs]

Calcium Hypothesis The formulation of the calcium hypothesis of brain aging and dementia began to take shape in 1982 by this author in parallel with the efforts to build the NIA portfolio of extramural research program in basic neurobiology of aging. The original hypothesis [1984] was highly speculative; with little data or circumstantial evidence for support. The original hypothesis proposed that sustained deregulations in the homeostasis of cytosolic calcium concentrations represents the '*final common pathway*' for neural dysfunctions/death associated with brain aging and dementia. The primary objective of this first attempt to develop a '*unified theory of brain aging and* dementia' was to redirect the primary focus of research [in the early 1980s] away from *descriptive* studies towards *molecular mechanisms* of neural functioning. Since 1984 the hypothesis has been revised several times on the basis of additional stronger evidence to support or warrant modifications. The last version published in 1994 proposed six interrelated postulates; one of these suggested that a 'a small change in calcium concentration sustained over a prolonged period [e.g., due to chronic hypo-perfusion] will result in similar neuronal damage as a large change in calcium concentration over a shorter period [e.g., stoke or multi-infarct]. Although the calcium hypothesis never gained wide support as other theories, it has had a small cadre of supporters. In the future hypothesis may gain wider acceptances as links are established with other theories e.g., impaired cerebromicrovascular perfusion, glucose hypometabolism, and evidence suggesting that mutations in presenilin genes alter calcium homeostasis as a crucial predisposing factor in formation of β-amyloid plaques and neurofibrillary tangles. (Khachaturian 1984, 1989, 1994; O'Neill et al. 2001; LaFerla 2002)

Amyloid Hypothesis While the cholinergic hypothesis was at the height of its popularity, studies using the tools of protein chemistry and molecular biology succeeded in extracting and sequencing the highly insoluble amyloid fibrils of the neuritic plaque proteins [senile plaques – SPs]. The 1984 discovery of the exact amino acid sequence of amyloidogenic peptide known as the amyloid β protein (Aβ), forming the building blocks of the amyloid fibrils in the neuritic plaques, (Glenner and Wong 1984a), was a significant turning point.. They demonstrated that the Aβ deposits, around meningeal blood vessels, isolated from the brains of people with Alzheimer's and Down syndrome (trisomy 21), were pathologically identical. They speculated that Aβ, as a gene product, may arise from a gene on chromosome 21. Within a short period, the parent gene encoding the precursor protein was cloned. The Masters and Beyreuther team purified and sequenced the peptide (A4), which they recognized as a smaller cleaved fragment of a larger precursor protein (APP) (Masters L, et al. 1985a).

These studies on the precise protein structure of Aβ protein, and the subsequent genetic studies, were important for shepherding in new investigators, opening the research floodgates. The studies on the molecular biology of Aβ provided a significant scientific hook for efforts to identify potential therapeutic targets for drug development. Since the mid-1980s, the advances in the basic science of normal aging brain, as well as the progress in the neurobiological mechanisms of AD, resulted from the infusion of new scientific talent, tools, ideas, knowledge, and experiences from other fields.

By the late 1980's, the "amyloid hypothesis" branch of the river became a torrent. In retrospect, some of the heated scientific debate and positions taken reflect the profound ignorance about many aspects of the disease. For example, Masters and Beyreuther were erroneously arguing that Aβ was the building block of PHFs and NFTs. Today, controversies about the function of Aβ and APP continue indicating the remaining challenge in sorting out the complex biology and the need to pursue lines of research contrary to the prevailing orthodoxy.

Shortly after Glenner's discovery, the race to identify the pathogenic mutations in genes that encode the Aβ began. Colin Masters, Konrad Beyreuther and collaborators sequenced the beta-amyloid precursor protein (APP), which was another significant event for the emergence of the amyloid hypothesis, along with Glenner's discovery. Cloning of the gene encoding the Aβ in the plaques, (the 40-42 amino acid fragment of the larger peptide made of ~ 700 amino acids) showed that the APP is encoded on chromosome 21. John Hardy, Alison Goate and Christine Chartier-Harlin found the first mutation in the APP gene on chromosome 21; involving the early onset form of AD. Soon after this discovery Christine Van Broeckoven and collaborators reported finding the Flemish mutation at codon 692 of APP, linked to early onset AD and cerebral haemorrhage (Goldgaber et al. 1987a; Kang et al. 1987; Robakis et al. 1987b; Tanzi et al. 1987; St. George-Hyslop et al. 1987, 1992; Schellenberg et al. 1992; Goate et al. 1991). The APP mutations on chromosome 21 affect only a small number of families. Therefore, the question of whether there might be other independent causative factors was answered in 1995 by the discovery of the presenilin 1 gene (PS1) on chromosome 14 (by Peter St. George-Hyslop) and the discovery of a homologous gene presenilin 2 (PS2) on chromosome 1 (by Shellenberg). Jerry Shellenberg and Tom Bird linked this locus to familial AD in Volga German pedigrees. As it often is the case in science the stage P. St. George-Hyslop of the PS1 gene was set by the earlier studies in the 1970s by Jean-François Foncin (Salpêtrière), who was one of the early investigators to start studying an Italian pedigree (family N) which became a precursor to the later discovery of the chromosomal localisation.

Since the discovery of these mutations, studies with transgenic mouse models have shown that animals with these mutations produce excessive Aβ. The next important challenge was to find out what Aβ did and how the production and accumulation of this abnormal protein was involved. Eventually these findings helped to establish animal models of AD amyloidosis. One of the early explorations of this question (Carl Cotman and Dennis Selkoe) indicated possible neurotrophic properties of the protein. Bruce Yankner's research showed that the actual toxicity of Aβ depended on the specific amino acid sequence. Bart De Strooper and Christian Haass independently found the relationship between PS1 and gamma-secretase activity and with Notch. Further research revealed that the Aβ peptide is formed by alternative cleavage of the APP molecule by β-secretase and a second cleavage at one of two sites by ɣ-secretase, the fragment left within the transmembrane domain is released as Aβ (Sisodia et al. 1990, Vassar and Citron 2000; Selkoe 2001)

In the 1990s, the amyloid theory became a powerful driving force that dominated the direction of research. The amyloid cascade hypothesis postulated that the primary event is the deposition of Aβ, which leads to astrocytosis, microglial reactivity, and the development of tangles. One of the most important developments was the production of an "animal model" of AD pathology. The first report of a transgenic model was

a false alarm based on fraudulent data. Subsequent models particularly those with cognitive deficits [developed by Karen Hsiao Ashe] have proven extremely useful. These animal models became important tools for testing novel treatment strategies as well as hypotheses about the molecular events in the abnormal synthesis, cleavage, aggregation, toxicity, and clearance of Aβ. The availability of animal models with Aβ pathology was one of the crucial factors leading to the formulation of the approach to treatment by vaccination (*immunizing against A*β).

However, the first human trial with this revolutionary approach was halted because of the development of meningoencephalitis in some patients. Nonetheless, the logic of the approach opened the door for totally new lines of research. Further studies are needed to understand the mechanism of Aβ clearance after immunization. Such knowledge will enable the design of specific vaccination strategies to target Aβ plaques without inducing any adverse events (Schenk et al. 1999). One of the novel immunotherapeutic approaches to AD is the idea of a peripheral amyloid "sink" (David Holtzman and collaborators). They have shown that peripherally administered anti-Aβ antibodies decrease brain Aβ deposition suggesting a change in Aβ equilibrium between brain and plasma.

Although this theory has a large following, definitive proof that Aβ initiates the degenerative cascade is lacking. The uncertainty has fueled a significant controversy concerning the importance or contributions of Aβ burden induced damage compared to that caused by NFTs or tau pathology. The argument between the two camps is reminiscent of the medieval religious wars. More importantly, these intellectual and scientific conflicts have stimulated new lines of research and novel insights. The skepticism concerning the Aβ hypothesis stems from: a) the spatial mismatch between amyloid deposition and tangle pathology, b) observations by several investigators (e.g., Terry, Masliah and others) that synapse loss has substantially stronger correlations with the severity of the dementia than plaque counts, c) transgenic mice that over-express mutant human APP develop numerous amyloid deposits but do not develop intracellular NFTs or extensive neurodegeneration, d) the relationship between "amyloid count" and severity of dementia is not found in some autopsy confirmed cases.. The likelihood of a link has been suggested by enhanced Tau phosphorylation in the vicinity of Aβ in vitro and in vivo. The hypothesis has been reinforced by the development of significant neurofibrillary pathology and degeneration in cortical and subcortical brain regions in transgenic mice expressing both mutant human Tau and APP (Busciglio et al. 1995; Lewis et al. 2001; Lee et al. 1991; Goedert et al. 1988). However, the story is not simple or straight forward. Christine van Brockhoven recently reported that a PS-1 mutation causes a Pick's disease phenotype including FTD tau pathology, but no Aβ deposition, thereby prompting further consternation about how to connect the dots linking Aβ to all the other downstream pathologies initiated by Aβ. This finding implies the need to "stay tuned" for further insights into pathologies that have yet to be fully deciphered.

Tau Hypothesis Studies of the chemical structure of twisted strands of neurofibrillary tangles (NFTs) began in the 1960s. The earlier EM characterization of NFTs by Kidd, and Terry, were followed by the discovery of new molecular probes by Peter Davies in 1983. Some of the antibodies Davies discovered while screening for differential reactivity of AD pathology vs. normal (e.g., Alz50/MC1) proved to be important for studying the molecular details of NFT pathology. Another facet of the controversy in the late 80s

and early 90s was about the composition of PHFs. During this period Peter Davies, an influential voice, was arguing that PHFs were anything but tau, while others suspected neurofilaments as building blocks of PHFs.

In 1985 J.P. Brion and André Delacourte were among the first suggest that tau might be the main component of neurofibrillary tangles. Soon after, in 1988, Michel Goedert and collaborators cloned the cDNA of PHF-tau. The question what actually are the constituents of PHF was finally resolved by two newcomers to the field [Virginia Lee and John Trojanowski] who isolated PHFs from AD brains and sequenced the protein bands, showing unequivocally that they were tau. However, it took time for these data to be accepted; but eventually this finding determined the focus for subsequent studies of the biology of PHFs and NFTs in AD, as well as in other FTD tauopathies (Vassar and Citron 2000).

Studies on the composition of the paired helical filaments (PHFs) in the NFTs of AD steadily grew into one the major branches of the 'river.' with the discovery of new clues about the pathology ; e.g., abnormal form of tau (PHFtau) as the true identity of PHFs (Vassar and Citron 2000; Selkoe 2001). The primary component of NFTs is the highly phosphorylated aggregate of microtubule (MT)-associated protein τ (tau) that "self-associates" into paired helical filaments (PHF-τ). Tau protein normally binds to and stabilizes microtubules. These slender tube-like proteins form a skeletal network that provides structure and organization within nerve cells. Two distinct changes occur in tau in Alzheimer's disease: a) phosphorylation is increased, in terms of the number of sites and the extent of phosphorylation at certain sites and b) conformational changes occur in folding, or formation of dimers or oligomers of tau.

In the early 1980's Eva and Eckhard Mandelkow had begun working on tau protein in the context of microtubule structure and biology, but not specifically on Alzheimer-related questions. However, when the link between AD and tau became apparent they switch over to the problems of hyperphosphorylation of tau. They were among the first to identify phosphorylation site, corresponding kinase [CaMK II] that caused abnormality in microtubule structure and a method to generate "Alzheimer-like" phosphorylation from a brain extract. (Steiner et al. 1990).Their other significant contributions was the discovery of the effects of phosphorylation on tau-microtubule binding and the aggregation of PHFs. These discoveries led to the realization that tau can have deleterious consequences for the cell long before effects on microtubule instability takes place. Tau pathology leads to neuronal dysfunction [eventually cell death], probably through excessive phosphorylation. Highly phosphorylated tau can clog up the microtubule surfaces making them inaccessible to motor proteins. The resulting failure of tau to regulate the MT stabilizing function leads to inhibition of anterograde transport which causes starvation of synapses. Thus phosphorylated tau is unable to perform normal functions critical to the survival of a nerve cell. (Busciglio et al. 1995).

The phenomenon of highly phosphorylated tau has been observed in human autopsy studies as well as in animal models. One of most significant findings in neuropathology report by Braak and Braak in 1991 indicating that: a) neuronal damage may actually start many years before any clinical signs (symptoms) are apparent, b) pathological changes proceed in stages, and c) changes in tau (NFTs) are among the earliest signs (Braak and Braak 2000). The accumulation of somatic NFTs and dendritic neuropil threads (NTs), or tau pathology, now has become an essential component for

the postmortem diagnosis criterion; replacing the "Khachaturian" and "CERAD" criteria (Mirra et al. 1991; Braak and Braak 1991; Consensus recommendations for the postmortem diagnosis of Alzheimer's disease 1997). It has become apparent that AD may be a heterogeneous disorder, resulting from a variety of etiologic factors leading to a syndrome complex sharing distinct clinical and postmortem features. There is a general consensus that an AD-like dementia characterized by Lewy bodies (LBs), rather than by SPs and NFTs, is a disorder distinct from AD, known as dementia with LBs (DLB). The most common subtype of AD, known as the LB variant of AD (LBVAD), has an abundance of SPs and NFTs as well as significant LB pathology in neocortical and limbic regions.

Two contributions to the study of tau pathology were the discovery of a gene encoding tau protein and the development of an animal model with the human tau gene (H1) (Cathy Andorfer and Peter Davies hTau mice). Tau protein is encoded in a single gene with six splice variants predominantly expressed in adult axons (Lee e al. 2001; Buee et al. 2000). A variety of mutations occur in the human tau gene, one of which can cause Fronto Temporal Dementia (FTDP-17). Further, mutations in the tau gene have now been shown to cause a familial form of dementia similar to AD. Additionally, α-synuclein proteins are the building blocks of the LBs in neurons of the Parkinson's disease brain, as well as those developing DLB and LBVAD. The discovery of the gene encoding phosphorylation of synuclein proteins (tau mutations) on chromosome 17 further reinforced the role of tau pathology in a variety of neurodegenerative disorders. Some of the investigators that made substantial contribution to this area of research included: Kirk Wilhelmsen, and Bernardino Ghetti, Michael Hutton, John Trojanowski, Virginia Lee, Maria Spillanti and Michel Goedert.

Energetics – Metabolic Hypothesis One of the earliest hypotheses for dementia was the notion of the 'hardenings of the arteries' as the primary cause of the disease but idea of brain vascular involvement gradually fell out of favor and was discarded as a viable hypothesis. However, the hypothesis that impairment in cerebromicrovascular perfusion, oxidative metabolism, glucose hypometabolism or mitochondrial dysfunction play a crucial predisposing role in the etiology of dementia has been steadily gaining support. The two important lines of evidence that have strengthened this hypothesis are: a) epidemiological data showing strong relationships between vascular disease, hypertension, or diabetes and increased risk for dementia and b) neuroimaging studies with PET showing specific regional reductions glucose utilization. Some of the people that have been instrumental in the development of the metabolic hypothesis have include Flint Beal, Siegfried Hoyer, John Blass, Davis Parker Suzann Kraft, William Markesbery, Mark Mattson; the key contributors to the more vascular aspects of the hypothesis have included Vladimir Hachinski, Raj Kalaria, J.C. De La Torres, M.M. Breteller, Bo Siejo and Arnold Scheibel just to mention a few (De La Torre 2000; Blass 2000; Beal 2000).

Recently Allen Roses and Ann Saunders have proposed a new version of the energetics/metabolic hypothesis based on the results of a 24-week randomized clinical trial of *rosiglitazone maleate*, an insulin sensitizer and peroxisome proliferator-activated receptor (PPARgamma, PPARγ) agonist, in the treatment of AD. Exploratory analyses of the data has shown the possibility of an interaction between treatment efficacy and presence or absence of the APOE4 allele. If subsequently confirmed, this interesting

and potentially important preliminary finding would provide the first clinically relevant evidence to support the hypothesis (Roses and Saunders 2006). Interest in the role of various forms of dysfunctions in energy production or utilization in the mitochondrial functions or ATP production has formed the basis for a work group lead by Nancy Wexler, Leon Thal, Flint Beal, Ann Young, John Trojanowski, Carl Johnson and others to start exploring common mechanisms [re: dysfunctions in mitochondria, protein misfolding] among some neurodegenerative disorders such as HD and AD.

Risks – Susceptibility Genes Critical challenges in the early 1990s included early identification of people at risk, and well-validated antimortem markers. The observations that molecular changes and neurodegeneration begin several years before measurable clinical changes [e.g., Braak's neuropathological findings and the results of David Snowdon's Nun study] opened the door to the search for antimortem diagnostic markers and provided implicit validation for the concept of a prodromal stage e.g., MCI (Petersen 2000). The discovery of the first susceptibility gene, APOE on chromosome 19, (Allen Roses and his team in 1993) generated enormous excitement. This study demonstrated that an apolipoprotein E (ApoE) allele, known as the ApoE ε4 allele, which encodes a variant of a cholesterol transport protein, is a major risk factor for sporadic AD. Initially, this finding was received with a high degree of skepticisms, but the basic elements of the research were confirmed rapidly and widely. The concept of susceptibility genes modulating the age of onset, either alone or in concert with some other factor, had a profound effect. Now the onset of the disease could be attributed to a biological process; thus offering the possibility of interventions to delay or slow progress. A flood of papers confirmed the Roses' findings that the apolipoprotein E4 allele increases the risk by two-to threefold in heterozygotes [one copy of ε4 allele] define here or page 4] and up to ninefold by age 80 in homozygotes [two copies of ε4 allele] (Corder et al. 1993). More recently other susceptibility genes have been reported but, not validated [e.g., chromosome 12 by Peggy Pericak-Vance; HLA-A2 allele on chromosome 6 by Payami; chromosome 9, chromosome 10, chromosome12A2n by Tanzi].

The search for the genetic basis of Alzheimer benefited from the *knowledge* and *technical resources* of two unrelated ongoing initiatives. The fist was the team Jim Gusella established at MGH to search for the Huntington gene. The team included Rudy Tanzi and Peter St. George Hyslop and capitalized on their experience in the search for AD genes. The second initiative was the cell repository in Camden, NJ (supported by NIA and NIGMS), which supplied fibroblast cell lines derived from the Nova Scotia FAD families.

Synapse Loss & Apoptosis Among the array of abnormal changes in the AD brain, the most significant proximal events are loss of synapses, dendrite pruning and cell death. The goal is to account for the molecular mechanisms that disrupt or destroy the functioning or integrity of the elements critical to signal transaction. One of the early crucial issues was the relationship between loss of synapses and cell death and aging. In the mid-1980 two lines of research demonstrated that the healthy aging brain maintains the ability to form new synapses (Cotman) and maintains rich arborization of dendrites (Coleman). Several studies showed that, in contrast to robust synaptogenesis in the healthy aging brain, the evolution of AD is characterized by dramatic synapse and

neuronal loss: the principal correlate of cognitive decline in AD reported by several investigators (e.g., Terry, Masliah, Scheff, and Mesulam). The observations that AD is associated with increased cellular vulnerability to neuronal loss led to the recruitment of investigators from other areas of neuroscience with an interest in mechanisms of "programmed cell death." One of the questions fueling a large number of investigations has been whether "apoptotic-type" insults in local microenvironment of neurites might initiate (cause) selective neurite degeneration.

Neuroinflammation An often overlooked area of investigation is whether specific immune responses may contribute to the pathogenesis of AD. This line of research had only a handful of ardent proponents (Felicia Gaskin, Joseph Rogers, Patrick McGeer, Sue Griffith, Tuck Finch, Gulio Pasineti, and Dana Julian). Until the early 1990s, these investigators were the lone voices promoting the importance of inflammation (McGeer and McGeer 1995; Rogers and Shen 2000). Over the past ten years this proposition gained prominence primarily because of epidemiological studies indicating that non-steroidal anti-inflammatory drugs might reduce the risk or delay onset. Immunohistochemical and molecular biological investigations revealed the presence of chronic neuroinflammation in affected regions of AD brains. Neuroinflammation, a characteristic feature of disease pathology in several neurodegenerative disorders, appears to involve excessive glial (astrocytes and microglia) activation, with overproduction of proinflammatory cytokines (e.g., cytokine interleukin-1,IL-1, and chemokines), oxidative stress-related enzymes, acute phase proteins, and various components of the complement cascade. The deposition of Aβ and neurofibrillary tangles in AD is associated with glial activation, neuronal loss, and cognitive decline. The IL-1 gene polymorphisms appear to be associated with an increased risk of AD. The direct linkage of glial activation to disease pathology has underscored the importance of understanding the signal transduction pathways that mediate these critical glial cellular responses.

Other Promising Leads The history of AD research has a rich array of promising leads, ideas, theories and observation that have ended as: a) blind alleys, b) underdeveloped areas of explorations and c) unexpected new directions. One example of an early promising lead that did not materialize was the notion that aluminum might play a significant role in the pathogenesis of AD. In spite of early findings and substantial amount of research on the relationship between aluminum and brain lesions, this area has not proven to be fruitful. However, the generic idea of "toxins" [e.g., endogenous glutamate, other excitatory amino acids or other environmental neurotoxins] playing a critical role in the etiology of the disease has not been conclusively ruled out and remains to be a promising underdeveloped area of study. Another very early idea about the potential causes of the disease related to cerebro-vascular pathology [hardening of the arteries], which was discarded in until recently. Now based on epidemiological findings and more careful systematic clinic-pathological findings there is a resurgence of interest in the possible critical role of brain vascular abnormalities and dementia. A related topic to cerebral vascular pathology, with an equally long history of interest is the role of energy metabolism due to mitochondrial dysfunction and/or brain metabolic abnormalities. This area of research as is the case with brain vascular pathology remains promising but not fully exploited avenue of exploration. Finally the hypothesis that dementia might be due to a transmissible infectious agent also

has long history dating back to the 1970s. The idea that a transmissible "slow virus" might be involved in AD has its origins in the early research of Carleton Gajdusek team with scrapie, kuru, Gerstmann-Straussler-Scheinker (GSS) and Creutzfelt-Jakob disease (CJD); a rapidly progressive dementia. In the late 1970s and early 1980s this was one of the viable, but a long-shot, idea on the pathogenesis of AD. In this period the NIA began to support Stanley Prusiner's research on scrapie, based on the similarities of amyloid in AD and spongiform encephalopathy and the possibility that AD might be caused by an infectious agent. The theory of a transmissible infectious agent as the direct cause of AD has not proven to be correct after nearly twenty years. However, the NIA's investment in Prusiner's the research has yielded totally serendipitous rich returns vis-à-vis new insights about neurodegenerative processes. Now as a result of Prusiner's work on the "Prion" biology has totally new concepts on: a) some infectious agents contain no nucleic acid; thus differentiating them from all other known viruses, b) disease could be hereditary and infectious and c) the culprit protein might be self-replicating.

The NIA support of Prusiner's research and the eventual impact of his discoveries for biology in general and specifically for neurodegenerative disorders of the brain is perhaps the best argument against programmatic or narrowly target support of research.

Development of Extramural Research Program on Alzheimer – NIA/NIH

In the period shortly after WWII, the NIH began to emerge as a critical a *catalyst* in furthering biomedical research in very broad range of topics. The renaissance of great scientific fervor in the US after the war was primarily the results of: a) increases in the level of research support and, b) the import of scientific/technical know-how from other counties. In the 1950s, the NIH had begun to change from essentially a group of intramural research laboratories to an array of disease specific institutes. Most institutes expanded their research mission by supplementing intramural studies with investigations at academic institutions. This was the start of the extramural research/supported programs, via grants-in-aid to outside investigators. The National Institute for Neurological Disease and Stroke (NINDS) and the National Institute of Mental Health (NIMH) were established in the 1950s with the missions of finding solutions respectively for neurological and psychiatric disorders. The relatively rapid progress in AD research in recent years is primarily attributable to sustained investments in basic research on brain and behavior made by NINCDS and NIMH. The rich intellectual returns of these investments provided the scientific building blocks to the subsequent efforts in the 1970s and 1980s. The important consequences of the NIMH-NINCDS efforts were to: a) increase the number of talented investigators (through pre- and post-doctoral training programs) and, b) expand the base of extramural support for fundamental research.

The NIA was established in 1974 with an amorphous authorization to address the "*problems and diseases of the aged*". The implicit directive to NIA, by the US Congress, was to develop *interdisciplinary* research on *healthy (normal) aging* as well as *disorders of aging*. This provided the NIA a unique mandate that was substantially different than

the other categorical institutes at NIH, which had responsibilities for specific disease e.g., cancer, diabetes, heart, stroke etc. In 1977, this author was recruited to translate NIA's broad legislative directive into 'a blue-print for action'. The NIA strategic plan for a national program of research on the neurobiology of aging and Alzheimer's disease outlined the details of the scientific content, organizational structure, mechanisms of support, resources and infrastructure needs, and professional judgment budget estimates for a comprehensive program. In 1978, however, the task of implementing NIA's broad legislative mandate, i.e., solving '*the problems and diseases of aging*' faced a number of difficult hurdles [e.g., lack of funds, little or no academic interest in the topic, small cadre of investigators, the absence of a compelling scientific story, the lack of scientific credibility and inadequate resources/infrastructure].

The strategy for addressing these challenges required NIA to adopt a different model, for developing, organizing and managing the Institutes extramural program, than those used by other well established institutes at NIH e.g., NINCDS or NIMH. The complexities of the multi-facetted problem, such as contrasting normal "aging" from "diseases of aging", required that the program structure be based on the replica of a *systems research*. This approach to program development de-emphasized disciplinary "silos" and focused on building linkages for the integration of knowledge, skills and points of views across a wide rang of disciplines. The NIA's program development efforts stressed: a) vertically integration of basic research with clinical studies, b) funding mechanisms to promote collaborative research, e.g., program projects, centers, research consortiums, c) building-up resources and infrastructure for conducting longitudinal clinical research and/or clinical trials, e.g., ADCS, and d) developing the "capabilities" of the field for clinical research, including development of diagnostic criteria, standardization of assessment tools and the methodologies of clinical trials. Thus the NIA plan to develop the nascent fields of AD and brain aging stressed the importance of not only on mechanisms of support for investigator initiated projects but also initiatives that encouraged: *coordination*, *organization*, and *infrastructure building*.

Although NIA continued to share an interest in Alzheimer research with other Institutes, by the mid-1980's it had become the lead Institute at NIH; by acquiring administered responsibilities for nearly 70% of all Federal expenditures on AD. In the period since 1978–2006 the total Federal funding for Alzheimer's disease grew from less than $1.0 million per year in FY '78 to over $650 million per year in FY '06. The NIA acquired this leadership by assuming a more proactive role in: a) the recruitment of new investigators and/or programming collaborative program projects, b) the creation of novel mechanisms of support, c) building infrastructures and d) lobbying for specific Congressional mandates and new authorizations (e.g., Centers program) or targeted appropriations. In 1985, James Wyngarden, Director of NIH, directed NIA to establish the Office of Alzheimer's Research, as the NIH coordinating center for all Alzheimer's research. In 1986 the NIA extramural Neuroscience of Aging Program, which had begun in 1978, was reorganized as the current Neuroscience and Neuropsychology of Aging Program (NNA). This program now is one of three extramural components and administers nearly 50% of the Institute's grant budget.

Challenges & Barriers to Program Development

In 1978, task of launching a national initiative on neurobiology of aging and Alzheimer research faced a number of daunting hurdles. The process of *program development* involved the interaction of several variables and required inertia [work] to overcome impediments; the relationship of the key components is very similar to that in a *chemical reaction*. The *raison d'être* of NIA's efforts to mobilize the scientific enterprise was the acquisition of new "*knowledge*" to solve a looming public health problem. The ultimate "***product***" of the program was the discovery of a "*cure*" for Alzheimer's disease. However, the start of the process required "***Energy***" in form of massive funds to support research. Any large-scale national enterprise of discovery could not be initiated, maintained or, expected to make progress without the appropriate level of resources. Certainly the funding *of any particular project alone could not assure discovery of "a cure", however inadequate funds was, and still is, a virtual certainty for failure.* The second essential component for the process of building-up a program for the discovering a cure required "***Substrates***" in the forms of: 1) rich reservoir of fundamental knowledge about the disease, 2) critical numbers of talented/skilled investigators and, 3) adequate research infrastructure. The final crucial requirement of the process is the "***Catalyst(s)***" in the form of an advocate, facilitator or mentor. Throughout the process of program development a number of different actors played the important role of catalyst. Just to mention a few, these included prominent scientist, such as Lew Thomas, James Watson, Robert Katzman, Robert Terry & many others mentioned elsewhere in this article; Alzheimer Association, key legislators, foundations, individual patrons and science writers in the press.

Building a Budget The availability of funds determines whether a new program or an initiative can be launched. The struggle for additional funds is a chronic universal problem for all programs at NIH. However, increasing the budget for a new program at a new institute was particularly challenging. The first problem was that in the late 1970s the generic field of 'neurobiology of aging', and NIA's 'neuroscience program' in particular, did not have any semblance of credibility or a track record of success stories e.g., reports of 'major discoveries' or the 'promise of a cure.' The second dilemma for program development was the lack of effective grassroots advocacy by either an outside interest group or within the Congressional appropriation committees. These relationships with outside advocates and key congressional staff (or members of Congress) had to be established and cultivated. The third challenge was to erase the negative image of aging research and to remove the stigma of 'senility,' which impeded the progress of research on the disease. Members of Congress and their staffs, who were not well-informed about the clinical problem and the long-range public health implications had to be educated. The public attitude of a negative view of Alzheimer as a mental disorder had to be changed.

Stigma of Aging One of the most serious problems for program development stemmed from the lack of clear distinctions between the concept of "disease" and the construct of "aging". There was confusion about these two entities; and often were assume to be causally linked. From the initial identification of AD, there was no consensus on the nomenclature of "senility." "Senile dementia of Alzheimer's Type" or "pre-senile

dementia" were commonly used, and interchangeable terms. The expression "senility" often implied dementia and generally described the *de facto* cause of the disease without any experimental proof. A prevailing misconception regarded AD as a hopeless and untreatable mental condition, an inevitable consequence of aging. As a result, very little clinical or research interest was generated, and focus on brain diseases in late life was generally deemed a career-killer in academic medicine and science.

The extremely poor reputation of "aging research" was a major handicap for program development. Aging research was regarded as 'low-grade science' and had two unique and negative consequences for NIA. One was the unfavorable attitude or scientific bias against aging research proposals reflected in the discussions of study sections. Proposals on aging or Alzheimer's invariably received substantially poorer merit ratings than those assigned to other institutes. The other outcome of the image problem of the field was the difficulty in attracting new competent scientific talent. Only a handful of investigators worldwide explored the unknown area of dementing disorders.

The important challenge for NIA was to change not only the public opinion, but also the attitudes of scientists and clinicians towards aging and aging research. Specifically, the concept of "senility" required replacement with the fact that AD is a brain disease. The promotion of this goal focused on the idea that despite a consistent *correlation* between "*age*" and incidence of dementia, there is no evidence for a causal relationship between the biological process "*aging*" and AD. The maxim that dementia is not an inevitable consequence of aging was adopted as the prime scientific principle for program development. This was critical for the goal of shifting the focus of research away from descriptive studies toward the search for the underlying neurobiology.

Credibility of the Science During the early phases [circa 1978–1985] of building the neuroscience program one of the major impediments was the lack of promising leads that might catapult the research career of a prospective investigator. The strategy to surmount this hurdle was to "program" or cultivate new project/proposals by proactively seek out prospective investigators. The goal was to actively recruit the best potential investigators into the fold of aging and Alzheimer's research and to recruit scientists with special expertise or technical skills. Such programming involved presentations of challenging scientific problems or unresolved questions and providing potential investigators assistance in identifying research opportunities in neurobiology of aging/Alzheimer's disease or help in planning, organizing and preparing. The strategy of "***priming the pump***" by actively seeking out investigators from related fields and promoting multidisciplinary research teams began to payoff by the mid-1980s. Such efforts to actively recruit talented investigators were one of the essential steps in establishing the "credibility" of aging/dementia research. Confidence in the quality of the science was crucial for changing attitudes towards aging research in the scientific community, congressional staff, legislators and the press Thus, the early foci of program development were to: a) prepare a *compelling scientific story* to justify targeted budget increases for AD research, and b) cultivate advocates, within the scientific community, Congress, and Alzheimer's Association.

Some of these almost insurmountable obstacles began to crumble by 1981 when NIA's budget request to Congress started to include an ever more compelling case for Alzheimer disease research funds. One of the first breakthrough findings in this regard was of the cholinergic deficits, by Peter Davies; the *cholinergic hypothesis* of

Alzheimer's provided the basis for such a story; later the *amyloid* and *tau* stories were promoted. As the science advanced, more 'stories' could be told to justify further funding increases, such as Carl Cotman's discovery of the retention of synaptogenesis in the aging brain and Fred Gage's demonstration of neurite outgrowth of fetal transplants in old brains. The presentations to Congress began to emphasize the importation of modern neurobiology and cutting edge science into AD research, and presented Alzheimer's disease as a legitimate, specific neurodegenerative disease to combat the stigma of senility. Gradually research on brain aging and Alzheimer began to gain momentum leading to its current status as a vibrant and prominent field of research.

Catalysts The unique functions of catalyst in general, but especially the role NIH program staff as facilitators and mentors to prospective investigators, is very pertinent to the story of the trials and tribulations in *program development*. The accomplishments of the NIA program staff over the past 30 years, underscores the important role they have played in promoting, guiding, supporting and catalyzing Alzheimer's research. The Public Information Office of NIA and media in general became important allies of NIA program staff in getting the 'story' out to inform the public (Congress) and to increase the awareness of the problem. The task of "selling" research on brain aging and AD became substantially easier by the mid-1980's because: a) the NIA grant portfolio had grown and was much stronger b) the Alzheimer's Association became more active in public policy; c) Dominic Ruscio, the Association's lobbyist, was a highly effective ally, and d) the program began to cultivate and acquire a number articulate champions within the scientific community, grass roots organizations and the Congress. In this period other advocates for the cause appeared. Among these, the most notable NIA allies were: a) the Alzheimer's Association which began to fund peer-review research projects, b) the John Douglas French Foundation, which also funded new investigators through fellowships, c) MetLife Foundation began an awards program to recognize significant scientific contributions, and d) IPSEN Foundation in Paris, under the able leadership of Yves Christen began to organize seminal symposia and publish critical reviews.

Capacity & Team Building Contrary to the prevailing bias for individual investigator initiated projects (RO1s) at NIH, the NIA began to promote *interdisciplinary* research and *multidisciplinary* teams. The solution to enormously complex clinical problems required a systems approach involving the expertise of many specialties, subspecialties, and disciplines. Although the interdisciplinary approach strategy was believed to be effective for multifaceted problems the approach require extensive: 1) *funding*, 2) *infrastructure*, 3) *organization*, and 4) *coordination*. To address these challenges, the NIA organized a series of research planning workshops to develop strategies for team building and collaboration, creating clinical research infrastructure, and promoting standardize diagnostic procedures. After twenty years the benefits of "team science" are reflected in the sharing of data and samples fostered by the Alzheimer's Disease Centers network, Alzheimer Disease Cooperative Study and the National Alzheimer Disease Coordinating Center and the productivity of these groups.

Infrastructure for Clinical Studies In the early years the critical rate-limiting factors that impeding systematic clinical trials (therapy development) were the lack of:

1) promising therapeutic targets, 2) viable lead compounds, 3) well characterized postmortem brain tissue for molecular studies, 4) infrastructure to support longitudinal clinical studies, 5) consensus on diagnostic criteria, 6) standardized assessment instruments, and 7) expertise in clinical trials.

In the late 1970s, it was difficult to conduct clinical studies because one could not find AD patients at teaching hospitals, the usual site for clinical research. Most of the AD patients were in nursing homes which were not research friendly environments. To address the long-term strategic goal of developing treatments, it was necessary for NIA to build a) mechanisms for promoting collaborative research, b) the capability of the field to conduct longitudinal clinical research and clinical trials and c) infrastructure for clinical research. The NIA began to create the necessary national research infrastructure. The Alzheimer's Disease Research Centers (ADRCs), established in 1984, and the Alzheimer's Disease Core Centers (ADCCs), established in 1990, were central components of the research and capability infrastructure. These programs, referred to as the Alzheimer's Disease Centers or ADCs, provide the infrastructure for integrating clinical and basic science research and allowed the augmentation of a wide range of studies on the etiology and pathogenesis of AD. In 1991 the 'Satellite Clinics' program was established to fund outreach to underserved or rural patient groups. Now Satellites are an integral part of many ADCs. The Leadership and Excellence in Alzheimer's disease (LEAD) award was created in the late 1980's as a mechanism to: a) elevate the profile of AD research [will one million dollars per award] and, b) expand the field by create a formal mentorship between established senior investigators and new promising younger investigators.

Other strategies were required to address issues in the development of diagnostic criteria, standardization of assessment tools and the methodologies of clinical trials. The 'Alzheimer's Disease Patient Registry' Program (ADPR) was launched in 1986 to address the goal of developing standardized diagnostic assessments. This program included the Consortium to Establish a Registry for AD (CERAD) led by Al Heyman and Gerda Fillenbarum, the Mayo Clinic Registry led by Len Kurland and Ron Peterson, the Seattle site led by Eric Larson, the Mon valley project with Lu Kuller and Mary Ganguli, and Denis Evans's group in East Boston. The CERAD project was successful in establishing uniform methods for the diagnosis and assessment of AD because of the dedicated and effective leadership provided by Al Heyman and the cooperation of clinicians and investigators nation and worldwide. The ADPR program overall was instrumental in the development of assessment instruments and procedures but also in filling gaps the epidemiology of AD.

In the late 1980s and early 1990s, there were no effective treatments and the drug industry had little or no interest in drug development. To stimulate targeted therapy development activities, the NIA launched two related programs: 1) Drug Discovery Groups, in 1991(as program projects), to facilitate pre-clinical drug discoveries and 2) the AD Cooperative Study (ADCS) program in 1991, led by Leon Thal, to promote clinical studies of new treatments (particularly for drugs developed in academia or small biotechnology companies) as well as to design, test and evaluate new instruments or methods in clinical trials. In the intervening ten years, the NIA has launched additional infrastructure building and or cooperative research programs, including the National Cell Repository for Alzheimer's Disease (NCRAD), National Alzheimer's

Coordinating Center (NACC), Imaging, and Genetics initiatives (with the Alzheimer's Association).

Conclusion

Now, there is a general consensus that many of the advances in diagnosis, treatment(s), care and understanding the cause(s) would not have been possible without the creation of instruments, criteria, infrastructure and programs that support interdisciplinary research. Some of the spectacular clinical and research strides attributable in some measure to the NIA initiatives include:

- improvements in antimortem and postmortem diagnosis
- access to samples of blood, DNA, CSF and postmortem tissues from well-characterized patients for basic and clinical research
- advances in understanding the neurobiology of the normal aging brain, as well as the mechanisms of AD and related neurodegenerative diseases
- capacity to pool data on large cohorts of research participants through the NACC , for example, the landmark cooperative study assessing the diagnostic impact of the ApoE ε4 allele in the evaluation of dementia patients
- insights into the role of immune mechanisms, the complement cascade, proteases, glia, head trauma, etc. in the pathogenesis of dementing disorders (Khachaturian and Mesulam 2000).

Prospects for the Future

- Only *thirty years* ago Alzheimer's disease was regarded as a hopelessly untreatable condition. Except for a handful of investigators, the area attracted little interest and virtually no support for research.
- *Twenty five* years ago, the essential clinical infrastructures for longitudinal studies of well-characterized patients did not exist.
- *Twenty* years ago, ideas about "cure" and "prevention" were unconceivable; such things as diagnostic criteria, standardized assessment instruments, cadres of specialized professionals, memory disorder clinics, family support groups or outreach programs, all taken for granted now, were not fully developed.
- *Fifteen years* ago, the knowledge on biological underpinnings and the genes associated with the disease had not been identified.
- *Ten years* ago, animal models of the disease were not available.
- *Five years* ago, persons risk for the disease could not be identified and the concept of clinical trials to delay the symptoms was unconceivable.
- Until 2004, the Aβ protein, hallmark lesions of the disease, could not be directly visualized in patients.

Today, the field is on the brink of major breakthroughs that may lead to more effective treatments and, ultimately, to prevention. A great deal has been learned about the pathogenesis of neurodegeneration, after less than three decades. Novel intervention

strategies are being developed to ameliorate the neuro-toxicity caused by abnormal metabolic products and prevent processes that lead to cell death. A large number of clinical trials are underway, both industry and government (NIA-ADCS) sponsored studies, with widely-used drugs (e.g., antioxidants, anti-inflammatory agents, statins, vitamins and folate) that might reduce the risk of Alzheimer's disease. Intensive studies are underway on multiple fronts, from basic science to genetics to drug therapy to care giving.

The remarkable progress towards understanding AD and the improved the prospect of discovering disease modifying therapies will not have been possible without the: 1) worldwide network of investigators working closely and collaboratively, 2) research infrastructure established by NIA and, 3) the successful partnership between the NIA Alzheimer's Association. Now these partnerships need to be expanded to include industry, foundations and individual philanthropists. The goal for such public-private working partnership is to mobilize all the necessary national resource for a new initiative to discovery [and/or develop] of interventions to prevent the disease. Time is running out; the epidemic of AD will completely overwhelm the health care system; due to the substantial growth the numbers of people with AD. The demographic changes, resulting from the continuing increases in the life expectancy of the oldest-old, are going to have their full impact in 20 to 30 years from now. The projected costs in human suffering and lost opportunities will be incalculable and unthinkable.

Ultimately, investment in brain research is the only cost-effective means to avoid the pending public health catastrophe facing countries with aging populations. Now it is essential to reevaluate priorities in all developed countries with resources with the goal of significantly expanding the world commitment to support research on disorders of the aging brain. The ultimate aim of such an international initiative should be the reduction of the: ***duration of illness***, ***numbers at risk or affected*** by AD and, ***cost of care***. Fortunately, the necessary scientific leads and the technical information are at hand to launch a bold initiative. A delay in the onset of disabling symptoms will allow patients to continue functioning independently for longer periods. An initiative aimed at mobilizing the necessary resource to delay the onset of the disease by five years for all age groups over 65 would reduce nearly half the total number of individuals with the disease. But, we need to act quickly. In 20 years, attempts will be too little and too late, because the healthcare needs of nearly 8 million people will overwhelm the available resources. It is no longer a question of whether the scientific community has the knowledge base to insure the success of such an endeavor; it is more a question of whether political leaders and policy formulators around the world have the vision and the will to move forward.

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Anatomy & Neuropsychology

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Anatomical changes underlying dementia in Alzheimer's disease

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The severity of the clinical problem of Alzheimer's disease (AD) is astounding: the Alzheimer's Association estimates that 1 in 10 Americans is affected by someone with AD in their family. Despite decades of research and advances in the cell biology and genetics of AD, current medications provide only subtle symptomatic benefits. Studies of the natural history of the illness present an even more worrisome image: the pathologic changes of AD begin years prior to the emergence of overt clinical symptoms. New imaging modalities (PIB amyloid PET scans, quantitative MRI scans, etc.) and more sensitive clinical instruments are pushing the diagnosis of AD earlier in the course, leading to an explosion of recognition of the illness in its earliest stages that makes the need for disease-modifying interventions even more pressing. The key to successful prevention and treatment is understanding what causes dementia. The major theme of this chapter is to examine ideas about the pathological substrates of impaired brain function in AD, with special emphasis on those issues that may well prove amenable to therapeutic intervention.

Neuronal loss and neurofibrillary tangles correlate with degree of dementia

The AD brain contains innumerable plaques, tangles, neuronal loss and gliosis. The idea that neuronal loss and synaptic loss are associated with impaired neural system function seems straightforward. Clinical-pathological correlation data support the idea that these lesions, as well as neurofibrillary tangle formation, correlate relatively well with dementia severity or duration (Arriagada et al. 1992a,b; Gomez-Isla et al. 1997; Ingelsson et al. 2004; Masliah et al. 1994; Terry et al. 1991). Indeed, neurofibrillary tangles and neuronal loss parallel one another and occur predominantly in parts of the brain that appear to be most affected clinically, such as the medial temporal lobe memory-related neural systems.

While neurofibrillary tangles and neuronal loss affect the same neuronal populations to a great extent, the role of neurofibrillary tangles in neuronal death and dysfunction has remained in doubt. Recent data from a novel transgenic model that overexpresses the P301L tau mutation suggest that the link between neurofibrillary lesions and neuronal death is not as strong as had been supposed (Santacruz et al. 2005; Spires and Hyman 2005). The transgene is expressed on a promoter that can

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be turned off by oral administration of doxycycline. Innumerable neurofibrillary tangles occur and neuronal loss is widespread. However, when the transgene is turned off, neuronal loss halts but tangles remain. Surprisingly, the animals' performance on memory tests improves in this circumstance, despite the continued presence of tangles. Moreover, stereological assessments suggest that some neuronal populations are lost without developing tangles, whereas others form tangles readily but neuronal loss does not occur. Thus a dissociation between tangle formation and neuronal loss is apparent, confirming earlier observations in patients with AD (Gomez-Isla et al. 1997) of a discrepancy between tangles and neuronal loss. From a therapeutics perspective, this is an important result – blocking neuronal loss remains a vital goal, although neuroprotective strategies lag behind anti-Aβ and anti-tangle approaches.

Aβ appears to be a critical component of the pathophysiological cascade that leads to dementia

The observation that all known genetic causes of AD, including PS1, PS2, APP mutations, trisomy 21, and even apoEε4, cause changes in Aβ metabolism and elevated plaque deposition leads to the conclusion that Aβ is central to the disease process (Selkoe 2000). Yet how Aβ disrupts neural function remains uncertain.

Experiments published in the last several years have suggested different ways in which Aβ might mediate neural system failure. These studies focus attention on the hypothesis that Aβ-induced synaptic failure underlies neural system failure in AD, yet complexities remain: axonal, dendritic, and synaptic defects are each associated with Aβ in different experimental systems. These observations include: 1) defects in axonal transport lead to dystrophic axonal processes that may both precede plaque formation and accelerate AD plaque pathology (Stokin et al. 2005); 2) spine loss occurs prominently near plaques (Spires et al. 2005; Tsai et al. 2004); 3) soluble Aβ leads to diminished neuronal responsiveness (Kamenetz et al. 2003) and loss of cell surface glutamate receptors (Almeida et al. 2005; Snyder et al. 2005); and 4) infusion of (oligomeric) Aβ leads to alterations in LTP and memory-related behaviors in rats (Cleary et al. 2005; Walsh et al. 2002). Finally, and perhaps most importantly, immunotherapy trials in mice (Bacskai et al. 2001; Schenk et al. 1999) and humans [with Elan AN1792] led to Aβ clearance (Ferrer et al. 2004; Masliah et al. 2005a; Nicoll et al. 2003).

Do plaques cause neural system disruption?

Transgenic mice that overexpress APP have early behavioral changes, and these worsen with age as plaques develop (Hartman et al. 2005). In humans, although some plaques (especially diffuse plaques) are frequently found in patients without dementia (Arriagada et al. 1992a), AD patients' dementia occurs only after the brain has innumerable plaques, tangles, and neuronal loss (reviewed in Gomez-Isla et al. 2006). Yet, despite high levels of amyloid deposition, the number of plaques often corresponds quite poorly with the degree of dementia (Arriagada et al. 1992b; Ingelsson et al. 2004). We have interpreted these results in the context of a model built on the hypothesis that Aβ

causes focal alterations in pre- and post-synaptic elements, leading to local changes in circuitry near plaques and ultimately failure at a neural systems level (Gomez-Isla et al., 2006). Thus, from a neural systems perspective, plaques can be viewed as distributed lesions that disrupt connectivity of neural networks. This model is based on the observations that dendrites and axons have altered morphology and trajectories near plaques, both large dystrophic abnormalities immediately in the vicinity of plaques as well as a more subtle but clear set of changes that occur throughout the course of dendrites and axons in AD brain (Knowles et al. 1998, 1999). Nearly identical changes are present in Tg2576 mice after plaques have formed (Le et al. 2001). Axonal dystrophies are a prominent feature both near and distant from plaques and may even precede plaque formation (Stokin et al. 2005). Axon terminals of cortico-cortical projections are disrupted (Delatour et al. 2004). We suggested that abnormal dendritic trajectories would lead to delays or mistiming of activation of downstream targets during afferent volleys (Knowles et al. 1999). It has recently been elegantly demonstrated that exact timing of inputs is critical to distinguish between synaptic potentiation and depression (Wang et al. 2005). We reasoned that, if some of the axons and dendrites trajectories were abnormal, a synchronous signal would not arrive at the destination when a set of neurons tried to fire to activate a second neuronal structure (Knowles et al. 1999).

To test this prediction directly, we collaborated with Dr. Ed Stern, a cortical electrophysiologist. In this series of studies, transgenic APP animals were stimulated with an extracellular electrode in one hemisphere and intracellular recordings were obtained contralaterally to look at the functional activity of multisynaptic callosal projections from one hemisphere to the other (Stern et al. 2004). In control mice, the intracellularly recorded neuron showed normal resting potentials and spontaneous activity and fired at a predictable interval after the extracellular stimulus. In aged Tg2576 mice (after plaque formation), despite apparently normal resting potentials and spontaneous activity, contralateral stimulation frequently did not result in firing of the recorded neuron. In fact, there was an average 2.5-fold greater rate of response failure compared with age-matched non-transgenic controls. This effect correlated with the presence of dense core plaques and with the extent of alterations in neuronal process trajectories. We interpret these data to suggest that synchronous activation, necessary for normal information transfer between neural systems, was disrupted in Tg2576 mice after plaque deposition. Similar conclusions were drawn by Brown et al. (2005), who suggesting that synchronized network activity was specifically and selectively altered in a different APP transgenic model.

Relationship between dystrophic neurites and plaques

Inspection of the AD brain reveals innumerable neuropil threads, dystrophic and morphologically bizarre axons and dendritic profiles, and several subtypes of senile plaques. The most prominent plaque is surrounded by tau-immunopositive swollen, dystrophic neurites (frequently referred to as a neuritic plaque). Even "normal" appearing neurites often take bizarre geometries and trajectories, curving around plaques and losing the rigid, straight appearance normally characteristic of dendrites and axons (Fig. 1).

Two alternative hypotheses have been suggested to account for the close relationship between dystrophic axons and dendrites and amyloid deposits. Goldstein and his

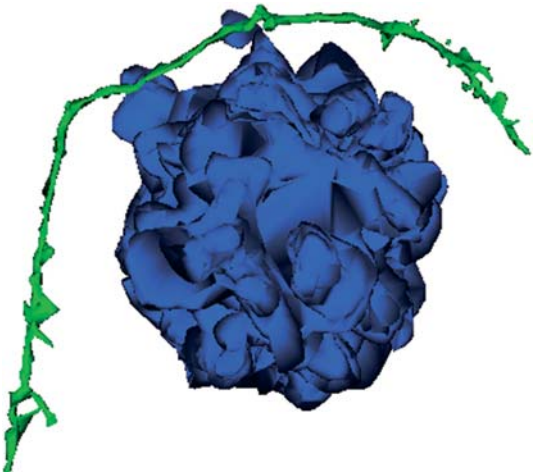

Fig. 1. A three-dimensional reconstruction of a plaque and a nearby neurite (filled with green fluorescent protein) from a Tg2576 mouse. This image, taken with a multiphoton microscope in a living mouse, illustrates the irregular surface and large surface area of a plaque and the dysmorphic nature of surrounding neurites. Note how the neurite courses around the plaque

colleagues (Stokin et al. 2005) hypothesize that axonal dystrophies precede and lead to senile plaques, and that axonal transport defects influence plaque deposition and lead to neural dysfunction. By contrast, we observe that axonal dystrophies resolve after application of anti-Aβ antibodies, suggesting that they are secondary to Aβ (Brendza et al. 2005). These observations lead to the possibility that axonal transport defects lead to axonal dystrophies and elevated Aβ production leading to plaque formation, and/or plaques (or locally high Aβ levels) lead to local alterations in axons, disrupting axonal transport. If so, this provides a unifying hypothesis for Aβ-induced alterations in neuronal structure and function.

Plaques vs soluble Aβ?

Morphological approaches emphasize a role for dense core amyloid plaques, whereas other experiments implicate soluble or oligomeric forms of Aβ. For example, we introduced green fluorescent protein (GFP) using adenoassociated virus (AAV) into young or aged Tg2576 mice (Spires et al. 2005) [or used a yellow fluorescent protein (YFP) cross APP mouse (Brendza et al. 2005)] and imaged the dendritic and axonal structures using multiphoton microscopy. Axonal dystrophies were obvious, both near and distant from plaques. Dendritic spine loss was also evident, with a dramatic loss of over one third of the spines in a zone within ~20 microns of the surface of the plaque, with loss proportional to distance from the plaque (Spires et al. 2005; Fig. 1). Generally similar results were reported by Gan, who also saw severe dendritic changes (Tsai et al. 2004). These results show that marked structural abnormalities in pre- and postsynaptic elements occur focally in the microenvironment around plaques. On the other hand, neuronal function has been shown to be affected by soluble or oligomeric Aβ. Malinow's laboratory transduced a subset of neurons with APP in slice culture and found depressed synaptic responses not only from the neurons transduced but also from its neighbors, suggesting that diffusible Aβ could depress neural function (Cirrito

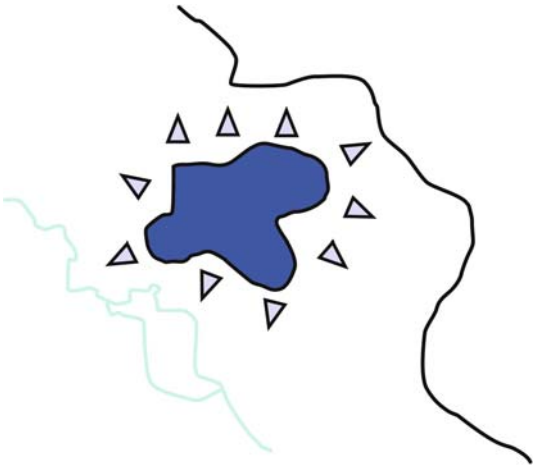

Fig. 2. Schematic representation of the surface of a plaque, which we postulate might act as a source of diffusible Aβ in vivo

et al. 2005a; Kamenetz et al. 2003). Injection of soluble oligomeric forms of Aβ alter long term potentiation (LTP) and are associated with impaired memory (Cleary et al. 2005; Walsh et al. 2002, 2005). These observations raise the question of the relative impact of plaques vs soluble (or oligomeric) forms of Aβ.

Do plaques act as a local source of soluble Aβ?

The arguments above suggest an "either-or" phenomenon highlighting a role for either plaques or soluble Aβ. However, these dichotomous ideas are not mutually exclusive. Instead, we propose that plaques might act as a source of soluble, potentially oligomeric Aβ into its immediate vicinity (Fig. 2). This idea is supported by our previous studies, in which we had proposed that a plaque grows to a certain mature size and then remains in a steady state with its environment (Cruz et al. 1997; Hyman et al. 1995; Urbanc et al. 1999). This hypothesis was confirmed by direct imaging of plaques in serial multiphoton microscopy studies, in which plaque size remained unchanged after formation for the duration of the imaging period, up to 100 days (Christie et al. 2001). These data are consistent with the hypothesis that the area immediately surrounding a plaque has a high local concentration of Aβ that is in a steady-state equilibrium with deposited amyloid in the plaque.

Thus synaptic, and neural system, dysfunction could come from alterations induced by plaques or by soluble Aβ, acting on axons, at synapses, or at the dendrite receiving the synapse (or a combination of all of these).

Summary

AD is a devastating illness in which individuals lose cognitive function. Recent studies suggest that a vital contributor to its pathophysiology is disruption of synaptic function

by Aβ, but critical holes in our knowledge remain. We can now directly observe structural and functional changes in axons and dendrites. By studying well-characterized mouse models of AD pathology and human AD (and "treated" AD), we will increase our understanding of the mechanisms of cortical dysfunction in AD and the extent to which these changes can be reversed. Exciting results are already being obtained using anti-Aβ immunotherapy, which can reverse both amyloid deposits and some of the neuronal alterations associated with amyloid overproduction (Bacskai et al. 2001; Brendza et al. 2005; Lombardo et al. 2003). These types of data are a strong reason for optimism as new therapeutic interventions reach patients with AD.

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Kelly Del Tredici (*left*) and Heiko Braak (*right*)

Staging of cortical neurofibrillary inclusions of the Alzheimer's type

Heiko Braak[1] and *Kelly Del Tredici*[1]

Alzheimer's disease (AD) is the most widespread degenerative illness of the human central nervous system. It is progressive and is not subject to remission. Central to the pathological process that underlies this disorder is the formation of proteinaceous inclusions in selectively vulnerable neuronal types (Goedert 1993; Goedert et al. 1997; Jellinger 1998; Dickson 1999; Trojanowski and Lee 2000). The pathological inclusions consist, for the most part, of abnormal tau protein and appear as pre-tangle material, somatic neurofibrillary tangles (NFTs), dendritic neuropil threads (NTs), and abnormal material in dystrophic neurites of plaques (NPs; Braak and Braak 1991; Braak et al. 1994).

Physiologically, the tau protein stabilizes microtubules. In AD, it initially becomes abnormally phosphorylated. The soluble and non-argyrophilic "pre-tangle" material fills the cell body and neuronal processes (Biernat et al. 1992; Braak et al. 1994). Eventually, cross-linking occurs and non-degradable NFTs/NTs appear that display a marked argyrophilia (Braak et al. 1994; Esiri et al. 1997; Trojanowski and Lee 2000; Uchihara et al. 2001b). Nerve cells that contain NFTs/NTs can survive for decades but probably with functional deficits. Following the premature death of involved neurons, the pathological material becomes extraneuronal and remains visible in the tissue. With time, these remnants are no longer immunoreactive for abnormal tau protein and their argyrophilia gradually becomes less pronounced (Braak et al. 1994; Cras et al. 1995; Bobinski et al. 1998).

NFTs/NTs do not necessarily or consistently accompany senescence and, therefore, are distinguishable from normal age-related changes (Braak and Braak 1997a; Esiri et al. 1997; Hyman 1997, 1998; Hyman and Trojanowski 1997; Thal et al. 2004). Furthermore, the pathological process singles out select neuronal types while bypassing others (Hyman and Gómez-Isla 1994). All cortical nerve cells prone to the development of the proteinaceous inclusions are projection neurons with a long and thin axon. Short-axoned projection cells, such as the spiny stellate cells of the fourth neocortical layer or the presubicular parvocellular layer, resist the pathology, and local circuit neurons also remain intact (Morrison et al. 1998; Braak et al. 2000), with the exception of chandelier cells, which occasionally develop soluble pre-tangle material but then vanish from the tissue without developing NFTs/NTs. The vulnerable neuronal types share a second feature, namely, their axons are unmyelinated or have only a thin myelin sheath. Cortical projection neurons with a heavily myelinated axon, on the other hand, are resistant.

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Pertinent examples are Betz cells in the primary motor cortex and Meynert pyramidal cells in the striate area.

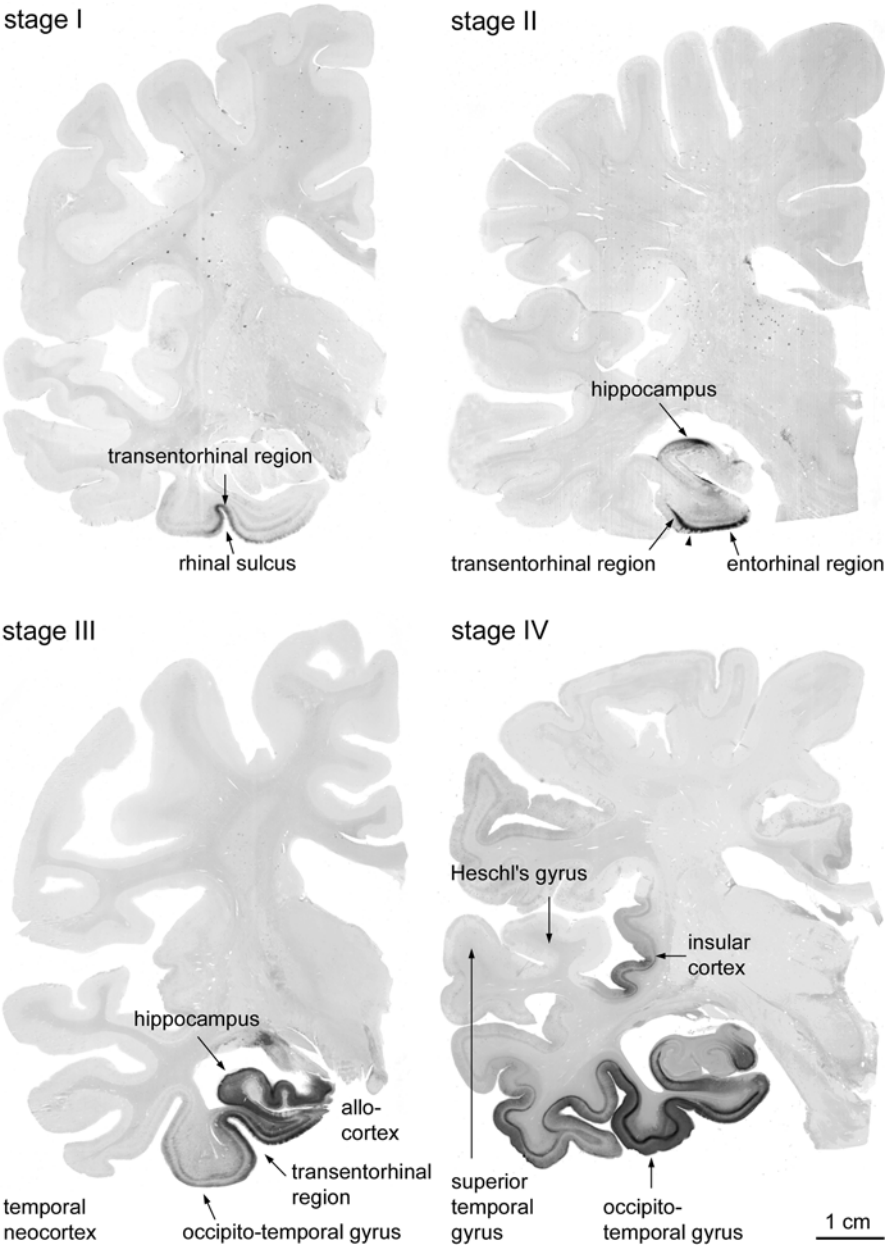
NFTs/NTs initially develop bilaterally and nearly symmetrically at predisposed cortical sites and in a few nuclei with diffuse projections to the cortex (Arnold et al. 1991). From there, they extend into additional cortical areas and subcortical nuclei (Figs. 1, 2, 3f,g). The sequence of changes in the topographical distribution pattern of the lesions is predictable and relatively consistent across cases. By pinpointing the location of affected neurons and the severity of the pathology, six neuropathological stages in the evolution of cortical NFTs/NTs can be differentiated (Braak and Braak 1991; Ohm et al. 1995; Duyckaerts and Hauw 1997; Hyman and Trojanowski 1997; Hyman 1998; Newell et al. 1999; Thal et al. 2004).

Stages I and II

The first cortical neurons to become involved are usually specific projection cells of the transentorhinal region, which is located in the rhinal sulcus found in anteromedial portions of the temporal lobe (Fig. 1, stage I). From there, the pathology reaches the superficial cellular layer (layer pre-α or layer II) and then the deep layer pri-α of the entorhinal region (Fig. 1, stage II; Braak and Braak 1992). Additionally, the first sector and/or second sector of the hippocampal formation become involved to a variable degree.

This neuronal damage slightly impairs the transmission of neocortical sensory information – via the transentorhinal region – to the entorhinal cortex and hippocampal formation (Fig. 3a). The overall negligible to mild destruction remains well below the threshold required for the manifestation of initial clinical symptoms (Grober et al. 1999) or even mild cognitive impairment. Stages I and II represent the pre-clinical period of AD.

Fig. 1. Stages I–VI of cortical neurofibrillary inclusions of the Alzheimer type in 100-µm hemisphere sections immunostained for abnormal tau (AT8, Innogenetics). In immunoreactions, the pathology can be seen even in paraffin sections with the naked eye (Braak et al. 2006). **Stage I**: involvement is slight and confined chiefly to the transentorhinal region, usually located on the medial surface of the rhinal sulcus (80-year-old female). **Stage II**: immunoreactivity additionally occurs in layers pre-α and pri-α of the entorhinal region. From the lateral border of the entorhinal region (arrowhead), the layer pre-α gradually sinks into a deeper position in the transentorhinal region. The lesions make headway into the hippocampal formation (80-year-old male). **Stage III**: the lesions extend into the high order sensory association areas of the basal temporal neocortex but usually not beyond the occipito-temporal and lingual gyri (90-year-old female). **Stage IV**: the third and fourth sectors of the Ammon's horn and a large portion of the insular cortex become affected. The involvement of the neocortical high order association cortex extends up to the medial temporal gyrus and stops short of the superior temporal gyrus. The primary fields of the neocortex (see the transverse gyrus of Heschl) and, to a large extent, also the premotor and first order sensory association areas of the neocortex remain intact (82-year-old female). Scale bar in stage IV applies to all hemisphere sections


stage I
transentorhinal region
rhinal sulcus
stage II
hippocampus
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entorhinal region
stage III
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allo-
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transentorhinal
region
temporal
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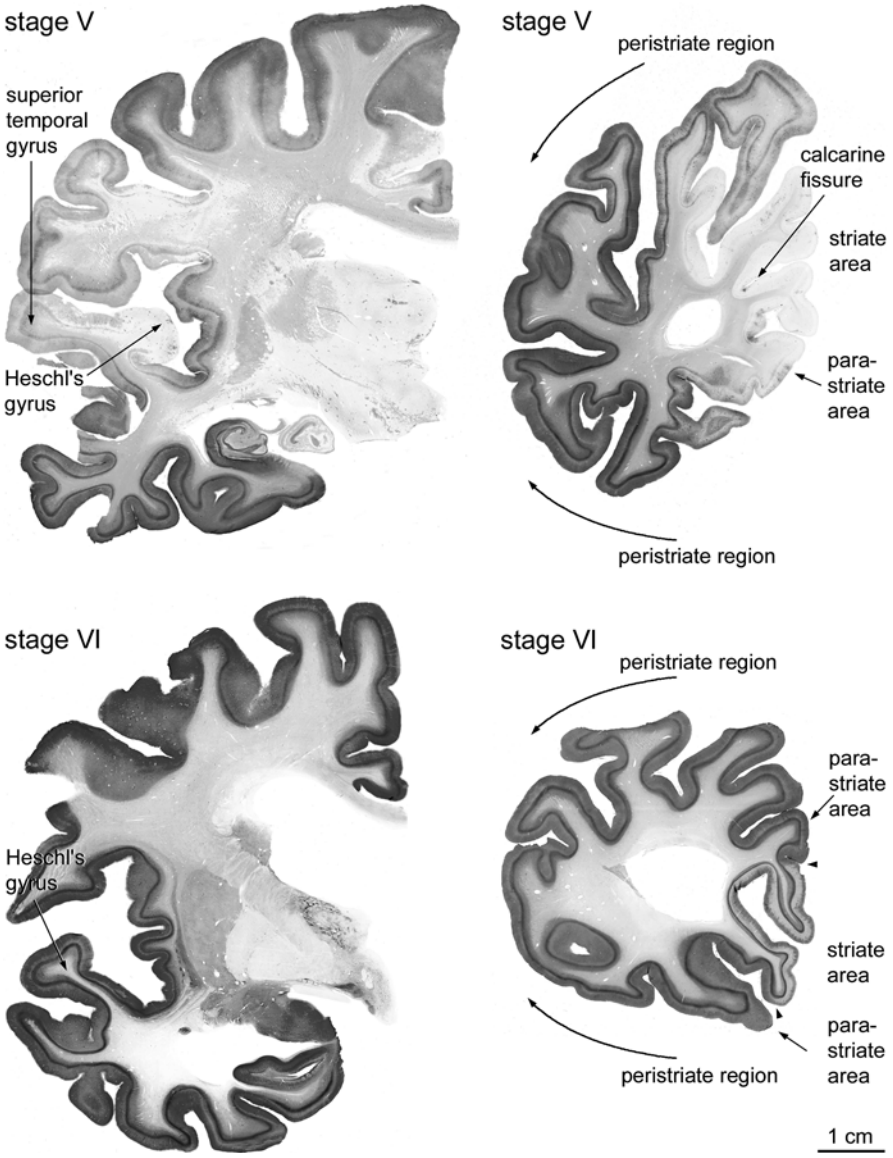

Fig. 2. Stage V: the pathology appears in the superior temporal gyrus and even encroaches to a mild degree upon the premotor and first order sensory association areas of the neocortex. In the occipital lobe, the peristriate region shows varying degrees of involvement, and lesions occasionally can even be seen in the parastriate area (90-year-old female). **Stage VI:** immunoreactivity is detectable even in the first order sensory association areas (e.g., the parastriate area) and the primary areas of the neocortex (e.g., the striate area, between arrowheads). Compare the superior temporal gyrus and transverse gyrus of Heschl at stage V with the same structures at stage VI (70-year-old female). Scale bar in stage VI applies to all hemisphere sections

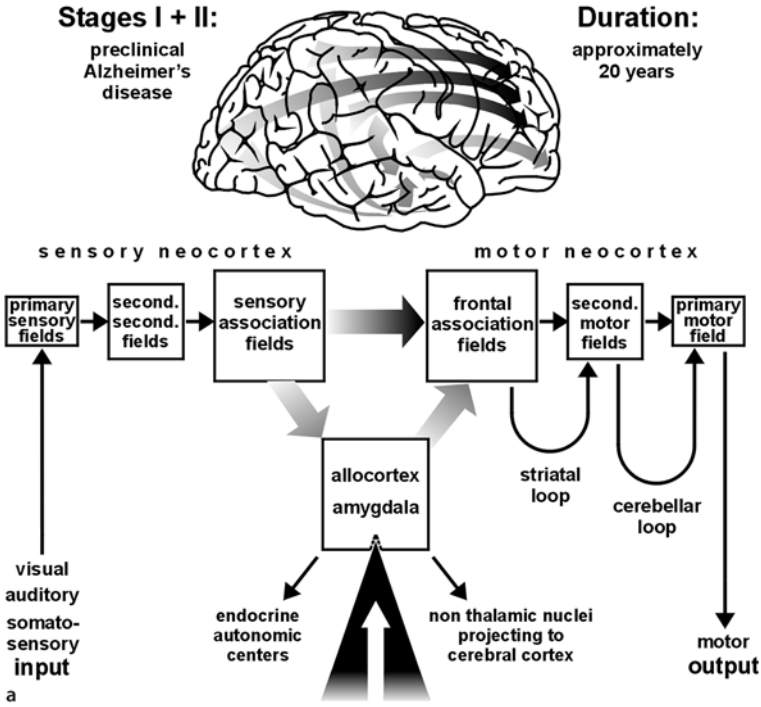

Fig. 3. **a** Initial changes in stages I–II slightly hamper the data flow from sensory association areas to superordinate centers of the limbic system (allocortex, amygdala). **b** Moderately severe lesions in stages III–IV disconnect the limbic centers from the neocortex. **c** The destruction in stages V–VI disconnects the sensory neocortex from the prefrontal cortex. **d,e** Neocortical myelination begins in the primary sensory and motor fields and progresses via first order sensory association areas and premotor areas to related high-order sensory association and prefrontal areas. With increasing distance from the primary fields, the average myelin content gradually diminishes and is minimal in the transentorhinal region. **f,g** An inverse relationship between the myelination process and neocortical destruction exists in AD. The first cortical lesions occur in the transentorhinal region and then extend into the adjoining high-order association areas. Eventually, the disease process reaches the neocortical first order association areas and primary fields. **h** Development of neurofibrillary pathology in non-selected autopsy cases (Thal and Braak 2005). White columns show the prevalence of cases devoid of AD-related intraneuronal inclusions, the shaded ones display the sequential appearance of the lesions (*light gray* – stages I–II, *dark gray* – stages III–IV, *black* – stages V–VI). A considerable number of individuals develop the initial lesions remarkably early in life

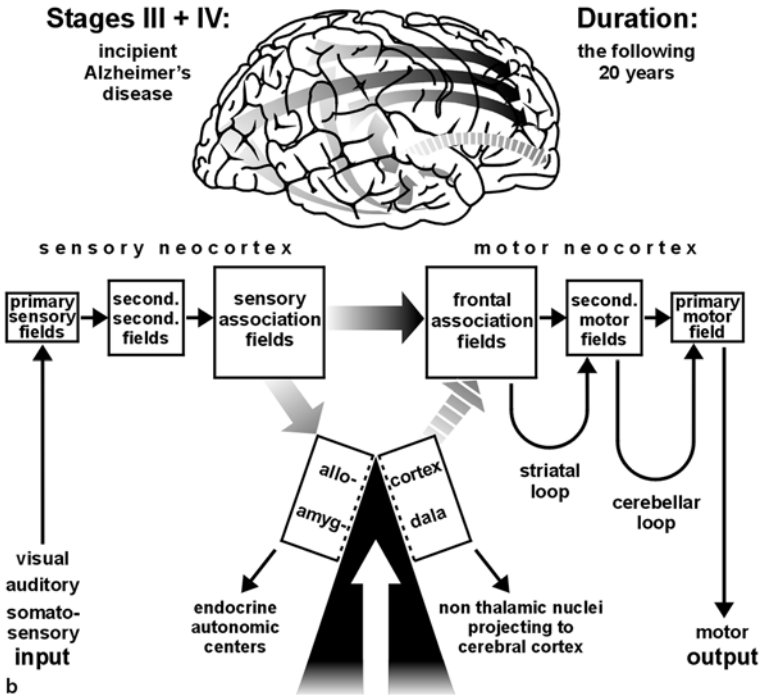

Fig. 3. (continued)

Stages III and IV

The lesions in previously involved sites worsen. From the transentorhinal region, the disease process encroaches upon the adjoining neocortex of the occipito-temporal (fusiform) and lingual gyri (Fig. 1, stage III). In stage IV, the disease process progresses more widely into neocortical high order association areas. In the temporal lobe, it gradually extends up to the middle temporal gyrus (Fig. 1, stage IV).

The tissue damage typical of the intermediate stages III and IV hampers the transmission of neocortical sensory information – via the entorhinal region and hippocampal formation (allocortex) – to the prefrontal neocortex (Fig. 3b). The clinical protocols of such individuals frequently make reference to slight impairment of cognitive functions, mild dysmnesia, and the presence of subtle personality changes. In some patients, the appearance of symptoms is still obscured by individual reserve capacities. Because initial clinical symptoms often become manifest in stages III and IV, these cases may be referred to as clinically incipient AD (Grober et al. 1999).

Stages V and VI

In stage V, the neocortical pathology fans out frontally, superolaterally, and occipitally, extending into additional high order association areas (Fig. 2, stage V). In the occipital

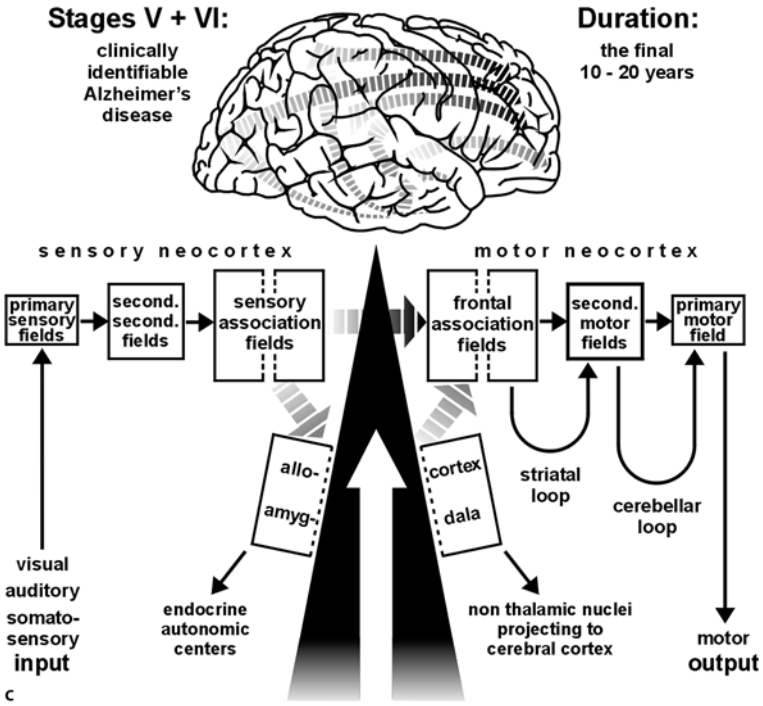

Fig. 3. (continued)

lobe, NFTs/NTs appear in the peristriate region (Fig. 2, stage V). In stage VI, even the secondary and primary areas of the neocortex become involved (Fig. 2, stage VI). The pathology passes through the parastriate area into the primary visual field, the striate area (Fig. 2, stage VI).

Persons with an extensive degeneration of neocortical sensory association areas and prefrontal areas (stages V–VI) present with severe dementia (Fig. 3c), and major deterioration of autonomic functions reflects the far-reaching destruction of limbic cortical and subcortical centers. In clinically diagnosed and neuropathologically verified cases of AD, the tau pathology takes its heaviest toll in the phylogenetically late-appearing and ontogenetically late-maturing high order association areas of the neocortex (Braak and Braak 1997; Grober et al. 1999).

The progression of the cortical pathology recapitulates the myelination process in reverse order

The sequential appearance of AD-related cortical destruction is the inverse of the cortical myelination process (compare Figs. 3d,e with Figs. 3f,g), thereby corroborating an earlier observation that regressive brain changes tend to repeat the maturation process but in reverse order (Rapoport 1988; Reisberg et al. 1992, 2003; Arendt et al. 1998; Mo-

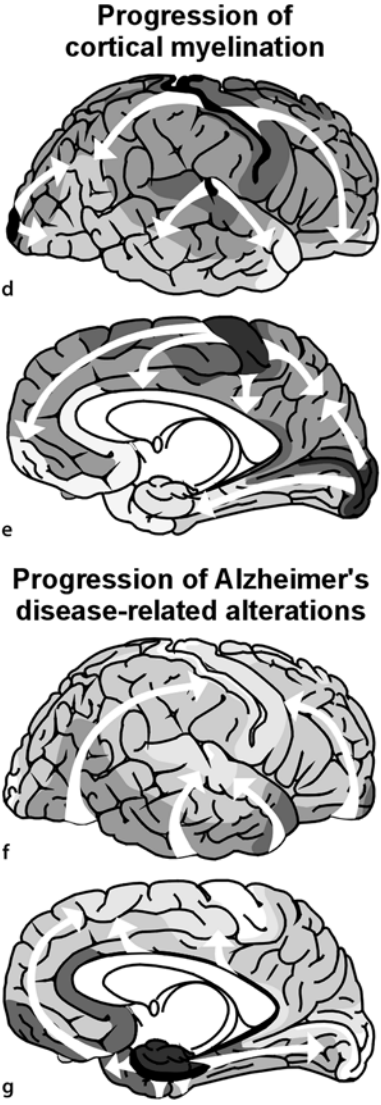

Fig. 3. (continued)

ceri et al. 2000). Whereas heavily myelinated areas (primary neocortical fields) remain virtually uninvolved, areas that undergo late myelination (transentorhinal region) are especially vulnerable and develop neurofibrillary lesions earlier in the disease process and at greater density (Braak and Braak 1996).

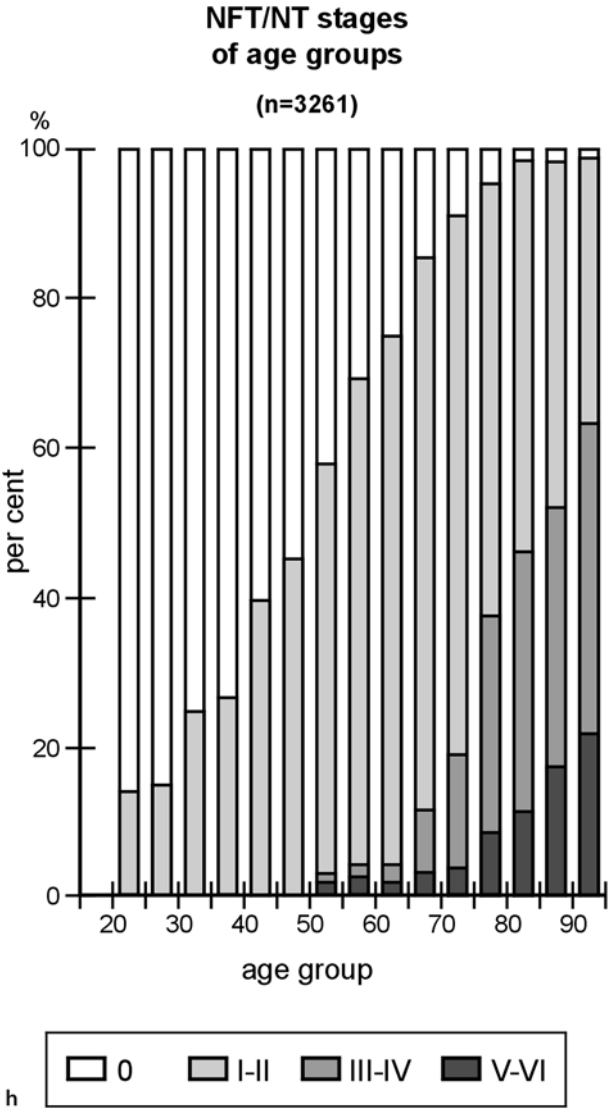

Fig. 3. (continued)

The relationships between age and AD-associated intraneuronal pathology

The percentages of non-selected cases at various stages and age groups display a continuum ranging from the first involved nerve cell to the destruction encountered in fully developed AD (Fig. 3h; Braak and Braak 1997; Thal et al. 2004). A certain percentage of individuals, even at a very advanced age, fail to develop the AD-related intraneuronal

inclusions (Fig. 3h). Considerable inter-individual differences exist with respect to the point at which the first affected nerve cells are detectable, but the initial lesions can appear remarkably early (Fig. 3h). In one study, 15–20% of cases in a group between 25 and 30 years of age already exhibited lesions corresponding to stage I (Braak and Braak 1997). Therefore, the onset of the pathological mechanisms that underlie AD is by no means confined to the aged or very old. Decades typically elapse between the beginning of the histologically verifiable process and stages of disease in which the lesions are widespread and sufficiently extensive for clinical symptoms to become apparent (Braak and Braak 1997; Thal et al. 2004).

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Charles Duyckaerts

Jean-Jacques Hauw

Of stains and brains: a brief account of how microscopic and clinical observations contributed to the understanding of Alzheimer's disease

Charles Duyckaerts[1] and *Jean-Jacques Hauw*[1]

Neurofibrillary tangles

At the eve of the 20th century, when Max Bielschowsky, using chemicals common in photography, described the silver technique to which his name is now attached, he believed that he had discovered a reliable way of staining the so-called "neurofibrils" (Bielschowsky 1902, 1903). Neurofibrils, described by Remak in 1838, make up a visible network of thin intracellular structures visible in the cell body and the processes (mainly the axon) of neurons. It is no wonder that Alzheimer, using Bielschowsky's method, described the abnormal accumulation of argyrophilic material seen in his first cases as "Fibrillenveränderung" (Alzheimer, 1911). He indeed believed that the neurofibrils of Remak were altered (Fig. 1). But what were these neurofibrils, seen with light microscopy using Bielschowsky's method? This has been a matter of debate since the advent of electron microscopy, with some authors stating that silver was deposited both on neurofilaments and on neurotubules and others believing that only neurofilaments were decorated (for review see Peters et al. 1976, p. 102). The hypothesis that neurofibrils were made of neurofilaments explains why neurofilament proteins were so actively sought in the neurofibrillary tangles (NFTs) when the nature of the lesions started to be probed with biochemical means. The original Bielschowsky method was performed on frozen section; a method adapted to paraffin was published by Yamamoto and Hirano (1986) and is still frequently used.

The tau revolution was announced with the discovery that the normal constituents of the neuron, present in the NFTs, were neither the neurofilament proteins nor the tubulins (Grundke-Iqbal et al. 1985a). Jean-Pierre Brion et al. (1985d) found that anti-tau antibodies labeled the NFT. The demonstration of NFT with anti-tau could well have been the first success of immunohistochemistry in the history of research on neurodegenerative diseases. Although the principle and the first immunohistochemical results were described in 1942 (Coons et al. 1942)), immunohistochemistry was not in common use in the neuropathology laboratories. The obsession with silver methods, the difficulty of the method or the lack of knowledge in immunology probably combined to prevent its use in degenerative neuropathology for nearly 45 years. It should be stressed that Bielschowsky's technique, in sharp contrast, was applied to neuropathology only four years after its initial description. The finding of tau accumulation in AD neurons was rapidly followed by the biochemical confirmation of abnormally phosphorylated tau in isolated NFTs or homogenized samples from Alzheimer's disease (AD) brains (Delacourte and Defossez 1986; Grundke-Iqbal et al. 1986a,b).

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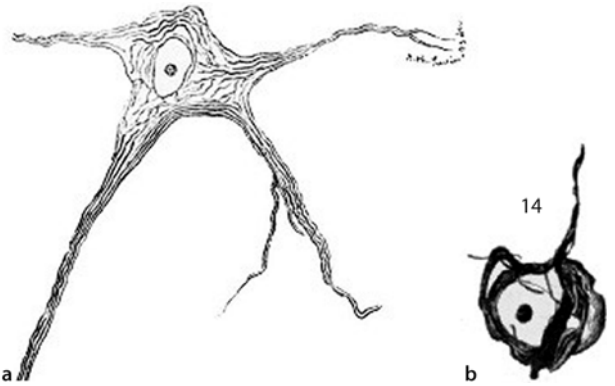

Fig. 1. Bielschowsky's technique. **a** One of the original pictures from Max Bielschowsky's paper(1903). Neurofibrils are perfectly visible in the cell body and in the processes of this neuron from the anterior horn. **b** A neurofibrillary tangle according to Alzheimer's pupil, Perusini (1910). The neurofibrillary tangle was thought to be an alteration of the neurofibrils, seen in a

The senile plaque

The existence of senile plaques was known before that of the NFTs. Emil Redlich (1898) called them "miliary sclerosis" and Oskar Fisher (1907), "miliary necrosis". The latter author thought that they were characteristic of senile dementia. The dense core of the senile plaque resembles a small ("miliary") focus of necrosis, but Alzheimer understood that it was "evidently formed by the deposition of a pathological metabolic product" (Alzheimer 1911). Fisher and Alzheimer observed the neuritic component of the plaque; Ramon y Cajal thought that the axonal component of the "plaques of Fisher" was the "genuine" expression of regeneration (Ramon y Cajal 1928, p. 735). The proof that the core of the plaque was not necrosis came with the use of Congo red, which exclusively stained the plaque core (Divry 1927). Congo red, an azo dye, was synthesized in 1883 by Paul Böttiger, working in the dyestuff chemical laboratory of the Friedrich Bayer Company. The red dye did not require a mordant to stain cotton (hence the term "direct" dye). Paul Böttiger sold his patent to AGFA, which associated the color of the dye with the name of Congo, probably for commercial reasons, suggesting that its brilliant color evoked that of the textiles then coming from the colonies (Steensma 2001). With Bennhold (1922) Congo red became the "specific" stain of the amyloid substance, hence the importance of the discovery of Divry, which placed AD among the amyloidosis-related disorders. Amyloidosis was actually first described in general pathology. One unique substance was initially thought to accumulate in organs (spleen, liver, kidney) or muscle of the heart or tongue, causing their hypertrophy and making them abnormally firm. Virchow had applied lugol, a solution of iodine, on a section of such a hypertrophied organ and found that it first turned deep brown and changed to blue when sulfuric acid was applied. Such a reaction had already been obtained with starch (amylos in Greek) – hence the term amyloid (= which resembles starch). In the quest for "the" amyloid substance, it was first discovered that it had the characteristics of a protein. But the search for that protein remained unfruitful for decades, due

to its insolubility; amyloid was indeed difficult to purify and thus to sequence. A new extraction method in pure distilled water (Pras et al. 1968) indicated that many peptides could become "amyloid," with all of them having the basic property, discovered by George Glenner, of being rich in β-pleated sheet structure (Glenner 1980; Glenner et al. 1973). Glenner also partly sequenced the amyloid peptide of AD and found it to be different from all proteins then known (Glenner and Wong 1984a). This identification led to the production of antibodies (Davies et al.1988) that beautifully labeled not only the senile plaques but also deposits that were Congo red-negative and interspersed between neurons, the so-called diffuse deposits (Delaere et al. 1990). Mutations of the amyloid precursor protein (APP) cause familial AD, suggesting that the disturbance of Aβ metabolism is the initial pathogenetic factor (the so-called "cascade hypothesis;" Hardy 1992, 1999, 2002).

Clinico-pathological correlation

The question about the relationship between the lesions and the clinical signs had been asked since the very first descriptions of the disease by Alzheimer himself (1911). He mentioned that there was apparently no link between the senile plaques and the cognitive deficit in senile dementia. He also observed that the general aspect of the cortex appeared nearly normal with Nissl stain. The conclusion that his reader must have drawn was the importance of the role played by the NFTs, the lesion that he had specifically described. However, the relationship with the clinical symptoms needed new methods to be uncovered. Roth et al. (1966) succeeded in revealing a quantitative relationship between the density of the lesions and the clinical signs by quantifying both the lesions and the clinical deficit and looking for their linear correlation. We and others used this statistical correlation to improve our knowledge of the progression and natural history of the disease. We found that NFT in the hippocampus and parahippocampal gyrus could occur without Aβ deposition in aging brains and were, therefore, probably the first lesions to appear. Diffuse deposits were sometimes observed in individuals that were considered intellectually normal (Delaere et al. 1990). They were generally found in cases that were less affected than those with Congo-red positive deposits. The neuritic component of the senile plaque and the NFTs were the lesions found in the most advanced cases .

Neuritic pathology in the hippocampal and parahippocampal gyri precedes diffuse, then amyloid deposits and finally neuritic pathology in the isocortex. This progression is not compatible with the cascade hypothesis, at least in its pure form, which predicts that the amyloid deposits are the initial lesions. The progression suggests, on the other hand, that the hippocampal-parahippocampal gyri (where neuritic pathology occurs first) and the isocortex (where amyloid deposits are first observed) do not behave similarly in AD. (Fig. 2)

The Aβ deposition seen in the various isocortical areas probably takes place within a very short time interval. In the cases where Aβ deposits are observed in the isocortex, they are usually present in *all* the areas, with no spared regions. The symptoms should, therefore, occur all at once, without progression, if they were directly caused by Aβ deposition. This is not the case: clinicians have indeed observed a regular increase in the severity of the cognitive deficit that has been divided into stages (Reisberg et

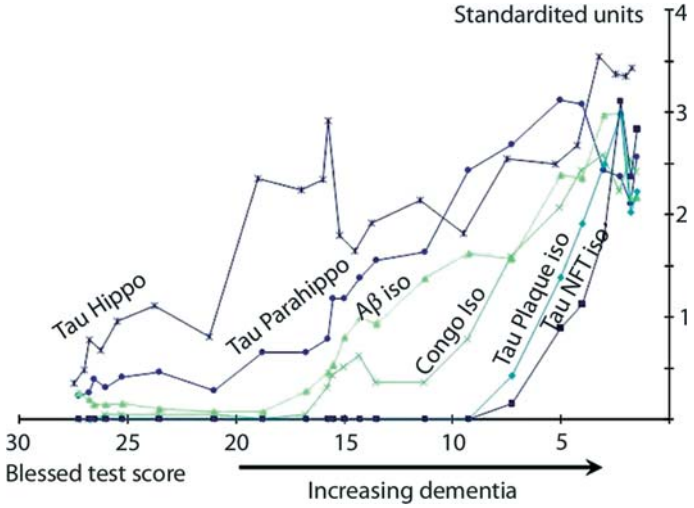

Fig. 2. The sequence of the lesions in Alzheimer's disease. BTS: Blessed test score. Tau Hippo: Density of tau-positive neurofibrillary tangles in the hippocampus. Tau Parahippo: Density of tau-positive neurofibrillary tangles in area TF-TH in the parahippocampal gyrus. Aβ iso: density of Aβ deposits in area 17. Congo iso: density of Congo red-positive plaque cores in area 17. Tau plaque iso: density of the senile plaques containing tau-positive neurites in area 17. Tau NFT iso: density of tau-positive tangles in area 17. *Curves* are running averages, one point being the mean of five successive cases and the cases being ranked according to their Blessed test scores

al. 1982) that appear to correspond to neurofibrillary progression (Delacourte et al. 1999; Duyckaerts et al. 1997a). NFTs indeed affect selectively one area after the other in a very stereotyped manner, so stereotyped in fact that the severity of the disease may be inferred from the topography of the neuritic pathology – a property that was used by Heiko and Eva Braak to devise their staging procedure (Braak and Braak 1991). It should be noted that their brilliant description was based on a somewhat old-fashioned technique. They used a silver method that, contrary to immunohistochemistry, was applicable to thick sections: antibodies do not diffuse in the whole thickness of the section whereas silver ions do. Because the density of lesions in a thick section is higher than in thin ones, the signal they give is stronger and may even be apparent macroscopically. Heiko Braak could thus illustrate his talks with real sections in which the lesions were visible to the naked eye. The silver technique that was used relied on "physical reduction," a procedure in which both silver ions and a reducing agent are mixed in the same solution, together with a protective colloid that hinders the first two components from reacting with each other (Gallyas 1971). For some unknown reasons, Gallyas' method beautifully labels Alzheimer changes as well as the alterations seen in progressive supranuclear palsy and corticobasal degeneration but does not stain Pick bodies (i.e., 3 repeats tau; Uchihara et al. 1998, 2000, 2001a). The mechanism that links the presence of NFT and the clinical deficit is not known. We have noticed that the density of the NFT in a given area is not an important factor. One single tangle appears to be associated with the dysfunction of the area where it appears (Duyckaerts et al. 1997a), suggesting that tangle formation is but the visible evidence of a hidden process

that causes the deficit. Using Bielschowsky's technique, Alzheimer detected not only the "true" neuritic plaques, i.e., those with tau-positive neurites that are statistically linked with the clinical deficits, but also the Aβ diffuse deposits that do not cause symptoms when isolated. This finding may be one of the reasons explaining the common opinion that "plaques" are not related to dementia.

What is the link between plaques and tangles?

Plaques and tangles, amyloid and neuritic pathologies, Aβ peptide and tau proteins are like the faces of Janus, indissolubly joined in AD. Neuritic lesions appear to be associated with the involvement of systems of connections: plaques occupy layers that are known to convey cortico-cortical connections (Duyckaerts et al. 1986), disconnected pieces of cortex do not exhibit tau pathology (Duyckaerts et al. 1997b), and progression of neuritic pathology follows anatomical pathways (Duyckaerts et al. 1992). However, Aβ deposition is observed in transgenic mice bearing the mutated gene of human APP, whereas tau pathology is, at the most, mild: NFTs are absent; the neuritic corona of the senile plaque is tau negative with most antibodies (Blanchard et al. 2003). Despite this apparent lack of tau pathology, the injection of anatomical tracers in the brain of transgenic mice showed that cortico-cortical fibers came into contact with the core of the amyloid plaques of the cortex and were dilated and grossly abnormal, whereas the thalamic fibers avoided them and kept a normal aspect in their vicinity (Delatour et al. 2004). Even in the mice, it therefore appears that the senile plaques are "innervated" and that this innervation involves specific systems of fibers.

The connection between Aβ and tau pathology remains elusive. The senile plaque, where Aβ deposits and neurites come into close contact, is probably the point where the two processes meet. Are the neurons whose axons enter into contact with the amyloid deposits those that develop NFTs? Do the tangles in the entorihinohippocampal complex seem to be the first to appear because of a funnelling effect, with the entorihinohippocampal complex being connected with so many large areas of the cortex, which cannot be sampled thoroughly that it is impossible to sample thoroughly and which could contain small amount of Aβ? Most neuritic plaques do have an amyloid core: is amyloid a requisite for neuritic pathology? Could preventing Aβ deposition impede tau pathology even in the hippocampus? These are but a few questions that will need new methods or new studies to be answered, with in vivo visualization of Aβ deposits being probably the longest-awaited one.

Michel Poncet

Presenile forms of Alzheimer's disease in 2006

Michel Poncet[1], *Olivier Felician*[1], and *Jean-François Pellissier*[2]

The relationship between neuropathological lesions present in Alzheimer's disease (AD) and senile dementia was quickly established. In 1909, based on his observation of Auguste D. and three other similar cases, Perusini noted that "the pathological process recalls main features of senile dementia; however, the alterations in the case described are more far reaching." (Perusini, 1909) The following year, Kraepelin attributed this condition to Alzheimer in the eighth edition of the Handbook of Psychiatry (1910). He made it clear that "although the anatomical findings suggest that we are dealing with a particularly serious form of senile dementia, the fact is that this disease sometimes starts as early as in the late forties." It was only later (Terry 1978) that senile dementia and AD became considered as a single common entity, placing this affection in the forefront of neurodegenerative diseases.

Our objective is to show that this unitary vision of AD deserves to be reconsidered and that the presenile forms of AD have distinct clinical and neuropathological features (severity and localization of lesions) that need to be taken into account.

Presenile AD and genetic background

Presenile AD refers in general to the form of AD in which the first symptoms appear before 65 years of age. Let us recall that, in this group, the autosomal dominant forms are over-represented. According to the study by Campion et al. (1999), the prevalence of the forms with presenile onset (here between 40 and 60 years of age) is approximately 40 per 100,000. There is a family history in 62% of the cases and a history compatible with a dominant autosomal transmission mode in 13% of cases. Finally, a mutation on the gene coding for the precursor of the β-amyloid protein (APP) or on the presenilin 1 gene was identified in 71% of the latter cases. These results taken together illustrate the strong influence and the heterogeneity of the genetic factors found in the early-onset forms of AD.

Presenile forms and clinical-pathological expression

The distinction between presenile and senile forms has been a matter of debate for a long time. Delay and Brion (1962) emphasized the relatively short period of evolution

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of the disease observed in the young subject (two to four years once the dementia criteria were reached). In addition, if the amnestic syndrome appears first, it is quickly accompanied by severe disturbances of instrumental functions (the "apraxic-aphasic-agnosic syndrome"), reflecting, according to the authors, "the most striking aspect of the disease." Finally, these authors observed that awareness of the disease was "oddly preserved for a long period of time in spite of dementia", leading patients sometimes to major depressive reactions. The authors contrasted these elements to those observed in the older subject suffering from "senile dementia." In the older subject, they noted a slightly longer evolution, severe memory impairments (sometimes with confabulatory reconstructions and false recognitions, corresponding to a "presbyophrenic" form), as well as the absence of typical language and praxic deficits.

Later work compared the neuropsychological profile of the disease forms of the young subject and the old subject. Concerning the presenile forms generally, longitudinal studies showed a shorter evolution of the disease (Jacobs et al. 1994). The neuropsychological profiles of early-onset and late-onset subjects were compared, particularly on linguistic variables. The majority of the studies observed an effect of age: language deteriorated earlier in the young subject (Seltzer and Sherwin 1983; Chui et al. 1985; Filley et al. 1986; Faber-Langendoen et al. 1988; Binetti et al. 1993; Imamura et al. 1998). A relative sparing of memory function was also reported (Binetti et al. 1993). Variable conclusions were obtained with regard to the examination of ideomotor praxis (Seltzer and Sherwin 1983; Reid et al. 1996).

MRI studies revealed a more important progression of cortical atrophy in the young subject (Woo et al. 1997). A very recent work compared the profile of cortical atrophy of two groups of subjects suffering from early-onset or late-onset AD using morphometric MRI (Frisoni et al. 2005). These two populations had an equivalent degree of severity of dementia and were compared with two groups of age-matched subjects. For the early-onset subjects, atrophy predominated in the temporo-parietal junction and, to a lesser degree, in prefrontal regions. For the late-onset subjects, atrophy prevailed in the hippocampical area as well as in the inferior and anterior temporal regions. Additional data were obtained using perfusion and metabolic imaging; parietal and posterior cingulate dysfunction was observed in early-onset subjects (Salmon et al. 2000; Sakamoto et al. 2002; Kemp et al. 2003).

In summary, these clinical and radiological observations lead the hypothesis that early-onset and late-onset forms have distinct underlying topographic distributions of lesions.

However, the neuropathological comparisons are scarce and have encountered methodological difficulties. Indeed, the brains available generally come from subjects who have died at the end of severe dementia, making any study of lesion progression difficult. Delay and Brion (1962) compared the presenile forms to the senile forms based primarily on quantitative differences. According to these authors, the lesions found in the older subject were similar to those observed in the young subject, but they were fewer and less diffuse. Recent studies came to similar conclusions (Ho et al. 2002). In our own experience, the lesion process appears definitely more important in the isocortex in the young subject, in terms of senile plaques and neurofibrillary tangle (NFT) density, but also in terms of neuronal loss. It is interesting to note that certain structures classically spared in late-onset forms (such as the primary visual cortex) were severely affected in presenile forms. Finally, differences relating to the neurotrans-

mission systems have also been reported. In the older subject, the cholinergic system appeared to be selectively affected within medial temporal lobe structures. In contrast, cholinergic disturbances were more diffuse in the younger subject and were more likely associated with dysfunction affecting other neurotransmission systems (Rossor et al. 1984; Ho et al. 2002).

These studies, whether clinical, neuroradiological or neuropathological, all suggest an earlier and more severe disruption of neocortical associative regions in the younger subject.

Alzheimer's disease forms with prevalent instrumental impairment

These forms begin with the insidious and progressive alteration of one instrumental function, in particular vision and language (Didic et al. 1999). The atypical character of their mode of presentation suggests that the lesion process has some intrinsic characteristics, in particular regarding its topographic distribution. It is striking to note that these forms generally appear in the young subjects.

Visual variants of Alzheimer's disease

These forms, which have been known for a long time, gained renewed popularity in the 1980s under the term "posterior cortical atrophies"(Benson et al. 1988). Clinically, they are characterized by visuospatial impairments, in the case of right hemisphere or bilateral lesions, and by the presence of one or several elements of the Gertsmann syndrome – agraphia, acalculia, digital agnosia and right/left confusion – in the case of left hemisphere lesions. Awareness of the deficits is often sharp. Standardized memory evaluation is often overshadowed by important instrumental deficits. Memory, however, remains generally preserved (particularly autobiographical memory). Neuroradiological examination indicates damage to occipitoparietal structures, most often bilateral.

The visual forms of AD rarely touch the older subject. For example, a clinical study carried out in our unit gathered 25 cases of patients who had presented with a neurodegenerative condition characterized by recent progressive visual defects. Within this group of patients, a sub-group composed of 12 subjects was characterized by a clinical and radiological evolution compatible with a diagnosis of probable AD. In this group, mean age of onset was 57 years ($\pm$8 years; Ceccaldi et al. 2002). In another study of four such cases where AD was confirmed at autopsy, the mean age of onset was 52 years ($\pm$3 years; Renner et al. 2004). Early onset was also present in the seven clinical and pathological observations (58 $\pm$ 6 years) reported by Tang-Wai et al. (2004). The originality of this last study was to compare the topographic distribution of the NFT and senile plaques (SP) in these patients with the topographic distribution in a group of 30 subjects who had presented with a classical form of AD (matched for age and gender). Concerning the SP, few differences were observed. However, a significantly greater density of NFT was present in the primary visual and associative cortices as well as in the inferior parietal region in those with the visual variant of AD. In contrast, the NFT density was significantly lower within superior and medial temporal lobe

structures. These observations highlight the importance of the relation between the topographic organization of NFT and the clinical expression of AD, as suggested by other studies (Delacourte et al. 1999). They also emphasize the clinical and pathological heterogeneity of presenile forms of the disease.

Linguistic variants of Alzheimer's disease

Prevalent linguistic impairments are not frequent in this disease and are rarely isolated. Once again, when they occur, these forms only rarely affect the very old person (Mesulam and Weintraub 1992; Galton et al. 2000). The speech difficulties generally resemble an aphasia described as "logopenic," characterized by a slow and hesitant speech in spontaneous expression, anomia upon presentation of pictures, difficulty at the level of repetition and syntactic comprehension, and contrasting overall with normal performance on tests of single-word comprehension and semantic matching (Gorno-Tempini et al. 2004). More rarely, language impairments mimic non-fluent aphasia or a form of fluent aphasia with prevalent semantic deficits (Galton et al. 2000). Secondary impairments concern visuospatial and visuoconstructional abilities, as well as moderate memory impairments in a clinical setting (although autobiographical memory is preserved). Autonomy is intact during a long period of time, as is a certain awareness of the deficits. Radiological examinations indicate abnormalities in the posterior temporal cortex and in the left inferior parietal lobe (Gorno-Tempini et al. 2004). At the neuropathological level, relative sparing of medial temporal lobe structures associated with a severe disruption of lateral superior temporal regions has been reported in a number of observations (Greene et al. 1996; Galton et al. 2000).

Other instrumental forms of Alzheimer's disease

More anecdotally, AD can begin with visuospatial and visuoconstructional disorders associated with left motor disturbances, suggestive of right parietal dysfunction (Crystal et al. 1982; Ceccaldi et al. 1995). Again, these atypical characteristics have essentially been reported in the young subject.

Conclusion

Contrary to the late-onset forms that are marked by slowly progressive impairments of memory, the clinical expression of AD in the young subject seems to be characterized by the early onset of important disturbances of instrumental functions. Moreover, atypical presentations, which are characterized by the progressive disruption of one instrumental function (visual or linguistic), appear to be more frequent in younger subjects. This pattern is likely due to certain underlying neuropathological specificities, such as the topographic location and spread of NFT within medial temporal lobe and isocortical structures. Thus, the clinical expression of AD in older subjects is probably strongly conditioned by the structural vulnerability of medial temporal lobe structures to the development of NFT during the course of aging. In contrast, NFT may not always be prevalent within medial temporal lobe structures in the younger subject,

as evidenced by the nature and heterogeneity of the clinical features. Furthermore, in some cases, NFT density even appears to be secondary in these regions whereas they abound in other neocortical regions. These phenotypical variations likely operate under the strong influence of genetic factors, which not only condition the sequence of pathological events but also the capacities of structural and behavioral compensation of each individual.

The commemoration of the centennial of the communication made in Tubingen invites us, in light of recent clinical observations, to step back to the work of Alzheimer and Kraepelin and consider the singularity of presenile forms of AD, which have been rather neglected by research over the last decades.

Cholinergic Deficit

Peter Davies

The cholinergic deficit in Alzheimer's disease

Peter Davies[1]

In the period immediately prior to publication of the first reports of cholinergic markers in the brains of patients with Alzheimer's disease, there was very little hard science going on in this field. This was before the recognition that Alzheimer's disease was the most common cause of dementia in the elderly, and it was generally thought to be a rather rare, pre-senile dementia. Having spent some time trying to do neurochemical analyses on the brains of schizophrenics, I became disillusioned by the subjectivity of the diagnostic criteria and looked around for a brain disease that was reasonably common and one in which objective, neuropathological diagnosis was possible. The late AJF Maloney convinced me that Alzheimer's disease would be a relatively easy problem to tackle, and so my systematic biochemical studies were undertaken in 1975. I was not aware at that time that two other groups in Great Britain were doing almost exactly the same things as I was, and the discovery of the cholinergic deficit in Alzheimer's disease was made independently and simultaneously by three groups: David Bowen and his colleagues in London (Bowen et al. 1976; White et al. 1977), Elaine and Robert Perry in Newcastle (Perry et al. 1977), and myself with AJF Maloney in Edinburgh (Davies and Maloney 1976). All three groups reported marked losses of choline acetyltransferase and acetyl cholinesterase activities in the brains of patients with histologically confirmed Alzheimer's disease, observations that have been repeated many times.

My own work was very much influenced by the work of Oleh Hornykiewicz on the dopamine deficiency in Parkinson's disease (Hornykiewicz 1970, 1971, 1973), and even in 1976, the therapeutic implications of the cholinergic deficiency were obvious. A psychiatrist I worked with went to an Edinburgh health food store and bought a supply of choline bitartrate with the intention of trying it in Alzheimer patients, even before the Lancet paper was published. Many similar attempts followed, with choline and later with lecithin (phosphatidyl choline), before cholinesterase inhibitors were tested and became the standard treatment. Without going into a review of the effectiveness of therapy using cholinesterase inhibitors, it is notable that we are still waiting for something better, 30 years after the initial reports on the cholinergic deficit.

The most dramatic effect of the early work on the cholinergic system was the vastly increased attention that Alzheimer's disease received from the research (and funding) community. The thought that Alzheimer's disease might be a specific degenerative disorder, akin to Parkinson's disease, and that it might be possible to develop rational treatments spurred a huge increase in basic science and clinical investigation. My own

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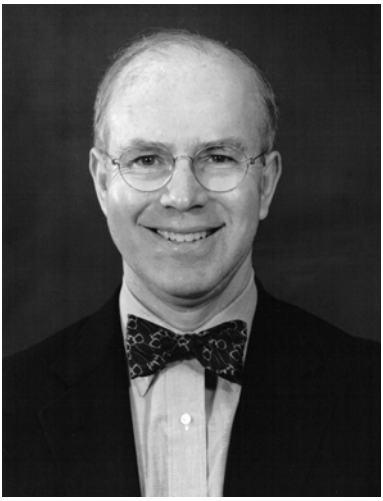
experience suggests that patients and their families have also benefited from having some treatment options, however effective, rather than none at all. My own research path from those early days led to a move from Edinburgh to New York in 1977 and the beginning of the obsession with understanding why and how cholinergic neurons are impacted by Alzheimer's disease.

Having the cholinergic neurons as the center of the research program, early efforts sought to find out what specific mode of cell death or cell dysfunction was taking place. A paper by Zipser and McKay (1981) suggested that monoclonal antibodies could be made to mark specific neurons in the leech, and I hypothesized that monoclonal antibodies might be able to specifically mark cholinergic neurons in brain tissue from Alzheimer cases. These antibodies would then provide probes for the molecular abnormalities occurring specifically in these cells. A senior colleague at the time called this a "stupid experiment." Antibodies were produced in mice using ventral forebrain homogenates from Alzheimer cases, and the resulting hybridomas were screened for the ability to discriminate normal and Alzheimer tissues. A very large number of antibodies have been produced in this way, and the best discrimination was found with antibodies that recognized various modifications of the microtubule associated protein tau (Wolozin et al. 1986). Of course, these antibodies did not just mark a specific degeneration of the ventral forebrain cholinergic neurons, but a much more widespread pattern of neuronal involvement. Because of this fact, our interest in molecular mechanisms also widened to include much of the cortex, including the hippocampus. However, it is still clear that these monoclonal antibodies do mark a type of cell dysfunction and death that, while not entirely specific to Alzheimer's disease, is absolutely characteristic of this condition. Tau abnormalities remain a defining feature of Alzheimer's disease and, as far as I am aware, are the earliest and most consistent signs of neuronal dysfunction in this condition. Whether or not tau abnormalities kill cells is, to some extent, irrelevant to me (but see Hutton et al. 1998; Spillantini et al. 1998c). I use the presence and nature of tau abnormalities as a sign that the process of Alzheimer's disease is underway. If tau abnormalities are absent, then whatever processes are ongoing, they are not Alzheimer's disease. This statement is not meant to be as dogmatic as it sounds: it is simply a way of focusing attention on the mechanism of cell dysfunction and death that occurs in the human disease. This is the mechanism I want to understand, and it invariably involves changes in tau.

This is not the place to review all the work on tau we and many others have carried out over the last two decades. My lab has made use of tau as a "reporter protein" in trying to identify the sequence of activation of various protein kinases or other signal transduction systems in the Alzheimer brain. Some of the most helpful series of papers in this effort were the "staging" papers from the Braaks (Braak et al. 1993; Braak and Braak 1995, 1997b). Reading these papers I realized that, most of the time, Alzheimer's disease affected the brain in a predictable fashion, "spreading" out from the hippocampus and ventral forebrain in an orderly fashion that could be used to great advantage in biochemical studies. In early-stage cases, it was possible to predict which regions would be involved and which regions would be apparently unaffected. However, if the disease was really progressive, it was possible to identify brain regions in these early cases that were on route to Alzheimer's disease *before* there was any sign of tau pathology. Of course, there are exceptions to the Braak staging scheme, cases in which the disease appears to begin somewhere other than the hippocampus and cases

in which the hippocampus is spared almost completely, but the majority of cases do appear to follow a consistent pattern of progression. Perhaps one of the great mysteries of Alzheimer's disease is what we mean when we say the disease "spreads." Why is there a seemingly orderly progression of the disease? What dictates this order, and what dictates which regions are affected when they are? The simple answer, probably the one that would be given by the majority of the participants at this meeting, would be Aβ. If this is true, it is not the visible amyloid deposits (Braak et al. 1989; Davies 1994) but some invisible species as yet undefined. Even if this proves to be the case (and I predict it will not), we will still have to unravel the mechanism by which this mystical Aβ species causes neuronal tau abnormalities and the subsequent cell dysfunction and death.



John H. Growdon

Acetylcholine in AD: Expectations meet reality

John H. Growdon[1]

Summary. The neuropharmacological consequences of finding evidence for deficient acetylcholine neurotransmission in AD have been complex. The initial optimism for a quick cure from choline or lecithin precursor administration, inspired by the success of levodopa in Parkinson's disease, quickly faded when put to the test. Nonetheless, the cholinergic hypothesis of memory dysfunction in AD was valid, and eventually it led to the introduction of AChEI drugs to increase acetylcholine transmission. Drugs of this class are the mainstays of current treatment for AD, even though their effects are generally modest. In the search for improved symptomatic and possibly neuroprotective treatments, acetylcholine may have an unexpected role. The observation that M1 and M3 receptor stimulation with cholinergic drugs drives APP processing into the α-secretase pathway adds a modern coda to the acetylcholine-AD story that is still unfinished.

Introduction

As my personal note on the history of Alzheimer's disease (AD) research, I would like to highlight a paper entitled "Increase in hippocampal acetylcholine after choline administration," by Madelyn J. Hirsch, John H. Growdon, and Richard J. Wurtman, (Hirsch et al. 1977) for sentimental reasons: it was my first paper on acetylcholine and it set the stage for investigations in AD that would occupy much of my subsequent career.

Neurologic Disease, Neurotransmitters and Neuropharmacology

In 1975, I joined Dr. Richard Wurtman's laboratory at the Massachusetts Institute of Technology as a postdoctoral research fellow. As a neurology resident, I had been intrigued by a clinical report that post-hypoxic intention myoclonus was linked to deficient serotonin neurotransmission (Lhermitte et al. 1972), which prompted me to develop an animal model of myoclonus produced by a serotonin neurotoxin (Stewart et al. 1976). I went to work in the Wurtman laboratory because he was a leading authority on serotonin metabolism, and I wished to learn more about the factors that controlled the synthesis and effects of this neurotransmitter. I knew that Wurtman and his colleague, John Fernstrom, had made a startling discovery: variations in plasma levels of the serotonin precursor amino acid tryptophan caused parallel changes in the amounts of serotonin synthesized in the brain (Fernstrom and Wurtman 1971). I had

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just joined the laboratory when Wurtman, along with a graduate student, Edith Cohen, published a paper showing that brain levels of another transmitter, acetylcholine, were regulated by the availability of choline, the natural dietary precursor for acetylcholine biosynthesis (Cohen and Wurtman 1976). These unexpected effects on brain of naturally occurring dietary constituents that are precursors for neurotransmitters raised the intriguing possibility that dietary substances could be used in the treatment of brain diseases (Growdon et al. 1977a).

Linking neurotransmitter abnormalities to neurologic disease was an exciting new approach to many neurodegenerative diseases (Moskowitz and Wurtman 1975). The most stunning example of neurotransmitter replacement treatment was, of course, Parkinson's disease: administration of the naturally occurring biochemical intermediate in dopamine synthesis, levodopa, produced dramatic suppression of many symptoms, including tremor and bradykinesia. A similar, although less dramatic, benefit occurred in post-hypoxic intention myoclonus following treatment with the serotonin biochemical intermediate L-5-hydroxy tryptophan (Growdon et al. 1976). As a prelude to determining whether cholinergic precursor treatment would benefit diseases linked to deficient acetylcholine neurotransmission, I thought it would be necessary to show that 1) the increase in acetylcholine induced by choline administration was in presynaptic terminals and thus available for synaptic release, 2) choline administration to humans would increase plasma levels of choline as it did in rats and 3) choline administration to humans would increase choline levels in brain or cerebrospinal fluid (CSF).

Our first paper examined hippocampal choline and acetylcholine levels after choline administration to answer the first requirement (Hirsch et al. 1977). The rationale behind this study was that acetylcholine in the hippocampus was largely confined to the axon terminals of the septo-hippocampal tract, and that acetylcholine was released from the hippocampus by septal electrical stimulation. Our study showed that a single interperitoneal injection of choline markedly elevated both choline and acetylcholine levels within the dorsal hippocampus, just as it did in the caudate nucleus and in the whole rat brain (Table 1). In a subsequent study, we showed that lecithin, the naturally occurring dietary source of choline, was more efficient at raising blood levels of choline in normal human volunteers than was a choline salt (Wurtman et al. 1977). Finally, in a separate clinical study, we confirmed that oral choline administration increased serum levels of choline and also produced substantial and significant increases in CSF choline levels (Growdon et al. 1977b)

The next step was to test precursor choline treatment in a neurologic disease linked to deficient acetylcholine tone. Tardive dyskinesia was one such condition. It had been known that intravenous administration of physostigmine decreased choreic movements whereas anti-cholinergic drugs such as scopolamine tended to worsen dyskinesia. We conducted a clinical trial in which we administered 8-20 grams per day of choline chloride to 20 patients with tardive dyskinesia, collected blood samples for choline measurements and counted the number of choreic movements over time. During the second week of choline ingestion, choreiform movements decreased substantially in five patients and moderately in four, whereas they were unchanged in 10 and worse in one (Growdon et al. 1977c). We found similar effects with oral doses of lecithin. Thus, administration of choline and lecithin to increase acetylcholine in brain was secure from a scientific standpoint. From a clinical perspective, both compounds

Table 1. Effect of choline chloride administration on choline and acetylcholine concentrations in rat hippocampus and caudate nuclei (modified from Hirsch et al. 1977)

Group	Choline (nM/g)	ACh (nM/g)
Control		
Hippocampus	27.29 ± 3.00	14.23 ± 2.95
Caudate	34.14 ± 2.31	48.70 ± 2.00
20 minutes after ChCl		
Hippocampus	42.60 ± 1.70^{a}	26.50 ± 2.70^{b}
Caudate	57.47 ± 5.50^{a}	59.15 ± 2.45^{b}
40 minutes after ChCl		
Hippocampus	37.48 ± 2.99	29.60 ± 3.04^{b}
Caudate	45.05 ± 3.53^{c}	63.16 ± 3.00^{b}

Groups of 10 rats received choline chloride (ChCl) (60 mg/kg, ip) or its diluent (water) and were killed 20 or 40 minutes after injection. Data are given as means +/− SEM.
[a] $p < 0.01$, [b] $p < 0.02$, [c] $p < 0.05$

had been tested in human subjects, both increased plasma and CSF levels of choline, and both had shown promise in treating a human disease associated with deficient cholinergic neurotransmission.

The Cholinergic Hypothesis of Memory Dysfunction in AD

Multiple and independent reports of a cholinergic deficit in AD (Bowen et al. 1976; Davies and Maloney 1976; Perry et al. 1977) appeared in 1976 and 1977 and signaled a new era in AD research. In addition to confirming the selective decrease in choline acetyltransferase (CAT) activity in AD brain, investigators reported preservation of intact muscarinic receptor sites. These observations raised the possibility that treatments designed to increase the synthesis or release of acetylcholine, or to block its subsequent hydrolysis, might be beneficial in treating AD symptoms. Prior pharmacological studies had already demonstrated the importance of intact cholinergic neurotransmission in memory functions. It had been widely known, for example, that anti-cholinergic drugs could impair memory and even produce amnesia. Drachman and Levitt extended this knowledge and showed that low doses of scopolamine produced a pattern of cognitive deficits that was qualitatively similar to those observed in demented patients (Drachman and Leavitt 1974). It was then shown that cholinergic agonists could enhance memory and reverse the adverse effects of scopolamine (Davis et al. 1978; Sitaram et al. 1978).

Pathologic studies also supported the cholinergic hypothesis of memory dysfunction and provided an explanation for the decreased CAT levels in AD brain. Decreased CAT activity in brains of AD patients correlated with estimates of dementia severity and with neurofibrillary tangle counts (Wilcock et al. 1982). Whitehouse et al. (1981) reported that the cholinergic neurons in the ventral forebrain, including the nucleus basalis of Meynert, whose axons project widely to neocortex and the hippocampus, were severely atrophic. Although atrophy of ventral forebrain nuclei is not unique to

AD, finding atrophic neurons was internally consistent with biochemical indices of decreased cholinergic transmission in the terminal projections of their axons. These three lines of evidence therefore formed the basis of the cholinergic hypothesis of memory dysfunction in AD. From a therapeutic standpoint, the parallels with Parkinson's disease were striking. In Parkinson's disease, neuronal loss in the substantia nigra pars compacta accounts for decreased production of dopamine; it is believed that deficient dopaminergic neurotransmission in the nigral projections to the striatum results in the characteristic extrapyramindal motor signs. Administration of levodopa to increase dopamine biosynthesis improves these motor signs. In AD, atrophy of the nucleus basalis of Meynert might be considered as a lesion comparable to the nigral damage in Parkinson's disease. As a result of the basalis lesion, there is deficient acetylcholine synthesis and reduced cholinergic neurotransmission in cholinergic axon terminal projections, especially to the hippocampus, which results in memory loss and perhaps other cognitive impairments. Could neurotransmitter replacement strategies similar to those used successfully in Parkinson's disease work to palliate cognitive impairments in AD?

Acetylcholine-related Treatments: The Reality

Precursors

In contrast to the beneficial effects in tardive dyskinesia, neither oral choline nor oral lecithin administration improved any aspect of cognition or behavior in AD patients. A study of lecithin administration vs. placebo conducted by Sullivan et al. (1982) is illustrative. We treated 18 AD patients with either lecithin or placebo according to a double-blind cross-over design. Lecithin administration increased plasma choline levels two- to four-fold ($p < 0.0001$) whereas choline levels returned to baseline during periods of washout or placebo administration. Despite the increase in plasma choline levels, no patient improved on any memory test during lecithin or placebo administration.

Acetylcholine Esterase Inhibitors (AChEIs)

An alternative way to increase acetylcholine neurotransmission is to block its hydrolysis and thereby prolong the intrasynaptic effects of released ACh. Physostigmine was the only AChEI in the formulary at the time, and its use was severely restricted because of the requirement for intravenous administration, as no oral preparation with a long duration of action was available. Early studies with AChEIs appeared more promising than precursor administration, and drugs of this class were finally developed and approved nearly 20 years after the discovery of the cholinergic deficit in AD (Lleo et al. 2006). There are now four AChEIs available; these drugs are the current standard of care even though they typically produce only modest clinical benefits (Greenberg et al. 2000). Since their introduction into clinical practice 10 years ago, it has become clear that this line of treatment is sub-optimal and that AChEIs have not become the levodopa of AD. Although the cholinergic deficit is still true, it is an incomplete account of the total AD pathology; correcting acetylcholine transmission is a bandage at best

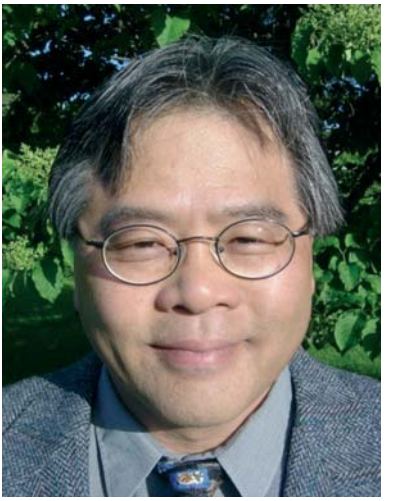
and has little or no effect on the overall progressive deterioration of this illness. To stop AD or slow its progression, the focus has shifted to neuroprotective strategies designed to counteract the underlying causes of AD.

Acetylcholine and the Amyloid Hypothesis

Amyloid deposition is an early event in brains of AD patients and defines much of the histopathology of AD. Amyloid plaques are composed of small peptide fragments called Aβ, which are derived by protolytic cleavage of a large transmembrane amyloid precursor protein (APP). Much of the research in AD during the past decade has centered on the molecular aspects of APP processing and its consequences. As described elsewhere in this volume, at least three enzymes are involved in APP metabolism: α-secretase, which cleaves APP within the Aβ sequence and generates non-amyloidogenic moieties, and another set of proteases (β- and ɣ-secretases) that generate Aβ1-42 and Aβ1-40 fragments, which are believed to be neurotoxic. Roger Nitsch joined the Wurtman laboratory in 1990 and soon established the initial and most direct link between acetylcholine transmission and amyloid metabolism. We discovered that HEK 293 cells separately transfected with the muscarinic M1 and M3 receptor subtypes increased α-secretase cleavage of APP within minutes of stimulating the receptors with the cholinergic agonist carbacol (Nitsch et al. 1992). In subsequent experiments, we showed that stimulating the M1 receptor subtype also produced a concurrent decrease in Aβ secretion (Hung et al. 1993). APP processing in wild type HEK cells, as well as those expressing the M2 and M4 receptor subtypes, was not affected by muscarinic stimulation, indicating that APPs secretion was specifically linked to the M1 and M3 receptor subtypes. We reasoned that if such a sequence were to occur in human beings, administration of an M1 agonist would be expected to decrease levels of Aβ in the central nervous system and possibly slow or even reverse the course of AD dementia. To test this hypothesis at the biochemical level, we administered the selective M1 and M3 agonist AF102B to 19 patients with the clinical diagnosis of AD and measured the CSF levels of soluble Aβ before and during drug administration. To determine the specificity of the AF102B effect, we administered two other drugs, hydroxychloroquin or physostigmine, to separate sets of AD patients. We found that treatment with AF102B lowered total Aβ levels in CSF by 22% in 14 of the patients, whereas levels increased slightly in three and were unchanged in two (Nitsch et al. 2000). The overall decrease in the group as a whole was statistically significant. CSF Aβ levels did not change significantly in the nine patients treated with physostigmine or in the 10 patients treated with hydroxychloroquin. These data provided evidence that specific activation of M1 receptors reduced total Aβ levels in CSF of AD patients. If this effect were to occur in the brain, M1 agonists might have long-term therapeutic benefits by lowering the Aβ load. This hypothesis is now being actively tested in transgenic mice. Caccamo et al. (2006) administered the selective M1 muscarinic agonist AF267B to a triple transgenic mouse that over-expresses both amyloid and tau. They found that AF267B reduced both Aβ and tau pathologies in hippocampus and cortex and that these changes were associated with improved performance in a spatial task. Further, they showed that the mechanism underlying the effect on Aβ pathology was caused by the selective activation of ADAM17, thereby shifting APP processing toward the non-amyloidogenic pathway. In contrast, the M1 antagonist dicyclomine exacerbated the Aβ and tau pathologies.

These pre-clinical and clinical experiments raise the possibility that drugs developed to stimulate specifically M1 and M3 muscarinic receptors might prove effective in lowering the Aβ burden in human brain and thereby slowing or even reversing the cognitive decline associated with AD.

Amyloid and Genetics



Cai'ne W. Wong

The Discovery of β Amyloid

Cai'ne W. Wong[1]

Dr. George G. Glenner died (too young) in 1995 due to complications of cardiac amyloidosis. If he had survived, perhaps he and I would be writing this story together. Instead it will only be my personal recollections of the work we did in 1982–85 that led us to the discovery of the Alzheimer's disease "beta protein," (Glenner and Wong 1984a) now also known as A-beta and beta-A amyloid.

How it started

My introduction to George Glenner was in 1982, when I interviewed for a job in his new laboratory at the University of California at San Diego (UCSD). He had recently moved to UCSD after two and a half decades at the National Institutes of Health in Bethesda, MD, where he had made a career of investigating human systemic amyloids. Now at UCSD, he was still focused on amyloid but on those found in the human central nervous system. His research goal was to identify the critical protein(s) that made up the amyloid deposits found in the brains of Alzheimer's disease (AD) patients. During the interview, I learned his research program was just beginning. The actual bench biochemistry would start when I did. This was clearly apparent since the two rooms that comprised the laboratory were empty with the exception of two large ultra cold Revco chest freezers. These freezers contained donated AD brains. The year before, George Glenner had created one of the first AD brain banks and it was to be a source of diseased human brains for us and other researchers. The mission of the brain bank was made known to the National Alzheimer's Disease and Related Disorders Association. Through this network and other AD support groups, brain donations were made to the brain bank by relatives, who had legal authority, of individuals suspected to have died of AD. The family would receive a no-cost diagnosis based on a postmortem neuropathological examination. The postmortem diagnosis, although not always 100% accurate, it was still the most definitive assessment for AD. In the months that followed, I would grow to appreciate the genius of that part of George Glenner's research plan and to realize how pivotal the brain bank would be to our success. Having ready access to AD brains with exceptional amounts of amyloid deposits was a tremendous advantage to our research.

We were a small research group, composed of George Glenner, Karen Rasmussen and myself. Karen was responsible for maintaining the brain bank and the myriad tasks associated with it. My responsibilities were to order equipment and supplies, to set up and maintain the new laboratory and to perform the biochemical experiments.

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In 1906, Alois Alzheimer, in a seminal presentation in Tübingen, Germany, made an association between abnormal amyloid deposits (which would later become known as neurofibrillary tangles, (NFTs) in the brain of Auguste D. and her dementia. This neurological malady would shortly become known as Alzheimer's disease. In time, two other forms of brain amyloid, neuritic plaques (NP) and cerebral vascular amyloid (CVA), would also be associated with the disease. In 1982, despite nearly eight decades that had lapsed since Alois Alzheimer introduced the disease to science, there was still nothing known of the amyloid etiology. Electron microscopy (Kidd 1963; Terry et al. 1964)) plus X-ray fiber diffraction studies (Eanes and Glenner 1968) showed that the amyloids were protein fibrils with a high content of beta-pleated sheet. The unknown etiology helped fuel a controversy over the role (if any) that amyloid played in the neuropathology of AD. To solve the ambiguity surrounding AD amyloid, George Glenner knew that it would be necessary that to identify the amyloid protein(s) identified. If nothing else, he speculated, there might be diagnostic potential in the identify.

Success, disappointment, then success and uncertainty

We began our investigation with CVA. In his histological examination and cataloguing of AD brains for the brain bank, George Glenner found that more than 90% of AD brains had amyloid deposits in blood vessel walls (Glenner 1983)). Of particular interest were the brains that had extensive deposition in the meninges. In these he saw the potential of obtaining enriched CVA preparations simply by stripping the meningeal membrane away from the cortex and thereby circumventing contaminants from that source. Once the meningeal membrane was stripped off and cleaned of residual cortex, it was finely minced and homogenized. The homogenate was centrifuged to collect a pellet. The pellet was composed of a large white bottom layer and a thin tan top layer. We examined the layers to determine which contained the bulk of the amyloid. This was done by making thin smears on microscope slides of each layer and examining them using the same Congo red histological stain technique used for examining brain sections. We found that the top tan layer was enriched for amyloid but was still contaminated with a significant amount of connective tissue-like debris. In a previous research position, I had used collagenase to perfuse rat livers to obtain single hepatocytes (Hatoff et al. 1985)). After a prolonged collagenase perfusion, the once lobed liver would become a shapeless bag of disassociated cells. When I suggested we try to remove the presumptive connective tissue contaminant by incubating the tan layer with collagenase, George Glenner immediately recognized the merit of that idea. As it would turn out, the collagenase was very effective at removing the bulk of the connective tissue contaminants without affecting the CVA. One of the hallmark properties of amyloid is the acquisition of apple-green birefringence after being stained with Congo red dye and viewed under a polarized light microscope. The collagenase-treated sample slide showed an almost unreal field of pure apple-green birefringence. George Glenner was known by those who knew him personally for the twinkle he had in his eyes. His eyes were exceptionally twinkling bright that day.

We then looked for a way to dissolve the CVA sample so that we could subsequently fractionate the components. Amyloids, as a rule, were known to be difficult to solubilize (Selkoe et al. 1982b). CVA would not be an exception to that rule. A number of

strong biochemical detergents, including SDS, were systematically tried at increasing concentrations, elevated temperatures, varied pH and incubation times. None of those experiments were successful. We then turned to chaotrophic denaturing agents and eventually found that prolonged incubation with 6 M guanidine-HCl under reducing conditions plus EDTA worked moderately well. Denaturation of the CVA was monitored by the loss of Congo red dye-mediated birefringence. We hoped that the denaturation was the result of solubilization. After removing the guanidine from the CVA supernatant by dialysis and concentration by lyophilization, we were able to resolve CVA proteins on SDS-PAGE. Comparing the protein band profile of the CVA sample to the profile of normal control brains meninges, we found a unique low molecular weight band at the electrophoretic front of the CVA sample lanes. We repeated the experiment with a higher percentage SDS polyacrylamide gel augmented with urea. This was done to resolve the low molecular weight band away from the electrophoretic front so we could determine the approximate molecular weight. There were other differences in the SDS-PAGE protein profiles but the low molecular weight band was the most salient, and that is where we focused our attention.

Now that we had a protein (or peptide) of interest, we scaled up the purification protocol by using a calibrated preparative G-100 Sephadex sizing column to resolve guanidine supernatants of CVA. The unique low molecular weight protein (or peptide) was recovered in the fractions centered at 4,200 daltons (CVA4200). The protein (or peptide) could be recovered from different CVA samples but not from control samples. CVA4200 was submitted for automated protein sequence analysis at the UCSD Weingart Protein Sequence Core Facility. The results were disappointing. There were multiple amino acid signals at every sequencing step, which suggested the sample had multiple proteins (or peptides) and/or ragged amino termini. It was clear that our purification scheme needed augmentation with an additional fractionation method.

Fortune, opportunity, and perhaps coincidence would collide next. Rob Nicholas, a friend and housemate, was a biochemistry graduate student at UCSD in Jack Kyte's laboratory. Rob and his colleagues were studying the structure-function of the Na^+-K^+ ATPase, a membrane protein with multiple transmembrane domains. I told Rob about our disappointing sequencing results and that we were looking for another purification scheme for small peptides. Rob then told me his lab had had a recent success at resolving small proteolytic peptide fragments from the transmembrane domains of the Na^+-K^+ ATPase using reverse phase HPLC and a newly formulated liquid phase. Perhaps, he suggested, it would work for fractionating CVA4200 as well. I was invited to use the Kyte laboratory HPLC system and was tutored by Rob in its operation. The results were immediate. On the very first run, three elution peaks were resolved. The second and third overlapped, eluting at 35% and 36% acetonitrile. After confirming that the results were repeatable with different CVA samples, fractions from the three peaks were submitted for amino acid sequencing. The first peak was called alpha and yielded no sequence information. The second and third peaks, called beta and gamma, respectively, yielded sequence information to 24 residues and the sequences were identical up to that point. Because of that identity, we renamed the second and third peaks beta-$_1$ and beta-$_2$. We expected approximately 38 residues based on a 4,200 dalton molecular weight. Amino acid analysis predicted 33. With only 24 amino acid residues, we knew we had only a partial sequence. With the partial sequence in hand, we enlisted the aid of Dr. Russell Doolittle, Research Professor of Biological Science at UCSD. He

was assembling one of the early protein-nucleic acid sequence databases. His computer search found no homology of the beta protein with any protein in any database. Excitement tempered with trepidation followed. Had the beta protein sequence been homologous with a known protein, particularly a human protein, we would not have felt so uncertain of what we had found. Previously, studies of human systemic amyloid proteins showed them to be derived from endogenous human proteins. However, Russell Doolittle had counseled us not to be too disappointed and overly concerned about the lack of homology, since all sequence databases (at the time) contained only a miniscule percentage of the expected number of proteins predicted to exist.

Uncertainty abated

Soon after we obtained the beta protein sequence from AD CVA samples, we obtained a similar sequence from a CVA sample of an adult Down's syndrome (DS, aka Trisomy 21) brain (Glenner and Wong 1984b). It was known that virtually every case of adult DS resulted in an AD-like dementia after 40 years of age. Microscopic postmortem examination of adult DS brains showed they also contained amyloid lesions indistinguishable from those found in AD brains. The DS result was an important finding in that it provided the first biochemical relationship between AD and adult DS. In addition, it further supported the possibility that the etiology of AD was located on chromosome 21. However, it did not provide an independent verification that the beta protein was a component of the CVA.

Vito Quaranta, a molecular immunologist at Scripps Clinic and Research Foundation, would help formulate the next step in our research. Vito Quaranta suggested that anti-peptide antibodies raised against beta amyloid could be used to immunohistochemically stain the CVA in AD brain sections. If the anti-peptide antibodies localized to the CVA deposits, it would provide an independent demonstration that the beta protein was a component of the CVA. Moreover, he offered to provide the necessary scientific guidance and invited me to perform the antibody production work in his laboratory. Doing so would circumvent the lag time of setting up our own laboratory for antibody production. Furthermore, his laboratory was only a 10-minute trip from the UCSD campus. We designed overlapping synthetic peptides to span the beta protein sequence. The synthetic peptides were coupled to keyhole limpet hemocyanin and used to immunize both rabbits and mice.

The antibody response varied for the different peptides used. We found that a synthetic peptide corresponding to the first 10 amino acids of the beta protein sequence was especially immunogenic in BALB/c mice. A mouse antiserum (OP1MS1) with the highest titer and specificity in ELISA studies was selected for immunohistochemistry experiments. The results were dramatic. The OP1MS1 antiserum specifically stained CVA deposits in both AD and adult DS brains (Wong et al. 1985). This finding provided the much-needed independent evidence that the beta protein was an integral component of CVA. Prior to obtaining the antiserum, George Glenner and I had an ongoing "bragging rights" wager as to whether the NPs and NFTs would also be recognized by it. George Glenner, with his always upbeat optimism, wagered that the antiserum would recognize not only the CVA deposits but also the NPs and the NFTs. I was less optimistic and hedged my bet by restricting my choice to the CVA. As it turned out, we

both lost that bet. The OP1MS1 antiserum recognized CVA and NP in AD and adult DS brains, but not NFTs. At face value, the results confirmed that the beta protein was an integral component of CVA and strongly suggested it was for NP amyloid as well.

When shown the results of the immunohistochemistry study, George Glenner had an uncharacteristically unrefined "HOLY SHIT" moment. Although George Glenner had wagered more, the clear and unambiguous results had surpassed his actual expectations. After carefully reviewing the experimental and control slides of the experiment, George Glenner remarked with his twinkling eyes and his widest grin, "Well, you can't win them all. Two out of three ain't bad. You had better get started on the writing." George Glenner did not say it specifically, but I took what he said to mean that we had a very good day. We had made progress towards teasing out the identity of two of the three amyloid deposits he had originally set out to find. We also learned something about the NFTs that we did not know before. The results suggested that NFTs were either composed of another peptide (or protein) or of the same peptide with the OP1MS1 epitope masked. It would be discovered later that NFTs are composed of hyperphosphorylated tau, a microtubule associated protein (Brion et al. 1985; Iqbal et al. 1989). While the OP1MS1 manuscript was in press, we learned that the collaborating teams of Colin Masters and Konrad Beyreuther obtained a peptide sequence from AD and adult DS NPs similar to the sequences we had found from AD and adult DS CVA (Masters et al. 1985a). Our immunohistochemisty studies (Wong et al. 1985) dovetailed with their finding.

Reflections

While preparing the 1984 beta protein amino acid sequence manuscript, George Glenner and I debated whether or not it would be too presumptuous to use the name "beta protein" with the obvious connotation to beta-pleated sheet and amyloid. At that time, there was no independent confirmation that our beta protein was amyloid. In the end, George Glenner, with his usual optimistic view of things, said, "Let's let it stand." In the following months, the beta protein would be confirmed as the amyloid peptide and the name "beta protein" would morph into the more definitive "beta amyloid," "beta-A" and "A-beta" by the AD research field.

The (beta) amyloid precursor protein (APP) gene would be cloned by four laboratories (Goldgaber et al. 1987a; Kang et al. 1987; Robakis et al. 1987b; Tanzi et al. 1987) and would be found on chromosome 21. At least one laboratory, and possibly others, would be aided by the beta peptide amino acid sequence. The APP turned out to be a transmembrane protein and beta amyloid a peptide fragment from part of the transmembrane domain. Rob Nicholas' suggestion of using a HPLC protocol developed to isolate protelytic transmembrane peptides to resolve CVA4200 was more prophetic than we could ever have imagined.[1]

The discovery of beta amyloid raised many fundamental questions, as witnessed by the extraordinary number of publications concerning it since. The other contributors to

[1] Rob Nicholas, now a professor of pharmacology at the University of North Carolina at Chapel Hill, was never publicly acknowledged for his crucial contribution to the discovery of beta amyloid. Hopefully, this will help correct that unfortunate oversight.

this volume have provided elegant and compelling answers to some of those questions and have, in turn, generated even more questions. Their work, along with that of many others, has extended, expanded and filled in the continuing beta amyloid story just as George Glenner had wanted.

I left George Glenner's laboratory in 1987 to attend graduate school at UCLA. In the years since, it has been a source of personal gratification to know that the work we did in 1982–85 opened a significant door for AD research and continues to be relevant to this day. It is rare to be part of something that has affected so many lives and I am grateful to have had the opportunity.

Colin L Masters

Konrad Beyreuther

Pathways to the discovery of the neuronal origin and proteolytic biogenesis of Aβ amyloid of Alzheimer's disease*

Colin L Masters[1] and *Konrad Beyreuther*[2]

Our contributions in the 1980s (Kang et al. 1987; Martins et al. 1986; Masters et al. 1985a,b) to the purification and N-terminal sequencing of the amyloid plaque cores (APC) of Alzheimer's disease (AD) and the discovery of its biogenesis from a neuronal precursor (the amyloid protein precursor – APP) by proteolytic cleavages (the β- and γ- secretases) need to be seen against the background of many years of prior research activity from a diverse range of individuals and groups.

Prior to the modern era – up to the mid 1960s

Prior to the mid-1960s, very little progress had been made in understanding the pathological significance and biochemical nature of the amyloid depositions either in the brain (the concept of amyloid dates from Virchow's description of cerebral corpora amylacea) or systemically. Von Braunmühl was among the leaders of the German school of pathologists who attempted to understand the "colloidal" nature of amyloid. The same school had developed the use of the cotton dyes, such as Congo Red, for the differentiation of amyloid from other proteinaceous deposits. The fact that abnormal degenerative and regenerative changes occurred in response to cerebral amyloid deposition was fully appreciated but the origin of the cerebral deposits remained enigmatic, particularly since some forms were clearly associated with small blood vessels, the amyloid congophilic angiopathy (ACA) of Pantelakis. The concept of a vascular or hematogenous origin of the cerebral amyloid plaque clearly arose during this period, and was promoted by the general (non-neuropathologically trained) pathologists who were used to evaluating the systemic forms of amyloidosis.

Beginning the modern era – mid-1960s to 1970

Three major intellectual streams emerged during the latter half of the 1960s. First, Friede described the histochemical reactivity of the AD "senile" plaque and observed

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* [This is an abbreviated version of a longer article written for the Alzheimer 100 Special Issue of the Journal of Alzheimer's Disease edited by G. Perry, J. Ávila, J. Kinoshita and M. Smith.]

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the enzymatic activity specific for acetylcholinesterase. This observation eventually developed into the cholinergic theory of AD and the current class of cholinesterase inhibitors that are useful in the symptomatic treatment of AD. Second, the emergence of the technology behind electron microscopy led to the "great tangle debate" between the schools of Bob Terry and Michael Kidd: was the Alzheimer neurofibrillary tangle (NFT) a paired helical filament or a twisted tubule? Third, and more productively, the unlocking of the structural basis of the systemic amyloid filament had begun.

Early studies on the structural and biochemical nature of the Alzheimer plaque – the 1970s

In the early 1970s, little progress was made in understanding the amyloid plaques in both AD and the "slow virus" diseases. Remarkably, the first attempts at purifying the APC came from James Austin's group in Denver, Colorado, in 1971-1972, but they had used formalin-fixed tissues. Nevertheless, their studies did reveal the presence of non-proteinaceous elements such as silicon.

In the first half of the 1970s, George Glenner had made spectacular progress in the biochemical elucidation of the AL types of systemic amyloid. Glenner, who died in 1995 at the age of 67 from the complications of cardiac amyloidosis, was among the first to obtain N-terminal sequences on the amyloid AL light chains. He had conducted his seminal studies at the NIH in Bethesda, having moved there in 1958.

Masters' first post-doctoral position was with EP Richardson in Boston in 1976–1977, continuing earlier studies on the AD/CJD amyloid plaques at a morphological level. In late 1977, Masters moved to the NIH laboratories of Carleton Gajdusek and Joe Gibbs, to continue the collaboration that had started in 1968. After some discussion with Joe Gibbs, it was agreed that Masters should start a project on purifying and characterizing the amyloid plaques from the human transmissible diseases (kuru, CJD and the Gerstmann-Sträussler Syndrome). Joe Gibbs, of course, knew of George Glenner's scientific reputation and of his presence on the NIH campus, and he suggested that Masters visit Glenner's laboratory. By this time, Glenner had begun to think about the AD-amyloid connection and had decided that the best approach would be to isolate the AD amyloid from the leptomeningeal vessels, but he had not commenced work on this subject. Masters and Glenner met on two or three occasions, and Glenner then drafted a research proposal, which he sent to Joe Gibbs. As a result of this, an intramural NIH conference was convened in 1978, at which Glenner, Masters, Gibbs and other intramural scientists presented ideas on how to approach the general methods involved in purifying the AD/CJD amyloids. From the very start, Glenner assumed that the AD amyloid was derived from the vascular compartment, in the same manner as had been identified for the AA and AL proteins.

Over the ensuing two years (1978–1980), Glenner and Masters met only on one or two occasions in Glenner's laboratory to discuss progress. At this stage, samples had not been exchanged between their respective laboratories at the NIH; neither was it clear that Glenner had actually begun dissecting any human AD brain tissues.

Masters' approach at the NIH was to try and adapt the known detergent–high salt extraction methods previously used for the purification of intermediate filaments, relying on the relative insolubility of the APC of both kuru and GSS brains. Because of

the scarcity of tissue samples and the low numbers of APC in these conditions, Masters also began using AD brains to establish the methodologies.

While these studies were going on at the NIH from 1978 to mid-1980, other connections and collaborations were being established. In the AD field, several groups were actively engaged in the purification of the NFT, including Dennis Selkoe in Boston and Henryk Wisniewski and his team at Staten Island (including Khalid Iqbal and Patricia Merz). On one of their many visits to Salem and Boston, Gajdusek and Masters dropped in unannounced to see Selkoe at his McLean laboratory, to check on progress with his NFT preparations. This would have been in 1979, and it was apparent at that time that Selkoe was not directly working on an APC purification strategy. In contrast, Pat Merz and Steve Bobin at Staten Island were very interested in the amyloid purifications in both scrapie/kuru/GSS and AD. Masters, Bobin and Merz set up a collaboration in which they shared protocols, samples, and techniques. This collaboration eventually resulted in a publication in 1983 (submitted in November 1982), which was the first to describe in detail some of the methods that had been jointly developed for the purification of amyloid from AD, GSS and scrapie frozen brains. This paper concentrated on the electron microscopic appearances of the different types of amyloid filaments, which at the time was a very controversial area because of the studies emerging from the Prusiner laboratory in San Francisco. In retrospect, it was evident that we had relatively "pure" preparations of scrapie/GSS amyloid in our laboratories at a time well before Prusiner had "pure" preparations of the prion protein (PrP). If we had been able to solubilize and characterize the scrapie/GSS amyloid protein at that time, it would have led us directly to the PrP protein, pre-empting Prusiner's later discoveries by several years.

Dramatic discoveries on many fronts in the 1980's

Masters left the NIH labs in the latter half of 1980 for a year in Heidelberg with Melitta Schachner before returning to Australia in 1981, where he recommenced studies on the sporadic and genetic cerebral AD/CJD amyloids. George Glenner moved to San Diego in 1982, having published a major review on the "β-fibrilloses" in 1980. His attempts to dominate the amyloid nomenclature debate with his emphasis on their β-pleated sheet structure were to be carried forward in his AD studies in California, where he found more ready access to AD brain tissues. During 1982-1983, Masters was in communication with Glenner, and samples of pure AD-APC were sent to him on the understanding that it was a collaboration in which he would perform X-ray diffraction studies. It was never clear what became of those samples, as results were never forthcoming from his laboratory. Also, in 1982, Prusiner visited Masters' Australian laboratory while attending the International Congress of Biochemistry in Perth, at which he set up the collaboration with Charles Weissman that was to lead to the eventual cloning of the PrP gene. During Prusiner's visit, Masters discussed progress in isolating the AD and GSS amyloid. Although Prusiner, at that time and for many years thereafter, maintained that his "prion rods" were distinct from amyloid fibrils and Merz's "scrapie associated fibrils," it came as a great surprise that he subsequently consulted with Glenner and published observations on the Congo Red negative birefringence of the aggregated prion rods.

In 1983, another surprising paper appeared from Michael Kidd, Mike Landon and David Allsop in the Nottingham Medical School, disclosing their method of AD-APC purification (discontinuous sucrose gradient with subtilisin pre-digestion) and showing that their total amino acid composition was different from the known AA/AL amyloid proteins. We had not been aware of competitors other than Glenner. Much later, we also learned that Alex Roher had also been working on purified APC. In subsequent discussions with Allsop and Landon, it was clear that they had made plans to determine the N-terminal sequence, but their chosen collaborator failed to deliver. Moreover, they apparently had not discovered a method to solubilize the APC, a precondition for determining the N-terminal sequence.

In retrospect, it was clear that Glenner had been very busy and productive during 1983 and 1984, as his two papers on the N-terminal sequence of the AD amyloid protein appeared in May and August 1984. As expected, he had confined himself to the amyloid extractable from the leptomeningeal vasculature, and his method required predigestion with collagenase and (partial) solubilization in 6M guanidine-HCl, followed by Sephadex G100 chromatography. He also found the amyloid was soluble in 88% formic acid for HPLC. In his first paper, he obtained an N-terminal sequence as far as residue 24, with a mistake at residue 11 (identified Gln instead of Glu). He named contents of the two G100 peaks "β_1, β_2peptide" after the "β-pleated sheet" configuration determined by X-ray crystallography (in contravention to the International Amyloidosis Nomenclature Committee rules, which required the A-"x" system). He predicted that the β_1/β_2 peptides would be derived from a unique serum protein precursor.

Masters first saw this paper when travelling to an EMBO-sponsored meeting on the Transmissible Spongiform Encephalopathies being organized by Alan Dickinson in Edinburgh. At this meeting, Konrad Beyreuther and Stan Prusiner were present: Beyreuther attended because of his association with Heino Diringer who had interested him in some of the properties of scrapie fibrils isolated in Diringer's Berlin laboratories; Prusiner was there with some important unpublished information on the N-terminal sequence of PrP. Masters approached Beyreuther, known for his expertise in amino acid sequencing, to help with his studies on the AD amyloid plaque cores. By that time, Masters had also determined their solubility in strong chaotropes, such as guanidine, and had discovered formic acid to be the most effective solvent (a tip derived from the previous generation of Australian wool protein chemists). Beyreuther readily agreed to collaborate, and Masters sent purified AD-APC to him and Gerd Multhaup for sequencing at the Institute for Genetics, Cologne. Our method for the APC purification now consisted of a pepsin digestion, Triton X100/high salt extraction followed by separation on a discontinuous sucrose gradient. Beyreuther and Multhaup were able to solubilize the AD-APC in formic acid and obtain a very ragged N-terminal sequence as far as residue 28 (four more than Glenner!). On SDS gels, dimers and higher order aggregates were readily observed of the 4kD monomer, which at the time, in conformance with the International Nomenclature rules, we referred to as "A_4" (and the oligomers as A_8,A_{16},A_{64}, etc, the "A" standing for either *Amyloid* or *Alzheimer*); see Fig. 1). We noted the pH-dependence of this aggregation process as being typical of protonation of histidines.

Glenner's next paper appeared while we were making rapid progress with our own analyses. He now referred to the "β_1/β_2peptide" as "the β protein," corrected

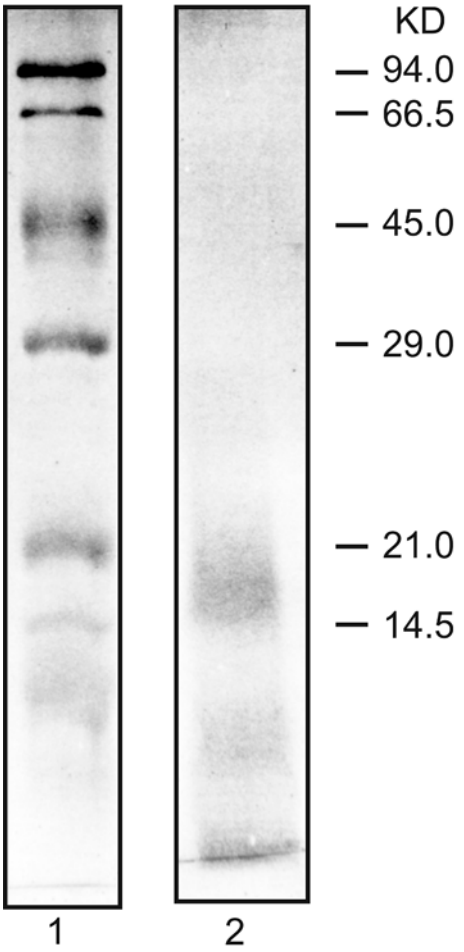
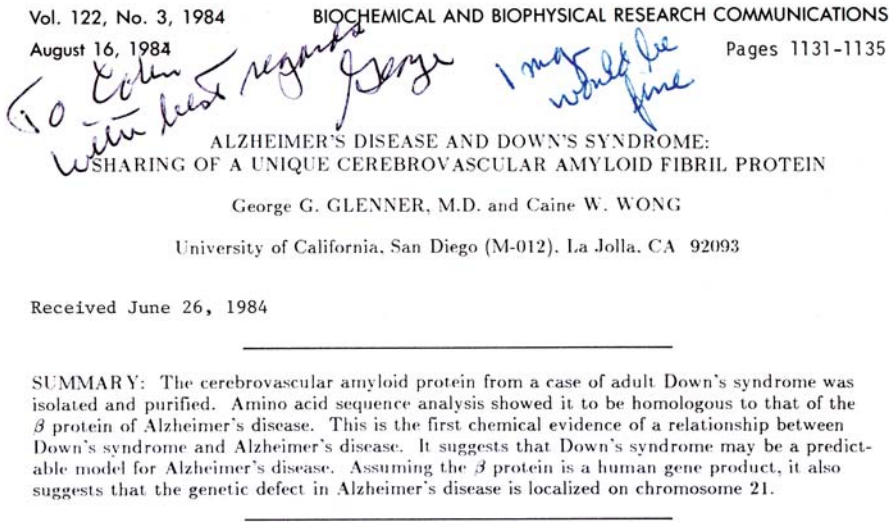


Fig. 1. Image of gel of native APC Aβ from Masters et al. (1985a) showing oligomers of Aβ (lane 2) of mass 8kd (A_8, dimer) and 16kd (A_{16}, tetramer?). These oligomeric species of Aβ have subsequently become the prime suspect in the quest for the "toxic species"

his sequencing error at residue 11, and predicted that, since the amyloid N-terminal sequence from a vascular preparation from a case of Down's syndrome was the same as from AD, there would be a gene defect on chromosome 21 responsible for AD.

By late 1984, we had assembled enough data from our APC studies to draft a manuscript that was submitted to PNAS, and accepted in January 1985. In the acknowledgments, we thanked Steve Bobin, Michael Landon and George Glenner for "helpful discussions." This statement was certainly true for Bobin and Landon, with whom we had developed cordial relationships. Glenner, however, maintained a very "stand-offish" attitude and even had the presumption to request further supplies of our purified APC (see Fig. 2 – "1 mg would be fine"!). Our PNAS paper was published in June 1985. Subsequent discussions with Glenner showed that he believed that we could never have obtained our results without reference to his 1–24 sequence. For many years thereafter, he maintained that the basic amyloid subunit was 28 residues in length. Initially, we ourselves were uncertain whether the N-terminal raggedness



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ALZHEIMER'S DISEASE AND DOWN'S SYNDROME:
SHARING OF A UNIQUE CEREBROVASCULAR AMYLOID FIBRIL PROTEIN

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SUMMARY: The cerebrovascular amyloid protein from a case of adult Down's syndrome was isolated and purified. Amino acid sequence analysis showed it to be homologous to that of the β protein of Alzheimer's disease. This is the first chemical evidence of a relationship between Down's syndrome and Alzheimer's disease. It suggests that Down's syndrome may be a predictable model for Alzheimer's disease. Assuming the β protein is a human gene product, it also suggests that the genetic defect in Alzheimer's disease is localized on chromosome 21.

Fig. 2. Image of a reprint sent to CLM from George Glenner, at some time in late 1984, with the inscription "1 mg would be fine," referring to the collaboration in which Masters had previously sent him samples of purified AD-APC for X-ray diffraction studies. Glenner was now requesting further supplies. No results ever came from this collaboration

was an artefact of the preparative method or caused by non-specific degradation of material remaining in situ for extended periods.

The most important question that needed to be addressed in early 1985 was the origin of the cerebral amyloid. We immediately set out to raise antisera to the purified and fractionated APC and to a variety of synthetic peptides of A_4 (the Aβ peptide, as it subsequently became known). Using these antisera on AD brain sections, we were privileged to be the first to see the full extent of amyloid deposition in the human AD brain – a major revelation to the eyes of a classically trained neuropathologist! The Nottingham group had drawn attention to the similarity in amino acid composition between APC and NFT preparations, and we were very surprised to find similar (but more ragged) N-terminal sequences from our own NFT preparations. Even more surprising, some antisera raised to both native and synthetic APC/A_4 reacted with a subpopulation of NFT in situ. All of the antisera that reacted with APC also strongly reacted with the vascular amyloid. We also observed that the APC might have a non-proteinaceous component. From these observations we made several bold predictions, including that the A_4(Aβ) subunit would be of neuronal origin, would consist of about 40 residues, and would be derived from a precursor protein. The concept of a neuronal origin of an intracerebral amyloidogenic protein (diametrically opposed to the prevailing views of Glenner, Frangione and Wisniewski) received further support from the studies of Ghiso and Frangione, who showed that a neuronally derived protein, cystatin C, was the cause of a rare Icelandic congophilic angiopathy. But the more compelling evidence for the neuronal origin of the AD-APC/ACA was to come eventually from the cloning of the Aβ precursor protein (APP) itself (Kang et al. 1987). Once we had determined the N-terminal sequence of the AD amyloid, it was clear that the major challenge ahead was

to use this information to derive a cDNA clone to uncover the precursor protein. This came to fruition in the second half of 1986 (Kang et al. 1987), when the APP gene was sequenced, disclosing the proteolytic origin of Aβ. Our studies at that time indicated that the C-terminus of Aβ was around positions 42/43. The neuronal origin of the Aβ was also confirmed through studies showing high levels of APP mRNA expression in the brain.

Our observations in 1985–1986 that the AD brain is under severe oxidative stress (Martins et al. 1986) were the first to suggest that the accumulation of Aβ in the AD brain might cause damage through some redox-active chemistry. This is currently one of our major strategies directed at therapeutic interventions in AD, in which we have increasing evidence that the Aβ fragment itself is driving the oxidative stress through metal-catalyzed oxidation. In some sense this closes a loop of investigation that has occupied us over the past several decades.

John Hardy

The Amyloid Hypothesis: history and alternatives

John Hardy Ph.D.[1]

Summary

In this centenary review, I outline two emerging hypotheses for us to consider if anti-amyloid approaches fail to have significant clinical impact.

The amyloid hypothesis of Alzheimer's disease, which was first explicitly proposed on the basis of genetic data by my colleagues and me in 1991 and 1992 (Hardy and Allsop 1991; Hardy and Higgins 1992) and, contemporaneously, by Selkoe (1991) and was implicit in the earlier work of Glenner and Murphy (1989) and Masters and Beyreuther (1987), has become the dominant philosophy driving research into the disorder.

While there has been much debate about whether the amyloid hypothesis is close to correct, until recently no coherent alternatives had been put forward that explain the genetic data. In this brief review to mark the 100th year of Alzheimer's lecture, I thought it would be more interesting, rather than merely outlining the amyloid hypothesis again (see Hardy and Selkoe 2002), to discuss two recent suggestions that offer coherent alternatives. I regard this as a valuable exercise at this time because several amyloid-based therapies are now in clinical trials, and if they are positive, we will feel the amyloid hypothesis is correct: if they are not, clearly, we should rethink our approaches to the disease.

The strength of the amyloid hypothesis is that it is consistent with the genetic findings: the autosomal dominant mutations in APP and in the presenilins all alter APP processing such that more Aβ42 is produced. Down's syndrome individuals, except those who are not trisomic for APP, develop Alzheimer pathology and those individuals who have a duplication of the APP locus also develop disease (Hardy 2006a). In addition, individuals with tau mutations develop tangle pathology and cell loss but not amyloid pathology, suggesting that tangle pathology is downstream of amyloid pathology. Mouse transgenic work has been completely consistent with the simple view outlined in Fig. 1.

The major weakness of the amyloid hypothesis, from a basic science perspective, has been the continued failure to identify the biochemical pathway that links amyloid to tangle formation. Transgenic experiments suggest there is a relatively direct link, and limited experiments in cultured neurons from mice in which the MAPT locus is deleted suggest that tau is needed for amyloid toxicity (Rapoport et al. 2002). However, work in this area has progressed slowly, and while we might have expected that there would be a rather direct link between amyloid and tau, none has yet been found.

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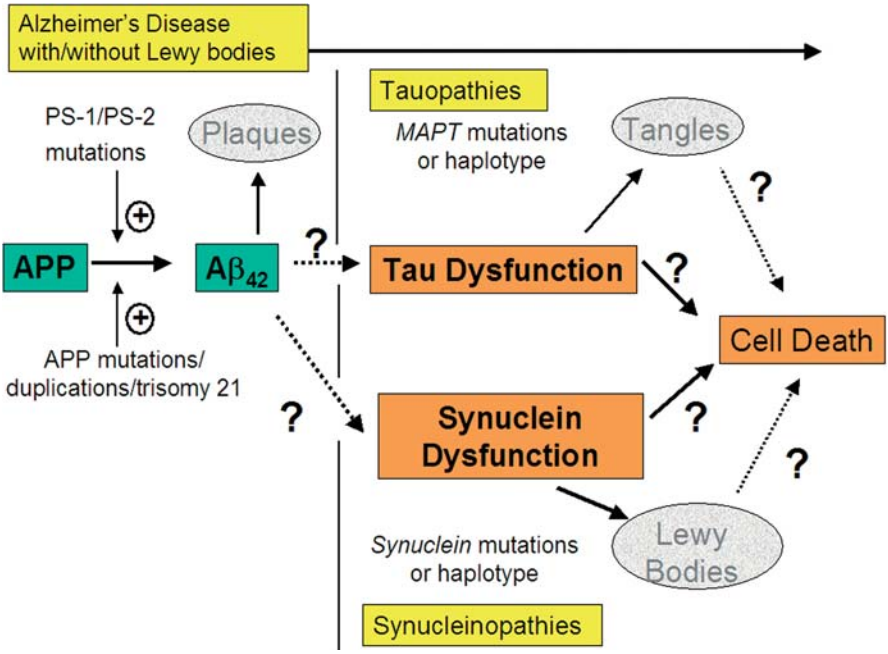

Fig. 1. The outline of the relationship between Aβ, Tau and α-Synuclein according to the amyloid hypothesis

Two emerging hypotheses have been suggested as alternatives to the amyloid hypothesis, which I will call the *presenilin inhibition hypothesis* and the *physiologic Aβ hypothesis*, which I outline below.

Presenilin inhibition hypothesis

This hypothesis has been suggested, in slightly different forms, by Shen (Beglopoulos and Shen 2006) and by Sambamurti (Sambamurti et al. 2006). These authors note that all the pathogenic presenilin mutations are, to some extent at least, a loss of its function as γ-secretase. The evidence that this is so is quite compelling. Presenilin knockdown increases Aβ42 production (Refolo et al. 1999); the mutation homologous to the *sel-12* Notch loss of function mutation is an Alzheimer-causing mutation that increases Aβ42 production (Lewis et al. 2000). γ-Secretase has many substrates besides APP (Sambamurti et al. 2006); however, APP is the predominant substrate, and it is possible that APP is a competitive inhibitor for the metabolism of presenilin's other substrates. Perhaps the problem in Alzheimer's disease reflects a more general inhibition of γ-secretase: in APP mutation cases, including those involving APP duplications, perhaps the problem is competitive inhibition of γ-secretase and there is a more direct relationship between presenilin inhibition and tangle formation and cell death (Beglopoulos and Shen 2006).

This hypothesis is almost as consistent with the genetic data as the amyloid hypothesis. The Swedish mutation can be accommodated because that causes increased flux through the pathway, as can the effects of APP duplications (Rovelet-Lecrux et al. 2006), but it is difficult immediately to accommodate the London mutations. From a therapeutic perspective, the hypothesis would predict that β-secretase inhibition may do more harm than good since this may lead to an increase in substrate levels for γ-secretase. From a basic science perspective, the hypothesis suggests that the connection between APP and tangles may relate to other presenilin substrates and signaling pathways rather than to APP.

Physiological Aβ hypothesis

An implicit assumption of the amyloid hypothesis has been that Aβ is just an accident of APP metabolism and that amyloid deposition is a pathological process, not a physiological process. In fact, this view has, in many ways, been eroding for some time. First, it was assumed that Aβ would not be a normal product of APP metabolism, and it was seen as a surprise when this was found not to be the case. And then it was seen as surprising that Aβ had a depressant effect on neurons, although this depressant effect has been noted without any discussion of the possible function of synaptic depression. Perhaps, indeed, these effects are physiological.

The pathology of Alzheimer's disease, by definition, includes amyloid plaques and neurofibrillary tangles. However, amyloid deposition also occurs as pronounced amyloid angiopathy. The relationship between the angiopathy and the neuritic plaques has not been clear and has been disputed for many years. However, recent data have shown that most, if not all, neuritic plaques are centered on angiopathic blood vessels (Kumar-Singh et al. 2005). Cullen and colleagues (2006) and Falangola and colleagues (2005) have presented data, in concurrence with earlier suggestions, that amyloid plaques are the sites of microhemorrhages (Miyikama et al. 1982; Hardy et al. 1986). Previously, Weller and colleagues (1998) have suggested that Aβ drains from the brain's extracellular fluid compartment via the perivascular space.

Together, these finding can be used to suggest a novel view of the relationships between Aβ, amyloid angiopathy, neuritic plaques and neuronal damage with the following components:

1. the initiating events in Alzheimer's disease are usually microhemorrhages;
2. one result of these events, either direct or indirect, is to alter the structure of γ-secretase from the Aβ40 to the Aβ42 producing conformation;
3. Aβ42 acts as a quick sealant for the blood vessel;
4. Aβ42 also acts as an immediate synaptic depressant to reduce metabolic demand during recovery;
5. tangles and neuronal damage and death are consequences of the hemorrhages and oxygen deprivation rather than a direct result of the amyloid deposition.

Under this scheme, the switch between Aβ40 and Aβ42 is physiological, not pathological, whose purpose (from an evolutionary perspective) is to act as a rapid protectant from cerebral hemorrhages.

This hypothesis is not immediately compatible with genetic data on the APP and presenilin mutations. Clearly, the people with these mutations would have a predisposition to form amyloid angiopathy and plaques but, unless they caused vascular damage and subtle hypoxia, it is not clear why they would lead to tangle formation and neuronal death. However, it is compatible with a surprising diversity of previously disparate and unexplained findings:

1. the presence of high concentrations of APP in platelets where, one presumes, it is part of the sealing cascade;
2. the fact that ApoE2 is associated with cerebral hemorrhages (Woo et al. 2005). The E4 allele is associated with amyloid deposition and Alzheimer's disease, whereas the E2 allele is associated with a paucity of deposition and, therefore, with hemorrhages;
3. the fact that so many presenilin mutations and so many pharmacologic agents can shift the metabolism of APP from Aβ40 to Aβ42 suggests that both ɣ-secretase conformations are stable;
4. the side effect of Aβ immunization of meningioencephalitis, which only occurred in cases with Alzheimer's disease (Nicoll et al. 2003). Presumably this side effect could relate to the reopening of vascular damage: a pulling off of the scab.

Of course, this hypothesis is also consistent with the epidemiologic evidence suggesting a relationship between vascular disease and Alzheimer's disease (Launer 2005), although in many ways it resembles a more sophisticated retake of the old designation of Alzheimer's disease as a hardening of the arteries.

This hypothesis would suggest that strategies designed to improve vascular health would be the most profitable route to Alzheimer therapy and that treatments based solely on Aβ would be likely to have side effects related to cerebral hemorrhages

Conclusion

Aβ-modulating therapies are now in progress; if they work, well and good. If, over the next period, these therapeutic strategies are not successful, we will have to rethink our approach. These two hypotheses offer a start in this direction.

Alison Goate

Segregation of a missense mutation in the amyloid β-protein precursor gene with familial Alzheimer's disease

Alison Goate[1]

In 1991, little was known about the pathogenesis of Alzheimer's disease (AD). Earlier studies had demonstrated that plaques contain amyloid β (Aβ) and that neurofibrillary tangles were composed of paired-helical filaments of hyperphosphorylated tau (Glenner and Wong 1984a; Masters et al. 1985a; Grundke-Iqbal et al. 1986a). However, a major impediment to a more detailed understanding of AD was the absence of cellular or animal models of disease.

Mutations in APP cause AD and stroke resulting from cerebral hemorrhage

It has been known for more than 50 years that families exist in which AD has an early onset (< 60 years) and is inherited as an autosomal dominant trait (Familial Alzheimer's disease (FAD); Lowenberg and Waggoner 1934), but the techniques of molecular genetics only made analysis of these families feasible in the late 1980s. Initial studies of FAD focused on chromosome 21 because individuals with Down Syndrome all develop AD and Aβ is derived from a precursor, β-amyloid protein precursor (APP), that is encoded by a gene on chromosome 21 (Goldgaber et al. 1987b; Kang et al. 1987; Robakis et al. 1987b; Tanzi et al. 1987). However, the APP gene was quickly excluded in several families (Tanzi et al. 1987; Van Broeckhoven et al. 1987). At this time, FAD was assumed to be a homogeneous disorder; therefore, exclusion of APP in one family was thought to exclude the gene in all families.

A turning point in AD genetics was a multi-center investigation that analyzed many families and came to the conclusion that FAD exhibited non-allelic genetic heterogeneity (St George-Hyslop et al. 1990). Shortly thereafter, two papers reported linkage to the APP gene and a mutation in APP in a disorder called hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D; Levy et al. 1990; Van Broeckhoven et al. 1990). These papers led our group to re-evaluate the APP gene in our own series of FAD kindreds. We had previously reported linkage to chromosome 21 in these families (Goate et al. 1989). Segregation analysis of multiple markers along chromosome 21 in the largest of these families demonstrated a common disease haplotype in all affected individuals. Information from two unaffected individuals placed the disease gene between D21S1 and D21S17, a region that includes the APP gene. Exons 16 and 17 were sequenced first because these exons encode the Aβ peptide and because the mutation

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that causes HCHWA-D is in exon 17. This sequencing revealed a mutation that results in a missense mutation, V717I (Goate et al. 1991). This mutation was present in all affected individuals in the family but none of the unaffected individuals. Furthermore, it was absent from 100 unrelated normal individuals but present in a second, early-onset FAD kindred. The V717I substitution is conservative but its location, close to the C-terminus of the Aβ peptide, suggested that it might influence production of Aβ.

We made four predictions: 1) other FAD kindreds would be identified with APP mutations; 2) other FAD genes would be identified; 3) Aβ deposition is the central event in the pathogenesis of AD; and 4) regulatory variants in APP might lead to late onset AD.

Mutations in APP alter processing or the physico-chemical properties of Aβ

Eight months after our original report, we reported a second mutation in APP that caused FAD (Chartier-Harlin et al. 1991). This mutation was also at codon 717 but resulted in a V717G amino acid substitution. Based upon the two mutations, we hypothesized that FAD mutations in APP alter APP processing to enhance Aβ production and thus Aβ deposition. In the 15 years since the publication of these papers, 23 amino acid substitutions have been described in the APP gene (http//:www.alzforum.), 19 of which have been shown to alter Aβ metabolism in vitro or cause age-dependent Aβ deposition in vivo (reviewed in Selkoe and Podlisny 2002) (Fig. 1, Table 1). In vitro overexpression of APP FAD mutations has demonstrated that all mutations affect APP processing, leading to changes in the amount of Aβ produced, changes in the ratios of the Aβ species produced and/or changes in the physico-chemical properties of Aβ. The so-called Swedish mutation results in an APP molecule that is a better substrate for β-secretase, resulting in higher levels of Aβ (Citron et al. 1992). In contrast, FAD mu-

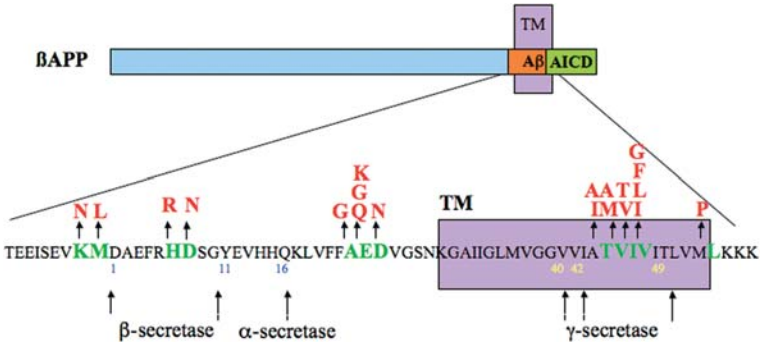


Fig. 1. Location of disease-causing mutations in APP. APP is a type 1 transmembrane protein. FAD mutations in APP are located within and flanking the Aβ sequence and close to the proteolytic cleavage sites within APP. FAD mutations are shown in red above the normal sequence of the protein. Numbers indicate the amino acid position within the Aβ peptide. The locations of the major proteolytic cleavage sites in APP are indicated by arrows below the sequence

Table 1. Pathogenic mutations in the APP gene (AU)

Mutation (number of families)	Phenotype	Age of Onset (years)
KM670NL (Swedish) (1)	AD + CAA (need to define?)	55
D678N (1)	AD	61.3
A692G (Flemish) (2)	AD / cerebral hemorrhage	45.9
E693G (Artic) (2)	AD	59.7
E693K (Italian) (3)	CAA	?
E693Q (Dutch) (4)	E693Q (Dutch) (4)	Cerebral hemorrhage
57.5		
D694N (Iowa) (2)	AD/CAA/ cerebral hemorrhage	62
L705V (1)	CAA	64
T714I (Austrian) (3)	AD	36.3
T714A (Iranian) (2)	AD	49.8
V715M (French) (1)	AD	51
V715A (German) (3)	AD	45.3
I716V (Florida) (1)	AD	53
I716T (1)	AD	36
V717F (3)	AD	41.2
V717G (1)	AD	55
V717I (London) (29)	AD	52.9
V717L (Indiana) (3)	AD	44
L723P (Australian) (1)	AD	56

Adapted from http://www.molgen.ua.ac.be/ADMutations/ and http://www.alzforum.org/res/com/mut/default.asp

tations located between APP714 and APP723 result in altered cleavage by γ-secretase (Suzuki et al. 1994). The effect of these mutations is more complex because the amount of Aβ and the ratios of the different Aβ species (Aβ37-Aβ43) vary with each mutation (Hecimovic et al. 2004). However, a common feature of all mutations seems to be an increase in Aβ42 relative to other Aβ species.

Five mutations have been reported within the Aβ sequence at residues APP692-694. These mutations are often associated with cerebral hemorrhage rather than AD (Levy et al. 1990; Hendriks et al. 1992; Nilsberth et al. 2001). Although these mutations are located near the α-secretase cleavage site and thus could alter APP processing, they are also within the Aβ peptide and thus alter the physico-chemical properties of the peptide, leading to increased protofibril formation (Nilsberth et al. 2001; Stenh et al. 2002).

Several of these mutations have also been used to develop transgenic animals (Games et al. 1995; Sturchler-Pierrat et al. 1997; Hsiao 1998). A consistent property of these animals is an age-dependent Aβ deposition. Another, striking observation coming from these mice is that overexpression of Aβ42 leads to parenchymal Aβ deposition, such as that seen in AD, whereas overexpression of Aβ40 leads to Aβ deposition primarily in the cerebral vessels (Herzig et al. 2004). Thus APPSwe, which results in higher levels of both Aβ40 and Aβ42, leads to both pathologies (Fryer et al. 2005), whereas APP717 mutations lead primarily to parenchymal Aβ deposition

(Games et al. 1995) and APP692 leads to Aβ deposition in the cerebral vessels (Herzig et al. 2004). Recently, duplication of the APP gene has been reported in several families. Consistent with data from transgenic mice, families that overexpress APP but do not have altered Aβ ratios have both cerebral hemorrhage and dementia (Rovelet-Lecrux et al. 2006).

Mutations in at least three genes can cause FAD

In 1995, mutations in two homologues now called presenilin 1 (PS1) and presenilin 2 (PS2) were reported in several large FAD kindreds (Sherrington et al. 1995; Levy-Lahad et al. 1995b; Rogaev et al. 1995). In vitro and in vivo studies have demonstrated that FAD mutations in PS1 and PS2 also lead to changes in γ-secretase cleavage of APP, resulting in higher Aβ42/Aβ40 ratios and early Aβ deposition (reviewed in Selkoe and Podlisny 2002). All known FAD mutations appear to alter APP processing to produce more Aβ, increase the propensity of Aβ to form protofibrils or alter the ratio of the Aβ species. Most early onset FAD kindreds appear to carry a mutation in either the substrate or the enzyme that generates Aβ. It is unclear how many other FAD genes there are because most large FAD kindreds carry a mutation in one of the three known genes.

A major focus of current genetic research is the identification of genetic risk factors for late-onset AD (LOAD). Currently, the only known genetic risk factor for LOAD is *APOE4* (Strittmatter et al. 1993a; Corder et al. 1993). However, only 50% of AD cases carry one or more copies of the *E4* allele, suggesting that there must be other risk factors.

Is Aβ deposition central to the disease process?

The third prediction has proven to be the most controversial. While it is clear that FAD mutations in APP result in increased Aβ deposition, it is unclear whether the deposition is itself pathogenic. Several alternative hypotheses have been put forward. Rather than the deposited Aβ being neurotoxic, some have suggested that the neurodegeneration observed in AD is caused by either soluble oligomers of Aβ, the build-up of C-terminal fragments of APP or abnormal signaling by the intracellular domain of APP (Neve and Robakis 1998; Walsh and Selkoe 2004b).

A key question for many years was whether LOAD also involves an Aβ-centric mechanism. Elegant transgenic studies have demonstrated that Apolipoprotein E (APOE) is required for Aβ fibrillogenesis (amyloid formation) and that APOE4 promotes Aβ deposition and amyloid formation (Holtzman et al. 2000a). The fact that all four known AD genes implicate Aβ and that APOE implicates Aβ fibrillogenesis directly provides support for the hypothesis that Aβ deposition is central to the disease.

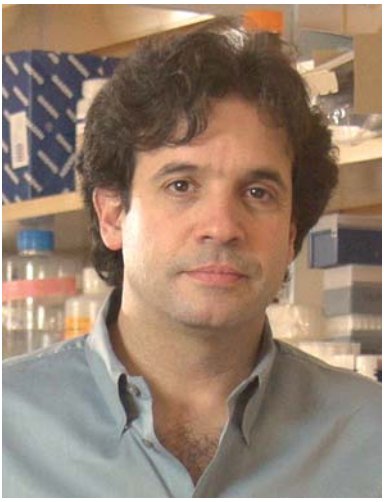
Can overexpression of APP lead to AD?

The fourth prediction was that variants in APP that altered the level of APP expression might also result in AD. Suprisingly, this hypothesis has not been rigorously tested.

Genetic linkage studies in LOAD families have reported evidence for linkage on the long arm of chromosome 21 (Myers et al. 2002). Despite these promising results, genetic analyses of the APP gene in LOAD have been very limited and inadequate to test the hypothesis. Thus, more than 15 years after the original report of a missense mutation in the APP gene causing early onset FAD, the APP gene remains a promising but untested candidate risk factor for LOAD.

Conclusion

Our report of a missense mutation in the APP gene that caused FAD provided an important turning point in AD research. This paper and subsequent papers provided information that has led to the development of cellular and animal models that recapitulate at least part of the AD phenotype. These models have greatly enhanced our understanding of the pathobiology of AD and have led to the identification of drug targets for AD and the development of drugs that are currently in clinical trials. Furthermore, the major predictions of this paper have withstood the test of time remarkably well.



Rudolph E. Tanzi

From the amyloid β protein (A4) to isolation of the first Alzheimer's disease gene: amyloid β (A4) precursor protein (APP)

Rudolph E. Tanzi[1]

Introduction

As life expectancy continues to increase, so will the prevalence and incidence of Alzheimer's disease (AD) in our elderly population; by 2050, as many as 14 million AD cases are expected in the USA, alone. AD is characterized by global cognitive decline in association with specific brain pathological lesions, neuronal loss, and synaptic pruning. The disease takes its name from Dr. Alois Alzheimer, a German psychiatrist who in the fall of 1906 suggested that specific physical aberrations in the brain were driving dementia in his female patient, Auguste D (Alzheimer 1907a). Alzheimer had been treating Auguste D since she was first admitted at age 51 to the Hospital for the Mentally Ill and Epileptics in Frankfurt for "frenzied delirium." Shortly after the patient's death at age 56, Alzheimer presented the results of his post-mortem examination of her brain at a meeting in Tubingen. He wisely took advantage of Camillo Golgi's new silver staining technique to examine the neurons in his patient's brain tissue. Alzheimer was not the first to describe the appearance of senile plaques (clusters he called "miliary bodies"); neither did he know that the core was made of amyloid, despite Virchow's description of "amyloid" decades earlier. However, with the help of Golgi's silver stain, Alzheimer does appear to have been the first to suggest that the plaques were associated with "dense bundles of fibrils" choking the inside of cortical neurons, i.e., neurofibrillary tangles, and that these lesions were the cause of dementia in Auguste D. Thus, the pathogenic mechanism presented by Alzheimer in 1906 can in some ways be considered the earliest form of the "amyloid hypothesis."

It was not until the 1960s that Robert Terry, Michael Kidd, Henry Wisniewski, and others would employ both light and electron microscopy to reveal the ultrastructural details of plaques and tangles (reviewed in Tanzi and Parson 2000). However, the question of primacy remained. Did plaques or tangles come first, and which lesion, if either, was killing off neurons? While these questions could not be immediately addressed, by the late-60s, Bernard Tomlinson, Gary Blessed, and Martin Roth provided the next boost for the emerging amyloid hypothesis when they suggested for the first time that dementia was correlated with senile plaque counts in the cerebral gray matter (Roth et al. 1966). Later, in 1968, these same investigators showed that over 60% of the demented elderly (the "senile") harbored the same lesions observed by Alzheimer in his

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pre-senile patient, Auguste D (Blessed et al. 1968). By 1970, as average life expectancy was hitting 70 years old, it became clear that AD was a prevalent cause of most cases of "senility." And, a growing amount of attention was being paid to the origins of senile plaques.

Discovery of the first AD gene: amyloid β (A4) Precursor Protein

The steadily emerging amyloid hypothesis received perhaps its greatest infusion of support in the early 1980s when Dr. George Glenner, an amyloidologist, entered the scene and argued that amyloid was the central player in AD pathology (Glenner 1981). By the summer of 1983, Glenner and his assistant, Cai'ne Wong, had started obtaining their first amino acid sequences from cerebral blood vessel amyloid isolated from a patient with Down syndrome (Tanzi and Parson 2000). In May, 1984, they published the first sequence of the 4 kDa peptide (called the amyloid β protein), which was found to be the major component of β-amyloid (Glenner and Wong 1984a). In a follow-up paper in August 1984, Glenner and Wong (1984b) showed the same amino acid sequence for amyloid β-protein deposits in both Down syndrome and AD and, since Down syndrome is caused by trisomy 21, made the prophetic statement that a genetic defect causing AD might be localized on chromosome 21. A year later, Colin Masters, who had teamed up with Konrad Beyreuther, reported that the amino acid sequences of senile plaque core proteins from AD and Down syndrome brains were virtually identical to the sequence published by Glenner (Masters et al. 1985a). The stage was now set for "reverse genetics" to potentially furnish the first AD gene and first molecular target for drug discovery.

Between 1980 and 1985, I had been working with Dr. Jim Gusella on a project that would become the first to identify and employ human DNA variants to localize a disease gene where no biochemical clues were available regarding etiology or pathogenesis of the disease. While considered quite routine today, this approach had never been used before, and the project had more than its share of "doubting Thomases." Yet, Jim Gusella believed (and convinced me) that if we could track the inheritance of a sufficient number of common DNA variants [restriction fragment length polymorphisms (RFLPs)] through families with the devastating movement disorder, Huntington's disease (HD), we might be able to find the location of that disease gene in the human genome. This finding would then pave the way for later pinpointing the exact genetic cause of the disease, all solely through genetics. When we started the project in 1980, only one human gene polymorphism was known, AW101 (amidst a handful of protein polymorphisms).

In the fall of 1980, we set out to identify as many human RFLPs as possible with the hopes of finding one that co-segregated with the onset of HD. The problem, Jim said, was that we might have to test hundreds, if not thousands, of RFLPs before we found one that revealed the location of the HD gene. As it turned out, not just luck, but *miraculous* luck was on our side. Among the first 12 RFLPs we pulled from the genome, one, which we had named "G8," was tightly linked to HD and mapped to the short arm of chromosome 4 (Gusella et al. 1983). Ten years later, we would learn that G8 sits less than 200 kilobases from the HD gene mutation. The odds against pulling out a RFLP so close to the HD gene mutation in a genome of three billion basepairs were greater than

15,000:1! And, if that were not lucky enough, even if we did not find G8, the very next RFLP that was randomly chosen, G9, was only 20 million basepairs away from the HD gene, which meant that, eventually, with more families, we would have found linkage to HD with G9, even in the absence of G8!

While working on the HD screen, I had also initiated a side project in Jim's lab in collaboration with Paul Watkins – an attempt to build the first complete genetic linkage map of a human chromosome. We chose the smallest one, chromosome 21, partly because of its role in Down syndrome (trisomy 21). We began isolating RFLPs from chromosome 21 (using chromosome 21-specific somatic cell hybrids) in an attempt to build a complete genetic linkage map that could be used to map features of Down syndrome. While pursuing the chromosome 21 genetic linkage map, I had read Glenner's prediction of an AD gene on chromosome 21 in his 1984 paper. In the meantime, Jim was able to obtain cell lines from a Canadian family with early-onset (< 65) familial AD (FAD) from Ron Polinsky and Linda Nee at the NIH. In the summer of 1984, after testing that family for linkage to markers on our chromosome 21 map, Jim had me bring the FAD-chromosome 21 genotype data to Michael Conneally's lab at the University of Indiana. There I would test for genetic linkage of chromosome 21 to FAD in the Canadian family, with the help of his graduate student, Peggy Wallace. The results were dismally negative. Meanwhile, Jim's lab had collected another FAD family of Italian origin with the help of Robert Feldman (Boston University) and Jean-Francois Foncin (La Salpetriere Hospital, Paris). I started testing our chromosome 21 markers as soon as I got back to Boston. But once again, by early 1985, we had found no signs of genetic linkage of chromosome 21 with FAD in the second family.

Around this time, Peter St. George-Hyslop had joined Gusella's lab as a post-doctoral fellow and assumed responsibility for the FAD-chromosome 21 linkage study, which was now extended to two additional kindreds, one from Germany and one from Russia (from Dan Pollen, University of Massachusetts). Meanwhile, by the fall of 1985, I had become a graduate student in the Neuroscience Program at Harvard Medical School. For my first rotation project, I had decided to employ a "reverse genetics" approach to isolating the gene responsible for the amyloid β-protein gene. For this purpose, I had joined up with renowned Down syndrome geneticist, Dr. David Kurnit, at Boston Children's Hospital. He set me up to work with his post-doctoral fellow, Rachael Neve, who had been constructing human cDNA libraries. My chromosome 21 map collaborator, Paul Watkins, and I then designed "best-guess" oligonucleotides to the amyloid β protein amino acid sequences published by Glenner and Masters.

To isolate the amyloid β protein gene, we employed what we called the "genomic window" strategy. This novel approach was based on comparative Southern blot analysis using various restriction fragment patterns. Briefly, we designed two non-overlapping oligonucleotides to the amyloid β protein amino acid sequence: the first, a 21-mer corresponding to amino acids 1-7, and the second, a 48-mer corresponding to amino acids 9-24. We then screened Rachael Neve's human fetal brain and fetal liver cDNA libraries and only pursued cDNA clones that hybridized independently to both oligonucleotides. We next picked cDNAs that hybridized to the same or overlapping sets of bands (on Southern blots containing human DNA cut with various restriction enzymes) as those detected by the two oligonucleotides used to screen the libraries. Next, just in case Glenner's prediction was correct, we asked whether any of those cDNAs mapped to chromosome 21, using human-rodent somatic cell hybrid cell lines

(from David Patterson, University of Colorado, and Margaret Van Keuren, University of Michigan). Two clones, FB63 and FB68, each detected a set of bands that matched subsets detected by the two screening oligonucleotides. Surprisingly, both clones mapped to chromosome 21, in agreement with Glenner's prediction. The two clones contained the same 1.1 kb EcoRI fragment and sequencing of FB68 by Susan Pagan revealed that this was a partial cDNA clone encoding a protein containing residues 3-29 of the amyloid β protein (Tanzi et al. 1987a). The final proof that the cDNA corresponded to a single copy gene on chromosome 21 came from hybridization to a whole genome somatic cell hybrid panel (from Gail Bruns, Harvard).

We next examined the expression profile for the gene, which we called the "β protein" gene, and found it be a 3.2 kb message ubiquitously expressed in all human tissues tested, with its highest expression in brain, heart, kidney, spleen, and pancreas. The gene was also expressed throughout adult brain, with highest expression in brain regions, A40, A44, A20/21, A10 and cerebellar cortex (Fig. 1). Next, we showed that an extra dose of the gene led to excessive amounts of message in Down syndrome patients, most likely explaining how these patients accumulate excessive amounts of amyloid β protein in their brains by middle age (Fig. 2). Finally, we used our chromosome 21 markers and RFLPs detected by FB68 to genetically map the β protein gene near marker, D21S1, from our chromosome 21 linkage map. In parallel studies, Peter St. George-Hyslop was continuing to test markers from our chromosome 21 map in the four FAD families, and together with genetic analyst, Jonathan Haines, had found evidence for genetic linkage of FAD to the region of chromosome 21 around the same marker, D21S1 (St. George-Hyslop et al. 1987a). Statistical significance for the linkage result derived primarily from the Italian FAD family. However, my own earlier analyses of the chromosome 21 markers in the Italian and Canadian FAD families had yielded only *negative* results. Two possible explanations for the discrepancy were 1) Peter had simply tested additional chromosome 21 markers in all four FAD families, and 2) Jonathan had employed the relatively new method of "multi-point linkage analysis," which tests several markers for linkage simultaneously and can, thus, yield different linkage results from the single locus analyses that I had carried out earlier.

Soon after our β protein gene [later renamed amyloid β (A4) precursor protein {APP)] cloning paper (Tanzi et al. 1987a) and the chromosome 21-FAD linkage paper (St. George-Hyslop et al. 1987a) were published in *Science*, I completed the definitive experiment aimed at asking whether the APP gene was linked to FAD in the four Massachusetts General Hospital (MGH) FAD pedigees by analyzing the segregation of APP gene RFLPs in all four families. The genetic linkage results were all negative. APP was clearly not the genetic culprit in these four FAD kindreds (Tanzi et al. 1987b). This finding meant that, even if there were a gene on chromosome 21 responsible for FAD in these four pedigrees, as purported in the St. George-Hyslop et al. (1987a) study, it was *not* APP. Later, these same four FAD pedigrees would be shown to actually be linked to chromosome 14 and to contain mutations in the presenilin 1 gene (Sherrington et al. 1995). Ironically, however, the most likely spurious multi-point linkage of these four FAD kindreds to chromosome 21 in 1987 (St. George-Hyslop et al. 1987a) had motivated other groups to analyze their own independent FAD families, some of which would be genuinely linked to chromosome 21, and which would later reveal pathogenic FAD mutations in the APP gene.

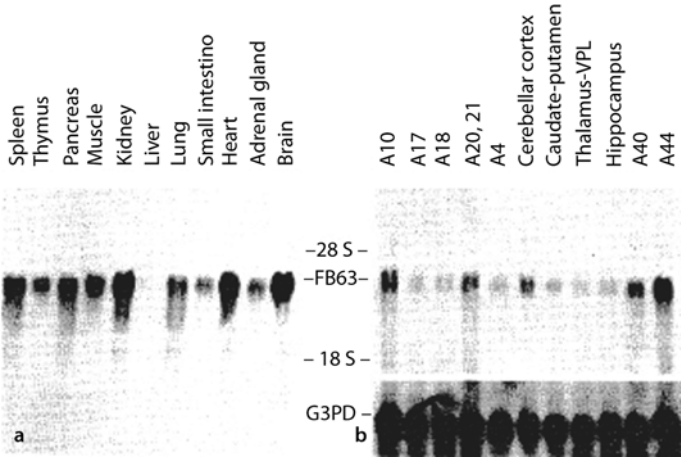

Fig. 1. Expression of APP (FB63) in (**a**) 20- to 22-week-old human fetal tissues and (**b**) adult brain regions (from Tanzi et al., 1987a)

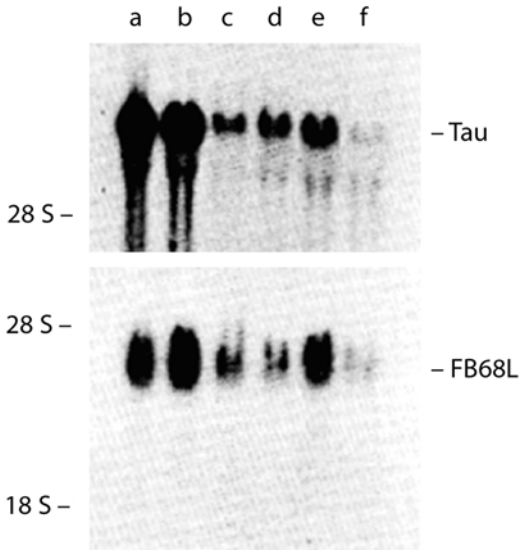

Fig. 2. Expression of APP (FB68L) and Tau in 19-week-old normal (lane a) and trisomy 21 (lane b) brain; adult normal (lane c) and AD (lane d) cerebellum; adult normal (lane e) and AD (lane f) frontal cortex (from Tanzi et al., 1987a)

Ultimately, the amyloid β protein (A4; Aβ) sequence was successfully employed by four different groups to isolate the APP gene (Goldgaber et al. 1987a; Kang et al. 1987; Robakis et al. 1987a; Tanzi et al. 1987a). However, only the Kang et al. study isolated the entire APP cDNA. This full-length APP clone revealed APP to be a type I integral transmembrane protein. Later in 1988, we discovered a novel transcript of

APP (APP751) containing an alternatively spliced exon encoding a Kunitz protease inhibitor domain. We found this to be the main form of APP in the periphery (Tanzi et al. 1988), and interestingly, the secreted portion of this form of APP had previously been identified as protease nexin II, which plays a key role in the coagulation pathway (Van Nostrand et al. 1989). To date, this is the clearest physiological role known for APP. As an interesting side bar to the APP cloning study, one of the cDNA clones that we had pulled out with our original oligonucleotide screen for APP turned out to be the gene that causes Wilson's disease, a copper toxicity disorder mainly affecting the brain and liver (Tanzi et al. 1993). Interestingly, the 48-mer oligonucleotide corresponding to amino acids 9-24 of the Aβ region was homologous to a sequence in the Wilson's gene encoding a copper-binding motif, thus explaining how this gene was fished out in the same experiment that landed APP. We would later show that the 9-24 amino acid region of Aβ also binds copper, which, along with zinc, drives oligomerization and aggregation of the peptide (Bush et al. 1994). Oddly enough, however, while the homologous DNA regions between the APP and the Wilson's disease gene both encoded copper-binding motifs, they were of two different types: the motif in APP was histidine-based whereas the motif in the Wilson's disease protein was cysteine-based. Yet, both types of copper-binding motifs in APP and the Wilson's disease protein were encoded in a single homologous stretch of DNA, in two different reading frames! To this day, is it is unclear whether this is due to simple coincidence or an example of evolutionary economy of function in the genome. Interestingly, APP contains another copper-binding site in its N-terminus, and this motif is uniquely conserved in the two other homologs of the human APP family that Wilma Wasco and I later identified, APLP1 (Wasco et al. 1992) and APLP2 (Wasco et al. 1993).

The first disease mutation in APP was reported in 1990 by Frangione, Van Broeckhoven, and colleagues, who after sequencing exons 16 and 17 of the APP gene (encoding the Aβ domain), discovered a mutation that caused hereditary cerebral hemorrhage with amyloidosis in a Dutch family linked to chromosome 21 (Levy et al. 1990; Van Broeckhoven et al. 1990). Sequencing of these same two APP exons in FAD families (that were genuinely linked to chromosome 21) then led to the discovery of the first FAD mutation in 1991 (Goate et al. 1991). Later, in the summer of 1995, St. George-Hyslop's group, in collaboration with our and other laboratories, reported that the original four MGH FAD pedigrees actually harbored mutations in the gene called S182 (now presenilin 1; *PSEN1*) on chromosome 14 (Sherrington et al. 1995). A month and a half later, in collaboration with Jerry Schellenberg, we first reported FAD mutations in the S182 homolog, STM2 (now called presenilin 2; *PSEN2*) on chromosome 1 (Levy-Lahad et al. 1995a). Collectively, the > 160 known mutations in APP and the presenilins account for roughly half of all cases of early-onset FAD and < 1% of all AD cases. However, studies of these three genes have arguably provided the most valuable clues we currently have regarding the etiology and pathogenesis of AD.

Summary and Future Directions

From Alois Alzheimer's clinicopathological assessment of Auguste D to the discovery of the amyloid β protein (A4, Aβ) to the cloning of the AD field's first molecular drug target, APP, to the identification of the pathogenic mutations causing FAD, the "amyloid

hypothesis" has continued to gather momentum. Moreover, functional studies of FAD mutations in APP and the presenilins in cell- and animal-based model systems have continued to lend strong support to the amyloid hypothesis. One updated version of the amyloid hypothesis, called the "amyloid cascade" hypothesis, posits that all AD pathology begins with excessive accumulation of Aβ in brain (Hardy and Higgins 1992). When one considers how Aβ accumulates in the brains of early-onset FAD patients, the common molecular phenotype for the vast majority of known early-onset FAD gene mutations does not involve increased production of Aβ but an increase in the ratio of $A\beta_{42}$:$A\beta_{40}$ (reviewed in Price et al. 1998). $A\beta_{42}$ is the more amyloidogenic and toxic species of the peptide that has recently been shown to impair synaptic plasticity (reviewed in Tanzi 2005). Many industrial AD drug discovery programs have assumed that FAD mutations simply serve to increase $A\beta_{42}$ levels and have, therefore, targeted $A\beta_{42}$ production. However, based on the known FAD mutations in APP and the presenilins, it is most likely not just absolute levels of $A\beta_{42}$ that drive AD pathology but the relative amounts, or stoichimetric ratio of $A\beta_{42}$ and $A\beta_{40}$. This ratio can be increased not only by elevations in $A\beta_{42}$, but also by decreases in $A\beta_{40}$. The latter most likely occurs as a result of loss of normal cleavage of APP by the presenilin/γ-secretase enzyme complex, e.g., owing to FAD mutations in either APP or the presenilins. Thus, in order to translate seminal genetic discoveries into effective therapies for preventing and treating AD, it will be important in future studies to more directly counter the exact molecular consequences of FAD mutations by medicinally suppressing the common biochemical phenotype of most FAD mutations. Specifically, we will need therapies that can safely and effectively decrease the ratio of $A\beta_{42}$:$A\beta_{40}$, either by reducing levels of $A\beta_{42}$, or perhaps even by increasing levels of $A\beta_{40}$. In summary, AD genetic studies over the past 20 years have provided us with the first tangible therapeutic targets for AD while also serving to endorse the earliest origins of the amyloid hypothesis, beginning with Alois Alzheimer's presentation of Auguste D in 1906. It is now critical to successfully translate these findings into effective therapeutics for stopping this dreadful disease, especially as life span steadily increases.

Karen Hsiao Ashe

Commentary on "Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice"

Karen Hsiao Ashe[1, 2, 3]

The Tg2576 mouse model was developed to study the molecular basis of memory loss in Alzheimer's disease (Hsiao 1996). The descriptions of the memory impairments, neuropathological abnormalities and biochemical analyses in Tg2576 mice were first presented in Chicago at IBC's 5th Annual Conference on Alzheimer's disease in June 1996. At that time the PDAPP mouse model had been shown to develop Congophilic Aβ deposits (Games 1995). Age-related behavioral and memory deficits had been demonstrated in several lines of transgenic mice that did not form Congophilic plaques (Moran 1995; Hsiao 1995), but little was known about memory function in mice that produced *bona fide* plaques. Because plaques that stain with Congo red dye are one of the neuropathological hallmarks of Alzheimer's disease, the demonstration that age-related memory impairment correlated roughly with the appearance of Congophilic plaques was hailed as a major advance in the field. Ironically, the Congophilic plaques that were considered to be a crucial feature of Tg2576 mice have been shown in subsequent research to have little or no impact on memory function.

Tg2576 mice are the most widely used model of Alzheimer's disease in the world, thanks to the joint efforts of the University of Minnesota and Mayo Medical Ventures to make them widely available, without scientific restriction, to investigators in both for-profit and not-for-profit institutions. Over 200 papers have been published from at least 60 independent laboratories using Tg2576 mice as a reagent. Because Tg2576 mice develop the amyloid plaques, dystrophic neurites, astrogliosis, microgliosis, oxidative stress and inflammatory cytokines that are characteristic of Alzheimer's disease (Irizarry 1997; Frautschy 1998; Smith 1998; Pappolla 1998; Benzing 1999), they have been extremely useful for studying these aspects of the illness. However, they lack neurofibrillary tangles, synaptic loss and neuronal loss (Irizarry 1997), which are prominent aspects of Alzheimer's disease. This lack led many scientists, rightly, to question the validity of Tg2576 mice as a model of Alzheimer's disease.

The value of Tg2576 mice as a model of Alzheimer's disease is only as good as our understanding of how well they mimic various stages of the illness. To appreciate the context in which these mice have helped us understand Alzheimer's disease, it has been useful to delineate the natural history of the disease. Alzheimer's disease has an insidious onset; we do not know precisely when it begins. Recent studies suggest that the disease begins earlier than it can currently be diagnosed, possibly even before neuronal loss has occurred. In this context, Tg2576 mice appear to represent individuals at high

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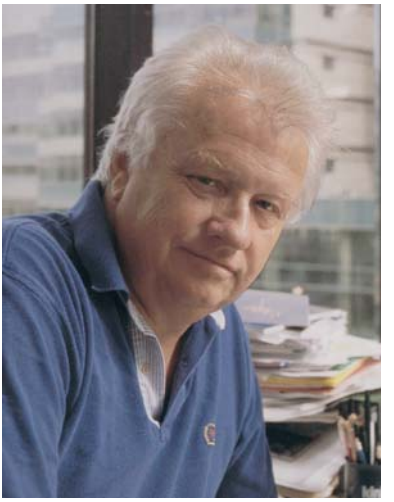
risk for developing Alzheimer's disease, at a stage before they have become demented or have lost neurons.

In Tg2576 mice, memory loss precedes amyloid plaque deposition by four to six months (Westerman 2002). After memory loss has developed, but before plaques have formed, memory function can be restored by passive immunization with anti-Aβ antibodies (Kotilinek 2002). These studies indicate that Aβ is involved in impairing memory in Tg2576 mice, but that plaques are not a major cause of the memory deficits. Recently, we purified from the brains of impaired Tg2576 mice a post-translationally modified species of Aβ that causes memory deficits (Lesné 2006). This species, a 56-kilodalton assembly of Aβ molecules called Aβ*56 (Aβ star 56), is also found in brain tissue of patients that died with Alzheimer's disease, suggesting an opportunity for developing diagnostics and drugs targeting a therapeutic target specifically responsible for cognitive impairment. The discovery of Aβ*56 in Tg2576 mice represents the culmination of 14 years of work in my laboratory to understand one aspect of the molecular basis of memory loss in Alzheimer's disease.



Donald L. Price

Vision for the future

Alzheimer's Disease: Pathogenesis, Models and Experimental Therapeutics

Donald L. Price[1,2,3], *Tong Li*[1,3], *Fiona M. Laird*[1,3], *Mohamed Farah*[1,3], *Alena V. Savonenko*[1], *Michael Lee*[2], *Juan Troncoso*[1,2,3], and *Philip C. Wong*[1,2,3]

Alzheimer's Disease (AD) is associated with progressive impairments of memory and cognition, genetic causes/risk factors, characteristic neuropathology and biochemistry, and dysfunction/death of specific subsets of neurons in certain brain regions/neural circuits. Disease-defining pathology/biochemistry include the presence of extracellular toxic Aβ42 peptides (oligomers) and intracellular protein aggregates of tau. Over the past several decades, investigators have taken advantage of advances in knowledge of the disease to design therapies for AD. For example, the demonstration of abnormalities of basal forebrain neurons (with cholinergic deficits in the cortex and hippocampus) led to the introduction of cholinesterase inhibitors for symptomatic treatments. Similarly, when information about involvement of glutamatergic systems in ventral-medial temporal lobes in AD was coupled with knowledge of roles of glutamate in excitotoxicity, glutamate antagonists were tried as treatments. Building on several observations by Glenner and by many geneticists regarding Aβ peptides and AD-related genes, investigators have generated a variety of models, particularly transgenic and knockout (KO) mice, that recapitulate some pathologies of AD or alter the expression of proteins critical to pathogenesis. Their models have proved to be of great value in understanding amyloid-related disease mechanisms, in identifying therapeutic targets, and in testing novel treatments. In this presentation, I will comment on these approaches, focusing on the roles of β- and γ-secretase activities in amyloidogenesis and the potential of these enzymes as therapeutic targets for future clinical trials.

In familial AD, mutant genes encoding the amyloid precursor protein (APP) or presenilins (PS1 and 2) influence the levels and/or character of Aβ peptides, which are generated via APP cleavages by the activities of β-secretase 1 (BACE1), and γ-secretase (the PS, Nct, pen2, Aph-1 multi-protein complex). Mice overexpressing mutant *APP/PS1* develop age-associated increases in brain levels of Aβ42, Aβ oligomers, neuritic plaques, and deficits in working memory. To gain insights into potential therapeutic targets, Dr. Phil Wong and colleagues targeted genes encoding proteins hypothesized to be critical for pro-amyloidogenic secretase activities. *BACE1 −/−* mice are viable and do not produce Aβ; moreover, *APPswe; PS1˙E9; BACE1 −/−* mice do not form Aβ deposits or plaques; neither do they show memory deficits. Thus, BACE1, the neuronal β-secretase, is an attractive target for inhibition as part of an anti-amyloidogenic treat-

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ment strategy. However, *BACE1* null mice exhibit abnormalities during performance of tests of cognition and emotion; the former phenotype is rescued by overexpression of *APP* transgenes, indicating that APP processing plays a role in memory/cognition. Clinicians will have to be alert to mechanism-based side-effects related to inhibition of BACE1 activity. More recently, lentiviral RNAi injection strategies and conditional expression systems have been used to influence BACE1 activities and levels of amyloid at various stages of disease. Parallel studies of γ-secretase have disclosed that PS1, Nicastrin (Nct), and Aph-1 (along with Pen-2) are key components of this complex and that lowering enzyme activity reduces production of Aβ. However, these manipulations are also associated with adverse events, including problems with gastrointestinal cells and lymphocytes in adults. Significantly, although *Nct*$^{-/-}$*APPswe; PS1*˙*E9* mice show reduced levels of Aβ in the CNS, they also develop skin tumors, which reflects the importance of the Notch1 signaling pathway in suppression of neoplasms of the skin.

In summary, studies of AD and genetically engineered models of Aβ amyloidosis (and the tauopathies), as well as investigations of other neurodegenerative diseases have greatly enhanced our understanding of pathogenic mechanisms, therapeutic targets, and potential mechanism-based treatments designed to benefit patients with AD and other neurological disorders.

Overview

The most common cause of dementia in the elderly, Alzheimer's disease (AD), is manifested by progressive loss of memory and by cognitive impairments (Brookmeyer et al. 1998; Cummings 2004; Wong et al. 2005). Over the next several decades, the number of affected individuals is predicted to triple. The syndrome is the result of abnormalities associated with dysfunction and death of specific populations of neurons, particularly those cells in neural systems participating in memory and cognitive functions (Wong et al. 2005; Whitehouse et al. 1982; Coyle et al. 1983; Price et al. 1998; Braak and Braak 1994; Hyman et al. 1984). The neuropathology is characterized by the presence of intracellular and extracellular protein or peptide aggregates: phosphorylated tau, assembled into the paired helical filaments (PHF) within neurofibrillary tangles (NFT) and swollen neurites; and Aβ peptides existing in extracellular β-pleated sheet conformations assembled into oligomers, in amyloid plaques (Wong et al. 2002; Lee et al. 2001). Inheritance of mutations in *APP*, *PS1* and *PS2* causes autosomal dominant familial AD (fAD), whereas the presence of the *ApoE4* allele is a significant risk factor for putative sporadic disease (Hardy 2006a). For many reasons (prevalence, lack of mechanism-based treatments, cost of care, and impact on individuals and caregivers), AD is one of the most challenging diseases in medicine (Cummings 2004; Wong et al. 2002, 2005; Price et al. 1998; Citron 2004; Selkoe and Schenk 2003a). However, extraordinary progress has been made in understanding the pathology, biochemistry and neurobiology of the disease, the clinical-pathological correlations, the value of diagnostic approaches, the mechanisms of AD, the utility of transgenic models of the genetic forms of the disease (Savonenko et al. 2006), and potential therapeutic opportunities. Discovery of involvement of the cholinergic system in AD has led to treatments based on inhibition of acetylcholinesterase, whereas demonstration of the disease in populations of medial temporal lobe neurons (where glutamate is a neurotransmitter)

has led to trials of antagonists of glutamate-mediated toxicity. More recent strategies are designed to, reduce production of Aβ, modify the nature (length) of Aβ peptides to shorter forms that are less likely to damage neurons, prevent formation of oligomeric species, enhance clearance of toxic peptides, and attenuate alterations in tau conformations leading to NFT. Herein, we briefly review some of the research. Future discoveries and development in these domains will lead to new disease-modifying therapies that will have a major impact on the health and care of the elderly.

Mild Cognitive Impairment and Alzheimer's Disease

Mild cognitive impairment (MCI) is characterized by memory complaints and impairments on formal testing. Amnestic MCI (aMCI) is usually regarded as a transitional stage between normal aging and early AD (eAD; Markesbery et al. 2006; Petersen et al. 2006; Jicha et al. 2006). Patients with AD show progressive difficulties with memory and other cognitive functions (Cummings 2004), leading in the late stages to profound dementia.

Histories, examinations, neuropsychological tests and laboratory studies (Cummings 2004; Nestor et al. 2004; Sunderland et al. 2003; Klunk et al. 2004b) are of value in establishing a diagnosis. Importantly, advances have been made in imaging and biomarkers. Magnetic resonance imaging (MRI) often discloses atrophy of specific regions of the brain (Cummings 2004; Nestor et al. 2004); positron emission tomography (PET) with ^{18}F deoxyglucose or single photon emission computerized tomography (SPECT) demonstrate decreased glucose utilization and early reductions in regional blood flow in the parietal and temporal lobes (Nestor et al. 2004). Following administration of brain penetrant ^{11}C-labeled Pittsburgh Compound B (PIB), which binds to Aβ with high affinity, PET discloses signal patterns that appear to reflect the Aβ burden in the brain (Klunk et al. 2004). In the cerebrospinal fluid (CSF) of patients with AD, levels of tau may be higher than in controls, whereas levels of Aβ peptides are often low (Sunderland et al. 2003). On the basis of studies of models of Aβ amyloidosis, it has been suggested that efflux of Aβ from brain to plasma may serve as a measure of Aβ burden in the CNS (DeMattos et al. 2002), and an inverse relationship appears to exist between the amyloid load (as assessed by PET amyloid imaging) and levels of Aβ in CSF (Fagan et al. 2005). In concert, these various approaches, as applied to patients, should increase the accuracy of diagnosis in earlier stages of disease and allow assessments of the efficacies of new anti-amyloid therapeutics.

Neuropathology and Biochemistry of AD

The clinical manifestations of AD stem from abnormalities occurring among populations of neurons in neural systems/brain regions essential for memory, learning, and cognitive performance. Damaged circuits include the basal forebrain cholinergic system, amygdala, hippocampus, entorhinal and limbic cortex, and neocortex (Whitehouse et al. 1982; Coyle et al. 1983; Braak and Braak 1994; Hyman et al. 1984; Markesbery et al. 2006; Petersen et al. 2006; Jicha et al. 2006; Braak and Braak 1991). In a recent study

(Markesbery et al. 2006), the character, abundance and distribution of the lesions (i.e., diffuse plaques, neuritic plaques, and tangles) were correlated with clinical signs in several cognitively characterized cohorts: controls and individuals with aMCI or eAD. There were no differences in the number of diffuse plaques between subject groups. In aMCI, tangles were significantly increased in the ventral medial temporal lobe regions as compared to controls; individuals with eAD showed greater numbers of NFT and neuritic plaques in both frontal lobes and temporal regions. Individuals with aMCI exhibited increased numbers of neuritic plaques in neocortical regions as compared to controls, but not as compared to cases of eAD. Memory deficits appeared to correlate most closely with an abundance of NFT in CA1 of the hippocampus and in the entorhinal cortex, leading the authors to conclude that tangles were more important than amyloid deposition in the progression from normal to MCI to eAD and that tangles in the medial temporal lobe play a key role in the memory declines in aMCI (Markesbery et al. 2006). Other studies (Petersen et al. 2006; Jicha et al. 2006) demonstrated that the majority of patients with MCI did not meet neuropathological criteria for AD; the data were interpreted to indicate that this syndrome reflected a transitional state in the evolution of AD. Because the regional distributions of NFT correlated most closely with the degree of clinical impairments from aged healthy controls to individuals with aMCI to cases of AD, the spread of NFT beyond the medial temporal lobe is thought to be linked to the development of dementia.

Cellular abnormalities within these neural circuits include the presence within neurons of conformationally altered isoforms of tau in the PHF comprising NFT, neurites, and neuropil threads (Lee et al. 2001; Goedert and Spillantini 2006), the presence of a variety of axonal pathologies, including varicosities and terminal clubs (also observed in aged, memory impaired Rhesus with Aβ deposits; Kitt et al. 1984, 1985; Martin et al. 1994; Selkoe et al. 1987; Stokin et al. 2005) an abundance of Aβ-containing neuritic plaques (sites of synaptic disconnection) in regions receiving inputs from these populations of neurons, decrements in generic and transmitter-specific synaptic markers in the target fields of these cells (Whitehouse et al. 1982; Coyle et al. 1983; Sze et al. 1997), local astroglial and microglial responses, particularly associated with plaques, and evidence of death of neurons, possibly by apoptosis. Thus, the clinical manifestation of aMCI and AD reflects disruption of synaptic communication in subsets of neural circuits associated with degeneration of axon terminals and, later, death of neurons in the brain (Whitehouse et al. 1982; Coyle et al. 1983; Braak and Braak 1991, 1994; Hyman et al. 1984).

In one hypothetical model thar mechanistically links Aβ and phosphorylated tau, Aβ42 species liberated at terminals oligomerize to form Aβ assemblies or Aβ-derived diffusible ligands (ADDLs), leading to synaptic damage (Wong et al. 2002, 2005; Selkoe et al. 2002). Subsequently, a retrograde signal (of unknown nature), which originates at terminals, triggers the activation of kinases (or the inhibition of phosphatases) in the cell body. Phosphorylation of tau at certain serine and threonine residues leads to conformational changes in tau associated with the formation of PHF and, eventually, NFT (Goedert and Spillantini 2006). Secondary disturbances of the cytoskeleton and alterations in axonal transport can, in turn, compromise the functions and viability of neurons. Eventually, affected nerve cells die (Goedert and Spillantini 2006) and extracellular tangles remain as "tombstones" of the nerve cells destroyed by disease.

Familial AD and Risk Factors

Established genetic factors implicated in AD include mutations in *APP* (chromosome 21), mutations in *presenilin 1* (*PS1*; chromosome 14) and *PS2* (chromosome 1), and the susceptibility allele of ApoE4 (chromosome 19; Price et al. 1998; Hardy 2006a; Ghiso and Wisniewski 2004; Bertram and Tanzi 2005). Autosomal dominant mutations in *APP*, *PS1*, or *PS2* usually cause disease earlier than occurs in sporadic cases, with the majority of mutations in *APP, PS1* and *PS2* influencing BACE1 and γ-secretase cleavages of APP to increase the levels of all Aβ species or the relative amounts of toxic Aβ42 (Ghiso and Wisniewski 2004). Individuals with duplications of *APP* (Rovelet-Lecrux et al. 2006) or with trisomy 21 (Down's syndrome; Hardy 2006a) have an extra copy of *APP* and develop AD pathology relatively early in life. The presence of A*poE4* predisposes to later onset AD and some cases of late-onset fAD (Bertram and Tanzi 2005; Corder et al. 1994).

A member of the *APP* gene family (*APP*, *APLP1* and 2), *APP* encodes a type I transmembrane protein that is abundant in the nervous system, rich in neurons, transported rapidly anterograde in axons to terminals (Lazarov et al. Buxbaum et al. 1998; Sisodia et al. 1993); its specific functions remain to be defined (Wong et al. 2005; Cao and Sudhof 2001). APP is cleaved by activities of BACE1 (β-site APP cleaving enzyme 1) of the +1 and +11 sites and by the γ-secretase complex at a variety of sites (see below) that generate the N- and C- termini of Aβ peptides, respectively (Citron 2004; Vassar et al. 1999; Cai et al. 2001; Selkoe and Kopan 2003; Iwatsubo 2004; Laird et al. 2005; Ma et al. 2005; Li et al. 2003). The *APPswe* mutation enhances many-fold the BACE1 cleavage at the N-terminus of Aβ (+1 site), resulting in substantial elevations in levels of all Aβ peptides. APP_{717} mutations influence γ-secretase cleavage to increase secretion of Aß42, which is the most toxic peptide. Thus, a variety of *APP* mutations alter the processing of APP and influence an increase in the production of Aβ peptides or the amounts of the more toxic Aβ42. In contrast, other mutations may promote local fibril formation and vascular amyloidosis (Ghiso and Wisniewski 2004). This information has been useful in creating transgenic models of amyloidosis (see Savonenko 2006 for a recent review of models).

PS1 and PS2 encode two highly homologous and conserved 43- to 50-kD multipass transmembrane proteins (Price et al. 1998; Sherrington et al. 1995) that are involved in Notch 1 signaling pathways critical for cell fate decisions (Selkoe and Kopan 2003). PS are endoproteolytically cleaved by a "presenilinase" to form an N-terminal ~28-kDa fragment and a C-terminal ~18-kDa fragment (Thinakaran et L. 1997); both fragments are critical components of the γ-secretase complex (Selkoe and Kopan 2003; Iwatsubo 2004)[3]. Nearly 50% of early-onset cases of fAD are linked to > 90 different mutations in *PS1* (Price et al. 1998; Hardy 2006a; Bertram and Tanzi 2005; Sherrington et al. 1995). A relatively small number of *PS2* mutations also cause autosomal dominant fAD (Price et al. 1998; Bertram and Tanzi 2005). The majority of abnormalities in *PS* genes are missense mutations that enhance γ-secretase activities and increase the levels of the Aβ42 peptides.

APP and Secretases

As described above, APP is cleaved by β-and γ-secretases, releasing the ectodomain of APP (APPs), liberating a cytosolic fragment termed APP intracellular domain (AICD), and generating several species of Aβ peptides. In the CNS (but not PNS), Aβ peptides are generated by sequential endoproteolytic cleavages by BACE1 (at the Aβ +1 and +11 sites) to generate APP-β carboxyl terminal fragments (APP-βCTFs; Cai et al. 2001; Luo et al. 2001 and by the γ-secretase complex (at several sites varying from Aβ 36,38,40,42,43) to form Aβ species peptides (Citron 2004; Iwatsubo 2004; Ma et al. 2005; Li et al. 2003). The intramembranous cleavages of APP-βCTF by γ-secretase releases an AICD (Cao and Sudhof 2001), which can form a complex with Fe65, a nuclear adaptor protein (Cao and Sudhof 2001). Fe65 and Aβ or Fe65 alone (in a novel conformation) can gain access to the nucleus to influence gene transcription (Cao and Sudhof 2001), a signaling mechanism analogous to that occurring in the Notch 1 pathway (Selkoe and Kopan 2003; Iwatsubo 2004; Barrick and Kopan 2006). It has been speculated that the AICD signaling pathway may play a role in learning and memory (Laird et al. 2005). In other cells in other organs, APP is cleaved endoproteolytically within the Aβ sequence through alternative, non-amyloidogenic pathways: α-secretase (TNF-alpha converting enzyme or TACE) cleaves between 16 and 17 (Sisodia et al. 1990); BACE2 cleaves between 19 and 20, and 20 and 21 (Farzan et al. 2000). These cleavages, which occur in non-neural tissues, preclude the formation of Aβ peptides and serve to protect these cells/organs from Aβ amyloidosis (Wong et al. 2001).

BACE1, encoded by a gene on chromosome 11, is transmembrane aspartyl protease that is directly involved in the cleavage of APP at the +11 > +1 sites of Aβ in APP (Vassar et al. 1999; Cai et al. 2001; Laird et al. 2005; Luo et al. 2001; Farzan et al. 2000). BACE1 is present in the CNS and BACE1 immunoreactivity is demonstrable in synaptic regions (Laird et al. 2005). Brain cells from *BACE1−/−* mice (Cai et al. 2001; Laird et al. 2005; Luo et al. 2001) do not produce Aβ1-40/42 and Aβ11-40/42, indicating that BACE1 is the neuronal β secretase. As compared to wild type APP, APPswe is cleaved approximately 100 times more efficiently at the +1 site, resulting in a greater increase in BACE1 cleavage products (elevating of cell Aβ species) in the presence of this mutation.

γ-Secretase, essential for the regulated intramembranous proteolysis of a variety of transmembrane proteins, is a multiprotein catalytic complex that includes PS1 and 2, Nicastrin (Nct), a type I transmembrane glycoprotein, and Aph-1 and Pen-2, two multipass transmembrane proteins (Selkoe and Kopan 2003; Iwatsubo 2004; Ma et al. 2005; Li et al. 2003; Goutte et al. 2002; Kimberly et al. 2003; Serneels et al. 2005). PS contains aspartyl residues that play roles in intramembranous cleavage, and substitutions of aspartate residues at D257 in TM 6 and at D385 in TM 7 are reported to reduce secretion of Aβ and cleavage of Notch1 in vitro (Selkoe and Kopan 2003; Wolfe et al. 1999). The functions of the various γ-secretase proteins and their interactions with each other in the complex are not yet fully defined. In one model, Aph-1 and Nct form a pre-complex that interacts with PS; subsequently, Pen-2 enters the complex where it is critical for the "presenilinase" cleavage of PS into two fragments. In concert, this complex is responsible for γ-secretase cleavages of APP, Notch, and a variety of other transmembrane proteins.

Genetic Models of Aβ Amyloidosis

In mice, expression of *APPswe* or APP_{717} (with or without mutant *PS1*) leads to an Aβ amyloidosis in the CNS (Savonenko et al. 2006; Mucke et al. 2000; Lesne et al. 2006). Mutant *APP*; *PS1* mice develop accelerated disease secondary to increased levels of Aβ (particularly Aβ42) associated with the presence of diffuse Aβ deposits and neuritic plaques in the hippocampus and cortex. Levels of Aβ peptides, particularly Aβ42, increase in brain with age (Savonenko et al. 2006; Borchelt et al. 1996, 1997; Jankowsky et al. 2004), and oligomeric species, variously termed ADDL's, Aβ*56, etc., appear in the CNS (Lesne et al. 2006; Cleary et al. 2005; Klyubin et al. 2005). Over time, mice carrying mutant transgenes exhibit Aβ deposits; swollen neurites develop in proximity to these deposits and neuritic plaques are associated with glial responses (Savonenko et al. 2006). Some lines of mice show evidence of amyloid in vessels (Calhoun et al. 1999). In forebrain regions, the density of synaptic terminals and several neurotransmitter markers (cholinergic, aminergic, glutamatergic, and peptidergic) are reduced. In some settings, there are deficiencies in synaptic transmission (Savonenko et al. 2006; Chapman et al. 1999). Moreover, some lines of mice show evidence of degeneration of subsets of neurons (Calhoun et al. 1998).

Behavioral studies of lines of transgenic mice, including those generated by Dr. David Borchelt (Savonenko et al. 2003, 2005a, 2006), disclose deficits in spatial reference memory (Morris water maze task) and episodic-like memory (repeated reversal and radial water maze tasks). At six months of age, *APPswe/PS1dE9* mice develop plaques, but all genotypes are indistinguishable from nontransgenic animals in all cognitive measures. However, in 18-month-old cohorts, *APPswe/PS1dE9* mice perform all cognitive tasks less well than mice of all other genotypes. In these animals, amyloid burdens are high and mildly statistically significant; decreases are detectable in levels of cholinergic markers (cortex and hippocampus) and somatostatin (cortex). The strongest relationships exist between deficits in episodic-like memory tasks and total Aβ loads in the brain (Savonenko et al. 2005b, 2006). Collectively, these studies suggest that, in *APPswe/PS1dE9* mice, some form of Aβ (ultimately associated with amyloid deposition) can disrupt circuits critical for memory. Episodic-like memory seems to be more sensitive to the toxic effects of Aβ. Some of these impairments have been linked to the presence of Aβ oligomers (see below) and can be reversed by antibody-mediated reductions of levels of brain Aβ (Lesne et al. 2006; Cleary et al. 2005; Klyubin et al. 2005. Although these transgenic lines of mice do not recapitulate the complete phenotype of AD, these animals are very useful subjects for research designed to examine the behavioral consequences of Aβ amyloidosis in CNS, to delineate disease mechanisms, and to test novel therapies (Savonenko et al. 2006).

Over the past decade, a variety of Aβ species, oligomers, and structural assemblies, ranging from monomers to amyloid deposits in neuritic plaques, have been suggested to play important roles in impairing synaptic communication. The pool of insoluble Aβ (or plaques) is believed to exist in equilibrium with peptides in interstitial fluid (Cirrito et al. 2003). Significantly, administration of antibodies in the periphery increases levels of Aβ in plasma, and the magnitude of this elevation correlates with amyloid burden in the cortex and hippocampus (DeMattos et al. 2002). In one study, a naturally secreted Aβ peptide was injected into the ventricular system of rats and LTP was inhibited in the hippocampus (Klyubin et al. 2005). The activity of the peptide was completely blocked

by the injection of a monoclonal Aβ antibody, a finding consistent with the concept that oligomers are the toxic moiety and that they are both necessary and sufficient to perturb learned behavior (Cleary et al. 2005; Klyubin et al. 2005) [63;64]. In this setting, active immunization was less effective in rescuing function and correlated most closely with the levels of antibodies recognizing oligomers (Klyubin et al. 2005). More recently, studies of TG2576 mice suggested that extracellular accumulations of a 56KD soluble amyloid assembly, termed Aβ*56, were purified from the brains of memory impaired mice (Lesne et al. 2006). Administration of Aβ*56 to young rats interfered with memory (Lesne et al. 2006).

The paucity of tau abnormalities in various lines of mutant *APP* mice may be related to differences in tau isoforms expressed in this species Xu et al. 2002). Early efforts to express mutant *tau* transgenes in mice did not lead to striking clinical phenotypes or pathology (Goedert and Spillantini 2006). More recently, mice overexpressing *tau* showed clinical signs, attributed to degeneration of motor axons (Lee et al. 2001). When prion or Thy1 promoters are used to drive tau_{P3O1L} (a mutation linked to autosomal dominant fronto-temporal dementia with parkinsonism), some brain and spinal cord neurons develop tangles (Gotz et al. 2001). Mice expressing $APPswe/tau_{P301L}$ exhibited enhanced tangle-like pathology in limbic system and olfactory cortex (Lewis et al. 2001). Moreover, injection of Aβ42 fibrils into specific brain regions of tau_{P301L} mice increased the number of tangles in those neurons projecting to sites of Aβ injection. A triple transgenic mouse (3xTg-AD), created by microinjecting *APPswe* and tau_{P3O1L} into single cells derived from monozygous $PS1_{M146V}$ knock in mice (Oddo et al. 2003), developed age-related plaques and tangles as well as deficits in long term potentiation (LTP) that appeared to antedate overt pathology. However, mice bearing both mutant *tau* and *APP* (or *APP/PS1*) or mutant *tau* mice injected with Aβ may not be ideal models of fAD because the presence of the *tau* mutation alone is associated with the development of tangles and disease.

Targeting of Genes in the Amyloidogenic Pathway

To begin to understand the functions of some of the proteins thought to play roles in AD, investigators have targeted a variety of genes encoding *BACE1*, *PS1, Nct* and *Aph-1*.

***BACE1-/-* Mice** These animals mate successfully and exhibit no overt pathology (Savonenko et al. 2006; Cai et al. 2001; Laird et al. 2005; Luo et al. 2001). *BACE1 −/−* neurons do not cleave at the +1 and +11 sites of Aβ, and the production of Aβ peptides is abolished (Cai et al. 2001; Laird et al. 2005; Luo et al. 2001), establishing that BACE1 is the neuronal β-secretase required to generate the N-termini of Aβ. However, *BACE1 −/−* mice show altered performance on some tests of cognition and emotion (Savonenko et al. 2006; Laird et al. 2005) see below); the former deficits can be rescued by overexpression of *APP* transgenes.

***PS1*$^{-/-}$ Mice** Embryos develop severe abnormalities of the axial skeleton, ribs and spinal ganglia, a lethal outcome that resembles a partial *Notch 1−/−* phenotype (Wong et al. 1997; Shen et al. 1997. PS1−/− cells show decreased levels of secretion of Aβ (Li et al. 2003; De Strooper and Saftig 1998) related to the fact that PS1 (along with Nct, Aph-1

and Pen-2) is a component of the γ-secretase complex that carries out the S3 intramembranous cleavage of Notch1 (Selkoe and Kopan 2003; Li et al. 2003: De Strooper et al. 1999). Without γ-secretase cleavage, NICD is not released from the plasma membrane and cannot reach the nucleus to provide a signal to initiate transcriptional processes essential for cell fate decisions (Selkoe and Kopan 2003; Barrick and Kopan 2006). Significantly, conditional *PS1/2* targeted mice show impairments in memory and synaptic plasticity in the hippocampus Saura et al. 2004), raising the question (posed by Jie Shen and colleagues) as to the roles of loss of PS function in neurodegeneration and AD.

***Nct*-/- Mice** Embryos die early and exhibit several patterning defects (Li et al. 2003), including abnormal segmentation of somites; this phenotype closely resembles that seen in *Notch1*$^{-/-}$ and *PS* 1/2$^{-/-}$ embryos. Importantly, *Nct*$^{-/-}$ cells do not secrete Aβ peptides, whereas *NctT*$^{+/-}$ cells show reduction of ∼ 50% (Li et al. 2003). The failure of *NctT*$^{-/-}$ cells to generate Aβ peptides is accompanied by accumulation of APP C-terminal fragments. Importantly, *Nct*+/− mice develop tumors of the skin, presumably related to reduced levels of signaling by Notch1, which appears to act as a tumor suppressor in the skin (Li et al., submitted for publication).

***Aph-1a*$^{-/-}$ Mice** Three murine *Aph-1* alleles (*Aph-1a*, *Aph-1b* and *Aph-1c*) encode four distinct Aph-1 isoforms: Aph-1aL and Aph-1aS (derived from differential splicing of *Aph-1a;* Aph-1b; and Aph-1c[45; 56]. *Aph-1a*$^{-/-}$ embryos show patterning defects that resemble, but are not identical to, those of *Notch1*, *Nct* or *PS* null embryos (Ma et al. 2005; Serneels et al. 2005). Moreover, in *Aph-1a*$^{-/-}$ derived cells, the levels of Nct, PS fragments and Pen-2 are decreased. There is an associated reduction in levels of the high molecular weight γ-secretase complex and a decrease in secretion of Aβ (Ma et al. 2005). In *Aph-1a*$^{-/-}$ cells other mammalian Aph-1 isoforms can restore the levels of Nct, PS and Pen-2 (Ma et al. 2005; Serneels et al. 2005)

Experimental Treatments and Therapeutics

In in vitro and in vitro models relevant to amyloidogenesis, the opportunity to ablate or knock down genes, to modulate cleavages and to influence clearance have set the stage for influencing Aβ production, cleavage patterns influencing peptide neurotoxicity, and promoting clearance and/or degradation of Aβ Citron 2004; Savonenko et al. 2006; Laird et al. 2005; Li et al. 2003; Lesne et al. 2006; Cleary et al. 2005; Klyubin et al. 2005; Walsh and Selkoe 2004a; Monsonego and Weiner 2003). It is not possible to discuss all the experimental treatments in mouse models of Aβ amyloidosis (Savonenko et al. 2006); below, we comment briefly on selected studies to illustrate experimental strategies directed at specific therapeutic targets that we predict will provide mechanism-based therapeutic benefits to patients with AD.

Reduction in β-Secretase Activity Significantly, deletion of *BACE1* in *APPswe; PS1˙E9* *(see note on p. 8)* mice prevents both Aβ deposition and age-associated cognitive abnormalities that occur in this model (Laird et al. 2005; Masliah et al. 2005a). Significantly, *BACE1−/−*; *APPswe;PS1˙E9*mice do not develop the Aβ deposits or the age-associated abnormalities in working memory that occur in the *APPswe;PS1˙E9* model of Aβ

amyloidosis (Laird et al. 2005; Borchelt et al. 1996; Singer et al. 2005; McDonald and Howard 2002). Similarly, *BACE1−/−* Tg2576 mice appear to be rescued from age-dependent memory deficits and physiological abnormalities (Savonenko et al. 2006; Ohno et al. 2004). Moreover, Aβ deposits are sensitive to *BACE1* dosage and can be efficiently cleared from regions of the CNS when *BACE1* is silenced at these sites (Laird et al. 2005). Inhibitors of β-secretase, conjugated to carrier peptides, are effective inhibitors in vitro and in vivo (following intraperitaneal injection into Tg2576 mice; (Chang et al. 2004). New approaches using conditional expression systems or RNAi silencing will allow investigators to examine the pathogenesis of diseases and to assess the degrees of reversibility of the disease processes (Laird et al. 2005: Singer et al. 2005; Ohno et al. 2004). The results of these approaches will provide a better understanding of the mechanisms that lead to diseases and aid in the design of new treatments. The above-described data indicate that BACE1 is a very attractive therapeutic target. However, *BACE1* null mice manifest alterations in both hippocampal synaptic plasticity and in performance on tests of cognition and emotion (Laird et al. 2005); the memory deficits but not emotional alterations in *BACE1*$^{-/-}$ mice are prevented by co-expressing *APPswe;PS1˙E9* transgenes. This discovery indicates that APP processing influences cognition/memory and that the other potential substrates of BACE1 may play roles in neural circuits related to emotion. These results establish that BACE1 and APP processing pathways are critical for cognitive, emotional and synaptic functions and that inhibition of β-secretase activity is an exciting therapeutic opportunity. However, future studies should be alert to potential mechanism-based side effects that may occur with BACE1 inhibition (Wong et al. 2005; Savonenko et al. 2006; Laird et al. 2005; Chang et al. 2004).

Inhibition of γ-Secretase Activity Both genetic and pharmaceutical lowering of γ-secretase activity decreases production of Aβ in cell-free and cell-based systems and reduces levels of Aβ mutant mice with Aβ amyloidosis (Li et al., submitted for publication). Thus, γ-secretase activity is a significant target for therapy (Wong et al. 2005; Ma et al. 2005; Li et al. 2003; Saura et al. 2004). However, γ-secretase activity is also essential for processing of Notch, which is critical for lineage specification and cell growth during embryonic development (Selkoe and Kopan 2003; Ma et al. 2005; Li et al. 2003; Wong et al. 1997, 2004; Shen et al. 1997; Wolfe and Kopan 2004). Significantly, one inhibitor of γ-secretase (LY – 411, 575) reduced production of Aβ but also had profound effects on T and B cell development and on the appearance of intestinal mucosa (proliferation of goblet cells, increased mucin in gut lumen and crypt necrosis; Wong et al. 2004; Milano et al. 2004; Barten et al. 2005). Moreover, as described above, *Nct+/- APPswe;PS1* δ *E9* (see note on page 8)mice show reduced levels of Aβ and amyloid plaques, but these mice develop skin tumors (Li et al., submitted for publication), presumably in part because reduced γ-secretase acts, via Notch signaling, as a tumor suppressor in skin (Nicolas et al. 2003; Xia et al. 2001). Thus, clinicians carrying out trials of this inhibitor will have to be alert to several potential adverse events associated with inhibition of this enzyme complex.

γ-Secretase Modulation by NSAID Compounds Retrospective epidemiological studies have suggested that significant exposure to NSAIDs reduces risk of AD (Anthony et al. 2000), an outcome initially interpreted as related to suppression of the well-documented inflammatory process occurring in brains of AD cases (Akiyama et al.

2000). However, more recent in vitro studies indicate that a subset of NSAIDs compounds in this class can modulate secretase cleavages (to shorter, less toxic Aβ species) without altering Notch or other APP processing outcomes (Weggen et al. 2001). Short-term treatment of mutant mice appears to have some benefit in terms of lowering Aβ and plaque pathology. This strategy is now being evaluated in clinical trials (Weggen et al. 2001).

Aβ immunotherapy Multiple lines of evidence, including lesions of entorhinal cortex or perforant pathway (Lazarov et al. 2002, 2005a; Sheng et al. 2002, 2003), indicate that removing the source of Aβ (re: lesioning cell bodies or axons/terminals transporting APP to terminals, respectively) significantly reduces Aβ in target fields. Similarly, increasing the local increase in levels of degrading enzymes (IDE and NEP) can facilitate cleavage and can reduce levels of Aβ (Carson and Turner 2002; Vekrellis et al. 2000; Marr et al. 2003; Leissring et al. 2003: Miller et al. 2003; Farris et al. 2003; Iwata et al. 2002, 2004).

However, to date, the most exciting findings regarding clearance of Aβ come from studies using active and passive Aβ immunotherapy (Selkoe and Schenk 2003a; Savonenko et al. 2006; Federoff and Bowers 2005). In treatment trials in mutant mice and in rodents injected with Aβ species (Klyubin et al. 2005; Monsonego and Hansen 2003; Hutton and McGowan 2004; Wilcock et al. 2003), both Aβ immunization (with Freund's adjuvant) and passive transfer of Aβ antibodies reduce levels of Aβ and plaque burden (DeMattos et al. 2001, 2002; Monsonego and Weiner 2003; Federoff and Bowers 2005; Hutton and McGowan 2004; Wilcock et al. 2003, 2004a; Bard et al. 2000; Kotlinek et al. 2002; Morgan et al. 2000; Dodart et al. 2002; Schenk et al. 1999; Oddo et al. 2004). Although, the mechanisms of enhanced clearance are not certain (Wong et al. 2005; Oddo et al. 2004), at least two not mutually exclusive hypotheses have been suggested: 1) a small amount of Aβ antibody reaches the brain, binds to Aβ peptides, promotes the disassembly of fibrils, and, via the Fc antibody domain, encourages activated microglia to enter the affected regions and remove Aβ (Schenk et al. 1999); and/or 2) serum antibodies serve as "a sink" to draw the amyloid peptides from the brain into the circulation, thus changing the equilibrium of Aβ in different compartments and promoting removal of Aβ from the brain (DeMattos et al. 2002; Cirrito et al. 2003; Morgan et al. 2000; Dodart et al. 2002). Whatever the mechanism, Aβ immunotherapy in mutant mice is successful in partially clearing Aβ, in attenuating learning and behavioral deficits in several cohorts of mutant *APP* mice, and in partially reducing tau abnormalities in the triple transgenic mice (Savonenko et al. 2006; Hutton and McGowan 2004; Kotlinek et al. 2002; Morgan et al. 2000; Dodart et al. 2002; Wilcock et al. 2004a,c; Oddo et al. 2004; Sigursson et al. 2004). However, several studies have documented that brain hemorrhages may be associated with congophilic angiopathy immunotherapy (Winkler et al. 2001; Herzig et al. 2004; Pfeifer et al. 2002). In individuals with congophilic angiopathy, the presence of amyloid could weaken vascular walls; potentially, removal of some intramural vascular amyloid could lead to rupture of damaged vessels and bleeding. Although mutant mice who received immunotherapy were not reported to develop evidence of meningoencephalitis, a subset of patients in a clinical trial did manifest these problems (see below).

Individuals receiving vaccinations with pre-aggregated Aβ and an adjuvant (followed by a booster), develop antibodies that recognize Aβ in the brain and vessels(Hock

et al. 2002). Unfortunately, although Phase 1 trials with Aβ peptide and adjuvant vaccination were not associated with any adverse events, Phase 2 trials detected complications (meningoencephalitis) in a subset of patients and were suspended (Monsonego and Weiner 2003; Masliah et al. 2005a; Schenk et al. 2004; Nicoll et al. 2003; Hock et al. 2003; Bayer et al. 2005). The pathology in the index case, consistent with T-cell meningitis (Nicoll et al. 2003), was interpreted to show some clearance of Aβ deposits, but some regions contained a relatively high density of tangles, neuropil threads and vascular amyloid. Aβ immunoreactivity was sometimes associated with microglia, and T-cells were conspicuous in subarachnoid space and around some vessels (Nicoll et al. 2003). In another case, there was significant reduction in amyloid deposits in the absence of clinical evidence of encephalitis (Masliah et al. 2005a). Although the trial was stopped, assessment of cognitive functions in a small subset of patients (30) who received vaccination and booster immunizations disclosed that patients who generated Aβ antibodies (as measured by a new assay), had a slower decline in several functional measures (Hock et al. 2003). The events occurring in this subset of patients illustrate the challenges of extrapolating outcomes in mutant mice to human trials. Investigators continue to pursue the passive immunization approaches and are attempting to make new antigens/adjuvant formulations that do not stimulate T-cell-mediated immunologic attack (Monsonego and Weiner 2003).

Conclusion

Over many years, investigators have more accurately defined MCI and early AD, developed diagnostic approaches and clarified the character and stages of pathology and related the findings to clinical signs. They have greatly enhanced our understanding of the mechanisms underlying the biochemistry of Aβ plaques and tau-related pathology. Following leads from human autopsy studies and from investigations of in vitro and in vivo models, investigators are now on the threshold of implementing novel treatments based on an understanding of the neurobiology, neuropathology, biochemistry, and genetics of this illness. Moreover, a variety of tools, including amyloid imaging and measure of Aβ flux between compartments, are now available to assess efficacy of treatment. It is anticipated that exciting discoveries over the next few years will lead to the design of new mechanism-based therapies that can be tested in models, and that these approaches will be introduced into the clinic for the benefit of patients with this devastating illness.

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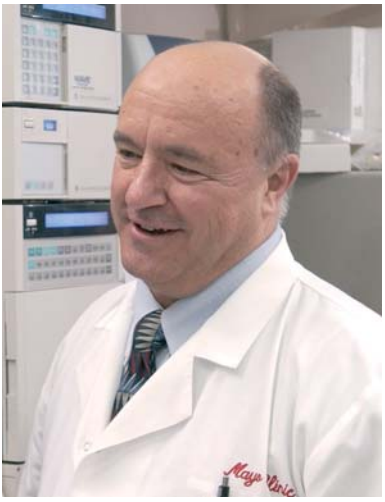
Because of space constraints, the citations are limited. Additional references relevant to the research can be found in Wong and Price (2005), Wong et al. (2002), Laird et al. (2005) and Savonenko et al. (2006).



Steven G. Younkin

Linda Younkin

Eckman

PS1, *PS2*, and *APP* mutations that cause Alzheimer's disease increase Aβ42

Steven G. Younkin[1]

Introduction

In 1996, my group published a manuscript in Nature Medicine (Scheuner et al. 1996)that showed that the *PS1*, *PS2*, and *APP* mutations linked to early onset familial Alzheimer's disease (AD) increase the highly amyloidogenic Aβ42 peptide. The important message of the manuscript was that, like the *APP* mutations on the carboxyl side of Aβ, *PS1* and *PS2* mutations selectively increase Aβ42. In this commentary on the manuscript, I depart from conventional scientific style and discuss the manuscript from the perspective we had in the laboratory as the experimentation progressed. To set the stage, I begin with a background section that discusses the line of investigation that led to and enabled the results reported. I tell the story behind this manuscript as I remember it, trying to get it right. But the recollections of everyone involved, both those in the laboratory and those outside in collaborating and competing laboratories, will certainly be different from mine. I hope this story is old enough by now that no one will care very much if my account seems erroneous or slanted in a way that appears self serving. The most important thing, after all, is that we collectively develop effective treatment for AD. I believe we are well on our way to this goal, but we have yet to accomplish it.

Background

In the mid 1980s, the amyloid in AD brain and meningeal vessels was isolated, solubilized, and sequenced. The amyloid in senile plaques was found to be composed of a then-novel peptide now referred to as the amyloid β protein (Aβ). In 1987, several different groups cloned the gene that encodes Aβ essentially simultaneously (Kang et al. 1987; Goldgaber et al. 1987; Tanzi et al. 1987), and they mapped it to chromosome 21. At that time it was known that patients with trisomy 21 (Down's syndrome) always develop AD pathology (senile plaques and neurofibrillary tangles) if they live past the age of 40. Subsequent work would show that the AD pathology that develops is associated with dementia. This seminal observation meant that there was a gene, or perhaps a combination of genes, on chromosome 21 that could cause AD when an extra copy was present (three chromosomes instead of two). When the amyloid β protein precursor gene (*APP*) mapped to chromosome 21, it was immediately obvious that the APP gene might be the gene or one of the genes on chromosome 21 that causes AD in

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patients with trisomy 21. If so, it would provide strong indication that the amyloid β protein, which invariably deposits in senile plaques in all forms of AD, plays a major role in AD.

At this time, there were two outstanding MD, PhD students, Todd Golde and Mark Palmert, working in my laboratory at Case Western Reserve University. Collectively, we decided to throw caution to the winds, to abandon the work the laboratory was funded to do, and to pore all of our energy and resources into studying the newly cloned APP. There was no guarantee that APP and Aβ were crucial in AD, but this line of investigation certainly held more promise than anything else. If we were serious about advancing AD research, we had to shift focus quickly and unequivocally.

Aβ is a secreted protein

Both Mark and Todd were fully supported by the NIH-sponsored Medical Scientist Training Program (MSTP) directed by Les Webster. To permit two of his coveted MSTP students to train simultaneously in my laboratory, which was largely unproven, was an act of enormous faith. I do not know why Les decided to back us, but I will always be grateful because his support enabled all of us to work to the limit of our ability. Within a few years, we learned that the APP was normally cleaved within the Aβ protein, causing the large extracellular domain to be secreted (Palmert et al. 1989). Joined by Steve Estus, we discovered that there was also APP cleavage that generated Aβ-bearing carboxyl-terminal fragments (Estus et al. 1992). Then, working with Mikio Shoji, in the summer of 1992, my laboratory and several others discovered that Aβ is normally secreted (Haass et al. 1992; Seubert et al. 1992; Shoji et al. 1992; Busciglio et al. 1993). I recall this five-year period as a very rewarding time. All of our results were obtained independently, but they were also obtained independently by other groups who were pursuing this same new line of investigation. So we had the joy of independent scientific discovery, which for me is the best part of research, but we also had independent confirmation so we knew we were on the right track. Importantly, we were not bickering very much about the results. Instead, the laboratories working in this relatively new area were reaching consensus and making scientific progress.

With the possible exception of Takeshi Kawarabayashi, Mikio Shoji works harder than anyone I know. When we were developing our assay to measure Aβ, there were times when we worked around the clock. My habit is to rise early, so I would get into the lab early to review the results Mikio had been getting all night long using synthetic Aβ. I would review those results while Mikio got a little sleep, we would discuss how best to pursue what he had, and Mikio would plunge immediately into the experimentation in the late morning. Over time, Mikio developed an assay for Aβ in which we immunoprecipitated with one anti-Aβ antibody, ran the immunoprecipitate down a gel, transferred to Imobilon, and labeled the 4-kD Aβ with a second antibody to a different site. I will never forget the day when I arrived to see the results Mikio had obtained using our new Aβ assay. In each of many CSF samples, from both AD cases and controls, there was a well-stained, 4-kD protein recognized by antibodies to two different sites in Aβ. What a moment that was! Aβ was no longer an internal peptide in APP that somehow assembles into amyloid fibrils in senile plaques. In that single

experiment, Mikio showed essentially unequivocally that Aβ is a secreted protein and that it is normally found in human CSF.

The discovery that Aβ is a secreted protein was an important turning point for two reasons. First, as described below, it enabled us to test the hypothesis that Aβ aggregation plays an important role in AD. Often called the amyloid hypothesis because Aβ aggregates to form the amyloid fibrils found in senile plaques, I refer to it here as the Aβ aggregation hypothesis, in deference to the growing realization that it is probably not amyloid fibrils per se that are the major culprit in AD but rather more mobile Aβ oligomers that may or may not be in the pathway that produces amyloid fibrils. Second, it opened the door to drug discovery. Once we knew that Aβ was normally secreted, it was possible to search in cell culture models for drugs that inhibit Aβ, and it was possible to search for the enzymes that cleave the APP to release Aβ, enzymes that would turn out to be excellent therapeutic targets for AD as strong support for the Aβ aggregation hypothesis was obtained.

Testing the Aβ aggregation hypothesis with APP mutations that cause AD

As the initial research on APP processing took place, others were making rapid progress on the genetics of AD. Many families were identified in which AD occurred at an early age (35-60 years) with fully penetrant, autosomal dominant inheritance. Termed early onset familial AD (EOFAD), the AD produced through this simple Mendelian pattern of inheritance constitutes a small, but highly informative fraction of all AD. Using new methods that had been developed to identify mutations that cause human disease in simple Mendelian fashion, several families were identified in which EOFAD was produced by mutations in the APP gene. The first mutations, which were identified initially by Alison Goate and John Hardy and then by others (Goate et al. 1991; Naruse et al. 1991; Yoshioka et al. 1991; Murrell et al. 1991; Chartier-Harlin et al. 1991), all occurred at the same location, several amino acids past the carboxyl end of Aβ (V/F,I,orG). Next discovered, in a large Swedish kindred, was a double mutation (Mullan et al. 1992) in the two amino acids just before the amino end of Aβ (KM/NL).

The Swedish KM/NL mutation increases Aβ If Aβ aggregation is an essential early event in AD, then mutations that cause AD must in some way foster Aβ aggregation. The simplest mechanism would be for the mutations to increase Aβ secretion, and the location of the APP mutations on either side of Aβ strongly suggested this could be the operative mechanism. So one good way to test the Aβ aggregation hypothesis was to determine if the APP mutations do, in fact, increase Aβ secretion. Using the technical infrastructure developed by Todd Golde in my laboratory, Dan Cai was quickly able to show that the Swedish KM/NL mutation increases secretion of Aβ (Cai et al. 1993). Working in Dennis Selkoe's laboratory, Martin Citron obtained the same finding and published it just before we did (Citron et al. 1992).

Mutations on the carboxyl side of Aβ selectively increase Aβ4

The results on the Swedish double mutation were encouraging. The problem was that we could not show that Aβ was increased by the EOFAD mutations on the carboxyl side of Aβ. By this time, we knew that most secreted Aβ has 40 amino acids (Aβ40) and that a small percentage have an two extra amino acids at its carboxyl end (Aβ42) (Dovey et al. 1993; Vigo-Pelfrey et al. 1993). We also knew from the work of Peter Lansbury's group and others that synthetic Aβ42 spontaneously assembles into amyloid fibrils far more rapidly than Aβ40 (Hilbich et al. 1991; Burdick et al. 1992; Jarrett et al. 1993). So we postulated (Cai et al. 1993) that the mutations on the carboxyl side of Aβ might selectively increase Aβ42. This postulated increase in Aβ42 could go undetected when measuring total Aβ, so we needed to develop a method for selectively measuring Aβ42 and Aβ40.

Toby Cheung, a PhD student in my laboratory, worked diligently to develop an effective but cumbersome method for measuring Aβ40 and Aβ42 by radiolabelling the carboxyl end of the molecule, isolating the Aβ, chipping off the radiolabeled Aβ36-40 or Aβ36-42 using cyanogen bromide, and then separating the labeled peptides by high performance liquid chromatography. This method was challenging to say the least. Based on the results with this method, both Toby and I were convinced that Aβ42 was, in fact, selectively increased by the EOFAD mutations. Whether we could convince skeptical reviewers of our finding was the question when Nobuhiro Suzuki joined the laboratory.

Nobu, who worked for Takeda Industries, was well versed in sandwich ELISA technology. Recognizing the potential importance of separately evaluating Aβ40 and Aβ42, he screened for and found a monoclonal antibody to Aβ40 and he also found one that he was pretty sure would selectively detect Aβ42. Through Takeda, Nobu asked to join my laboratory so that we could jointly explore the potential of his new monoclonal antibodies. As soon as he arrived, Nobu and I sat down to go over the rigorous data that he had assembled. Initially, I was hesitant about pursuing sandwich ELISAs. I emphasized the security of detection afforded by our gel-based system, which used two different antibodies that detected Aβ as a 4-kD gel band. I pointed out that with sandwich ELISAs there would be no 4-kD band to ensure that we were detecting Aβ. When Nobu explained he expected that his sandwich ELISAs could increase the sensitivity of detection by three orders of magnitude, my hesitancy vanished immediately. We would have to be careful to document the specificity of the ELISAs, but the possibility of such increased sensitivity had to be explored fast. We began that afternoon. Within a matter of months, we knew that the antibody to Aβ42 was sensitive and specific, we had sandwich ELISAs with remarkable sensitivity for Aβ40 and Aβ42 up and running, and they showed what Toby and I believed based on his more cumbersome method. The EOFAD mutations on the carboxyl side of Aβ selectively increased Aβ42.

One of the toughest decisions I ever made was the decision to have Dr. Suzuki be first author on the Science report describing this result (Suzuki et al. 1994). It was the right decision because of Nobu's immense, independent contribution, and Toby was recognized as an equal contributor. But Dr. Cheung worked hard and diligently to secure the initial indication that our hypothesis was right, so it was a shame he did not get to be first author.

Presenilin mutations that cause AD selectively increase Aβ42

Continuing work on the genetics of EOFAD established that the most important EOFAD gene was an unknown gene located on chromosome 14. If the mutations in this gene could also be shown to increase total Aβ or Aβ42, we would have strong additional proof for the Aβ aggregation hypothesis, and the case for Aβ as a therapeutic target might then be sufficient to galvanize a concerted effort by the pharmaceutical industry. It was, however, difficult to evaluate the effect of the mutations in this unknown gene. In our previous work, we had compared medium conditioned by transfected cells expressing mutant as compared to wild type APP. Since we didn't know the identity of the gene or the specific mutations that were causing EOFAD, we could not employ this approach.

Presenilin mutations selectively increase Aβ42 in fibroblast-conditioned medium

What we could do was to compare Aβ in mutation carriers as compared to non-carriers because genetic markers reliably linked to the unknown mutations were available to identify the carriers and non-carriers in specific families. Skin fibroblasts from carriers and non-carriers were available, so we decided to see if we could detect any difference in conditioned medium using the sensitive sandwich ELISAs we had developed for Aβ40 and Aβ42. Donalyn Scheuner, the postdoctoral fellow who spearheaded this project, was very ably assisted by Debbie Glass, a superb tissue culture technician with long experience who luckily became available just as this project was commencing. Working closely together to match fibroblast cultures from carriers and non-carriers as closely as possible, they worked for almost a year and finally obtained enough data to convince us that the chromosome 14 mutations were selectively increasing Aβ42.

Since skin fibroblasts senesce as they are passed in culture, it is possible that fibroblasts within skin may be heterogeneous with respect to how much Aβ they secrete, and getting cultures from carriers and non-carriers well matched with respect to cell density and number can be challenging. All of these are problems that we felt we had surmounted by carefully measuring many fibroblasts from many individuals, but we wanted a second line of evidence and we thought we might be able to get it by analyzing plasma from carriers and non-carriers.

Presenilin mutations selectively increase Aβ42 in plasma.

To determine whether we could detect the effect of EOFAD mutations in plasma, we had previously evaluated plasma from carriers and non-carriers in the Swedish kindred with the double mutation (KM/NL) on the amino side of Aβ. In this project, we worked closely with Lars Lannfelt and Malene Jensen, who drove around the Swedish countryside to collect the requisite plasma samples from non-carriers as well as pre-symptomatic and symptomatic carriers. Malene then visited our laboratory to participate in measuring the samples. I vividly remember hovering in the laboratory, making a nuisance of myself, to be sure we were running the assays, which were blinded, as well as we possibly

could. As we watched the color come up at the end of the assay, it was immediately obvious by eye that there were some samples with a distinctly higher level of Aβ40. When we broke code, every one of those samples was from a carrier; the Aβ40 levels in the carriers were completely separated from those in the non-carriers. We thought our ability to distinguish both pre-symptomatic and symptomatic carriers so clearly in human plasma was exciting news. The reviewers of the manuscript that was submitted disagreed, arguing that finding elevated Aβ in the plasma of carriers wasn't novel since we and others had already shown the same effect in transfected cultured cells. Because of this, the finding went unpublished until we included it in the manuscript focused primarily on *PS1/2* mutations (Scheuner et al. 1996) that is the subject of this commentary. The result from the Swedish APP mutation was critically important for our work on *PS1/2* mutations because it gave us confidence that we would able to see in plasma the effect that we had detected in fibroblast medium. When this proved to be the case, we had the results necessary for publication.

When it was finally published, this paper was for several years one of the most widely cited publications in the AD literature. Getting it published, however, was anything but easy. The paper would not have been possible had it not been for the fibroblasts and plasma provided to us by our co-authors from many well-known groups working on AD. We thought the data in the manuscript were convincing and this conviction was shared by all of the co-authors, some of whom worked closely with me reading reviews, drafting revisions, and writing rebuttal letters as the manuscript was considered by several high profile journals. For some reason, we could never convince all of the reviewers the manuscript should be published, and the editors decided they would not publish unless all reviewers agreed. Finally we sent about two inches of printed material (I probably exaggerate), which included our revised manuscript along with numerous reviews and rebuttal letters, to Adrian Isaacson at Nature Medicine, which was then a relatively new journal. It was a great relief when, at last, he decided to publish the manuscript.

One interesting point to make about the manuscript is that we knew the chromosome 14 mutations selectively increased Aβ42, and we submitted a manuscript to that effect before we knew the mutations were in the presenilin 1 gene. As the review process proceeded, the PS1 paper was published (Sherrington et al. 1995) and then the papers describing mutations in presenilin 2 (Levy-Lahad et al. 1995a; Rogaev et al. 1995). We then adapted our manuscript to identify the specific PS1 mutations we had already evaluated and included additional data on PS2 mutations collected by Chris Eckman, who had joined the laboratory.

Postscript

When this manuscript was published, it was clearly important for our group and others to determine if the results obtained from human plasma and fibroblasts could be duplicated in cultured cells transfected with the newly identified *PS1* and *PS2* mutations. I was very concerned that the effect we observed in material from subjects with one mutant and one wild type gene might not be evident when mutations were over-expressed in cultured cells with two wild type genes. If that had happened, the follow-up experimentation would have been far more challenging, and we might still

be arguing about whether these mutations affect Aβ42. Fortunately, it did not occur. Instead there was an avalanche of confirmatory data from our laboratory and many others, both in transfected cells and in transgenic mice. The collective weight of this evidence, along with the demonstration that aggregated Aβ is neurotoxic both *in vitro* and *in vivo,* launched the concerted effort now underway to treat AD by lowering Aβ, especially Aβ42, or otherwise preventing it from aggregating (e.g., with active or passive immunization). Although considerable intellectual effort and resources have been expended in this effort, multiple setbacks have left us with the frustrating truth that we still don't really know, even in principal, whether this approach to therapy is or is not going to be effective.

I had some reservations about preparing this commentary because I prefer pushing forward to looking back. These days my laboratory is focused on determining whether plasma Aβ40 and Aβ42 can be used as premorbid biomarkers for AD in the same way that LDL and HDL are used for heart disease. In addition, we are pursuing the complex genetics of AD, a change in direction almost as large as the change we made in 1987. Not too long ago, I was seriously concerned that the complex genetics of AD might be an intractable problem, but recently things have turned around. We are now finding that many genes in the Aβ processing pathway have multiple variants with modest effects that, when analyzed jointly in large case control series, show significant replicable association with late onset AD. As these genes and others are identified using the methods now being perfected, they should open new therapeutic possibilities much as the EOFAD genes have done. In addition, as the set of susceptibility alleles that influence AD are identified, they should help us to identify those who are at risk and make possible preventive therapy, which is apt to be the most effective way to curtail this disorder.

Edward H. Koo

Amyloid precursor protein, Alzheimer's disease, and Down's syndrome

Edward H. Koo[1]

Summary

The co-occurrence of Alzheimer's disease pathology in Down's syndrome individuals in the fourth decade of life has been known for almost eight decades. A recent study showing that some cases of Alzheimer's disease result from an extra copy of the amyloid precursor protein gene locus quite possibly brings to a definitive conclusion the idea that a modest increase in APP gene dosage in Down's syndrome is causative of the Alzheimer phenotype in these individuals. This commentary will trace some of the historical studies in the context of this new finding.

Introduction

2006 is the centennial anniversary of Alois Alzheimer's discovery of the disease that bears his name. It is also a year that will be remembered as the year in which the amyloid hypothesis gained one of the most compelling pieces of genetic evidence of the pathologic phenotype that can result from having three copies of the amyloid precursor (APP) gene (Rovelet-Lucrux et al. 2006). Indeed, this study showed that an extra copy of the APP gene results in the neuropathology of Alzheimer's disease (AD). This finding fulfills a series of predictions made between 20 and 60 years ago that a common genetic cause underlies the pathological similarities between AD and trisomy 21 [Down's syndrome (DS)] and, in so doing, provides some of the strongest evidence yet of the central role of APP in AD pathogenesis. This short commentary will review the historical evidence linking AD pathology in older DS individuals to APP.

Discussion

The defining pathology of AD was described in 1906, yet it would take six more decades before it was recognized as a common disease affecting the elderly. Meanwhile, Struwe briefly mentioned the finding of senile plaques in a 37-year-old DS individual ("mongoloid") back in a 1929 monograph (Struwe 1929). More careful studies in the 1940s reported senile plaques, neurofibrillary changes, and loss of neurons in DS individuals in the fourth and fifth decades of life. In a study of three DS subjects, Jervis was apparently the first to note that these changes were accompanied by mental deterioration

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in these individuals in their fourth and fifth decades of life (Jervis 1948). While these changes were known to be present in aged individuals, the author noted the uniqueness of the extensive pathological changes in relatively young individuals. Indeed, his own unpublished observations led him to conclude that affected DS individuals older than 35 years of age were susceptible to "early senile dementia." Furthermore, he concluded with foresight that "the finding of this peculiar tendency of mongloloid idiots to develop premature senile dementia may offer some clue with regard to the problem of pathologic aging of the brain. It justifies the hypothesis that certain etiological factors which play a role in mongolism may be similar to those responsible for some of the senile changes… (Jervis 1948)."

Studies performed two decades later, particularly those by Nathan Malamud, established that AD changes in DS were seen in all individuals beyond the age of 40 (Malamud 1972). Moreover, the AD neuropathology in DS was unique to trisomy 21, as it was not seen in mental retardation in general without DS. In the 1970s, the similarity of AD pathology was extended to the ultrastructural level (Burger and Vogel 1973). Therefore, even before the recognition of AD as the most common neurodegenerative disorder with a high prevalence in the mid-1970s, it was well established that the neuropathology of AD was fully recapitulated in older DS individuals. Indeed, Malamud and Hirano suggested that "… the chromosomal abnormalities in Down's syndrome might predispose to development of the neuropathologic changes characteristic of Alzheimer's disease (Malamud and Hirano 1974)."

The next important milestone was the sequencing of the amyloid protein in meningeal vessels of adults with DS by Glenner in the early 1980s (Glenner and Wong 1984a). This study was published in the same year as the seminal paper reporting the isolation and identification of amyloid β-protein (Aβ) from meningeal vessels of AD subjects. Equally prophetic in their second article was the statement in the abstract that "this is the first chemical evidence of a relationship between Down's syndrome and Alzheimer's disease… Assuming the beta protein [Aβ] is a human gene product, it also suggests that the genetic defect in Alzheimer's disease is localized on chromosome 21" (Glenner and Wong 1984b). As readers all know, the latter prediction was fulfilled in 1987 with the cloning of APP gene by several laboratories virtually simultaneously, using strategies that depended on the initial sequence characterization of meningeal amyloid reported by George Glenner and plaque core amyloid reported by Konrad Beyreuther and Colin Masters. Upon the discovery that APP was located on chromosome 21, the link to DS was immediately obvious. In fact, a study the same year suggested that APP duplication was detected in AD individuals with sporadic onset, a finding that apparently was never replicated (Delabar et al. 1987). Nonetheless, the questions that remained to be clarified were whether it was indeed duplication of APP that resulted in the invariant AD pathology in trisomy 21 individuals and whether extra APP gene dosage could be pathogenic in non-trisomic 21 individuals.

Two more series of discoveries set the stage for the 2006 report by Rovelet-Lecrux and colleagues. The first was the finding of mutations within APP that result in a rare autosomal dominant form of AD, thus placing APP at the core of AD pathogenesis, if only in a small subset of inherited AD cases. Second, fine mapping of genes duplicated in several individuals with partial trisomy, where some but not all chromosome 21 genes were duplicated, excluded APP and SOD1 genes in generating classical features of DS (Korenberg et al. 1990). Subsequently, a remarkable case report described a 78-year-old

woman with DS features due to partial trisomy 21 who at postmortem examination did not have any of the expected AD pathological changes in brain (Prasher et al. 1998). The segment of the chromosome that was duplicated in this individual excluded the APP gene, signifying that APP or possibly genes immediately adjacent to APP are necessary for the development of AD histopathology.

Finally, in January 2006, Rovelet-Lucrux and colleagues reported several independent duplications of the APP locus in different French families (Rovelet-Lecrux et al. 2006). Even more interesting was the finding that these families with variable autosomal dominant inheritance pattern showed neuropathology consistent with AD and severe congophilic amyloid angiopathy (CAA); the latter was likely the reason for the large lobar cerebral hemorrhages seen in some of the cases. In retrospect, it is ironic that the first APP mutation was not discovered in familial AD but in the hereditary cerebral hemorrhage with amyloid angiopathy, Dutch type, a particularly malignant form of amyloid angiopathy with early cerebral hemorrhages (Levy et al. 1990). With the finding of subsequent APP mutations, it is now known that these APP locus duplications can result in either classic AD or primarily CAA, or both. Taken together with studies of DS individuals, it can be concluded that duplication of the APP locus results in premature accumulation of Aβ in brain causing AD and CAA. These findings also provide compelling evidence that the invariant AD pathology in trisomy 21 individuals is due to the third copy of the APP gene. Put another way, a modest 50% increase in gene dosage is sufficient to drive AD changes in individuals in their fourth to sixth decades of life.

Thus, in reviewing the history of the studies into the development of AD pathology in DS, it is apparent that in the last six decades, a number of remarkably prescient predictions have been made by those examining the brains of elderly DS individuals. It is fortuitous that the year when we commemorate the centennial of Alois Alzheimer's publication is also the year that compelling, perhaps even definitive, genetic evidence of the central role of APP and Aβ in AD is reported. In turn, this brings to a satisfying conclusion the linkage between the development of AD pathology in DS individuals and the APP gene.

Acknowledgements. I wish to thank Drs. Peter Burger, Albert Heyman, and Stephen Vogel, who first introduced me to what has become a lifelong pursuit to understand the pathogenesis of Alzheimer's disease.

Takaomi C. Saido

Catabolism of amyloid β peptide and pathogenesis of sporadic Alzheimer's disease: towards understanding the underlying mechanisms

Takaomi C. Saido[1]

Biochemical characterization of Aβ-degrading enzyme(s) in vivo

Nearly a decade ago, we hypothesized that down-regulation of amyloid β peptide (Aβ) degradation may be a primary relevant cause for Aβ accumulation in sporadic Alzheimer's disease (AD; Saido et al. 1996; Saido 1998; Iwata et al. 2000). This hypothesis was made on the basis that there is little evidence supporting the up-regulation of Aβ generation upon aging prior to Aβ deposition in the brain, that sporadic AD patients seem to accumulate less $A\beta_{42}$ compared to $A\beta_{40}$ than familial AD patients (Lemere et al. 1996), and that aging generally accompanies down-regulation rather than up-regulation of enzyme activities (except for those associated with inflammation and other pathological processes).

To test this working hypothesis, we first set out to identify the major in vivo Aβ-degrading enzyme in the brain. Whereas the mechanism of Aβ generation had been examined in depth using molecular and cellular biological approaches (Citron 2003; Hartmann 2003; Ishiura 2003; Wolfe 2003), the mechanism of Aβ degradation remained elusive, not only because of the cellular topology but also because the complex structural organization of the brain tissue composed of various types of cells needed to be taken into consideration in analyzing in vivo Aβ degradation. Therefore, we started our series of degradation studies by establishing a novel in vivo experimental paradigm in which we injected synthetic, internally multi-radio-labeled $A\beta_{1-42}$ into the hippocampus of anesthetized live rats and analyzed the degradation process using high-pressure liquid chromatography directly connected to a flow-type scintillation counter (Iwata et al. 2000). Experiments using a panel of more than 20 peptidase inhibitors highlighted that a neutral endopeptidase family member, similar or identical to neprilysin, appeared to play a major role in the $A\beta_{1-42}$ catabolism because thiorphan, a well-characterized neutral endopeptidase inhibitor (Turner 2004), was the most potent inhibitor. In accordance with this assumption, short-term and long-term infusions of thiorphan into the rat hippocampus resulted in biochemical and pathological accumulation of endogenous Aβ, respectively. Dolev and Michaelson (2004) recently demonstrated that thiorphan infusion induces Aβ accumulation in an apolipoprotein E genotype-dependent manner, consistent with the human pathology (Saunders et al. 2000; Morishima-Kawashima et al. 2000).

The next task was to identify the major responsible Aβ-degrading peptidase among members of the neutral endopeptidase family. We made efforts to determine the molecular identity by using biochemical and molecular biological approaches and, consequently, predicted that neprilysin was likely to be the primary candidate (Takaki et al.

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2000; Shirotani et al. 2001). We subsequently confirmed our prediction by examining the degradation of radio-labeled Aβ in neprilysin-knockout (KO) mouse brains using their wild-type littermates as positive controls (Iwata et al. 2001). Neprilysin is a type II membrane-associated peptidase whose active site faces the lumen or extracellular side of membranes (Roques et al. 1993; Turner et al. 2001; Turner 2004). This topology is suited for the degradation of extracytoplasmic peptides such as Aβ. Using immunofluorescence microscopy, we confirmed that neprilysin is essentially exclusively expressed in neurons, not in glia, and that the peptidase, after synthesis in the soma, is axonally transported to presynaptic terminals (Fukami et al. 2002), presumably in a manner similar to the way APP is transported. Therefore, presynaptic terminals and nearby intracellular (lumen-side) locations are likely to be the sites of Aβ degradation by neprilysin (Iwata et al. 2005).

Reverse genetic identification and characterization of the Aβ-degrading enzyme, neprilysin

Based on the above observations, we examined the ability of neprilysin-KO mouse brains to degrade Aβ in vivo and we quantified the endogenous Aβ levels in the brains (Iwata et al. 2001). Due presumably to redundancy in the neutral endopeptidase family (Turner et al. 2001; Kiryu-Seo et al. 2000; Turner 2004), the KO mice show normal characteristics in relation to reproduction, development, and adult anatomy, to the best of our knowledge (Lu et al. 1995). The ability to degrade the radiolabeled Aβ was significantly reduced in the KO mouse brains. Consistently, both the endogenous $A\beta_{40}$ and $A\beta_{42}$ levels were elevated approximately two-fold, in a manner comparable to or even greater than what has been described in familial AD-causing mutant presenilin transgenic or knock-in mice (Duff et al. 1996; Nakano et al. 1999). More importantly, the elevation of Aβ levels was inversely correlated with the gene dose of neprilysin and thus with the enzyme activity. These observations suggest that even partial loss of neprilysin expression/activity causes the elevation of $A\beta_{1-42}$ levels and thus could induce Aβ amyloidosis on a long-term basis in a manner similar to that of familal AD-causing gene mutations. These results also indicate that the rate constant for the in-parenchyma degradation of $A\beta_{42}$ by neprilysin accounts for about 50% of all clearance activity, which includes transport of Aβ out of brain via cerebrospinal fluid and the blood-brain barrier.

In a similar manner, some of the other Aβ-degrading enzyme candidates were examined by a reverse genetic approach, i.e., by measuring the Aβ levels in the brains of KO mice. To our knowledge, neprilysin seems to be the dominant peptidase regulating the steady-state level of the primarily pathogenic Aβ species, $A\beta_{42}$, as do the pathogenic familial AD presenilin mutations. Endothelin-converting enzymes (ECEs) are interesting because they resemble neprilysin in structure, belonging to the same family of proteases (M13 family;Saido and Iwata 2006), and because they degrade Aβ in acidic intracellular compartments (Eckman and Eckman, 2003). Notably, not only neprilysin but also a number of other Aβ-degrading enzyme candidates, including ECEs and insulin-degrading enzyme (IDE), are zinc-requiring enzymes. Further to this, all the α-secretase candidates, a disintegrin and metalloproteinases (ADAMs) 9,

10, and 17 (Ishiura et al. 2003; Hartmann et al. 2003), which could contribute to reduction of Aβ synthesis, also require zinc for their proteolytic activities. Therefore, although too much zinc would obviously be harmful (Cherny et al. 2001), a heightened zinc deficiency is likely to be a negative risk factor for AD.

Neprilysin in aging and sporadic AD development

McGeer and colleagues reported that neprilysin mRNA levels are significantly reduced in the brain areas vulnerable to Aβ pathology in sporadic AD patients at a relatively early stage (Braak stage II) as compared to age-matched normal controls (Yasojima et al. 2001a, b). These observations are consistent with our hypothesis. Nevertheless, as it was still unclear whether reduction of neprilysin expression or activity preceded Aβ pathology during the course of aging, we examined the effect of aging on the expression/activity of brain neprilyisn using two methods: 1) a biochemical assay to measure neutral endopeptidase activity in the brain (the advantage of this method is that it is more quantitative than the second one, whereas the disadvantage is that the method fails to provide information regarding the local or spatial activity/expression of neprilysin); and 2) an immunofluorescence detection method using an anti-neprilysin antibody (Fukami et al. 2002). We observed that selective reduction of neprilysin expression upon aging occurs in the polymorphic cellular layer, inner molecular layer, and outer molecular layer of the dentate gyrus and also in the stratum lucidum of the CA3 sector of the hippocampus (Iwata et al. 2002). The differences were also proven to be statistically significant by quantitative image analyses. The results of the enzymatic quantification were also consistent with these observations; we observed a statistically significant reduction of approximately 10% of the enzyme activity per year in the entire hippocampus for two years and a 10% reduction in the neocortex in two years. If the 1.5-fold increase of $A\beta_{42}$ caused by the pathogenic mutations in the presenilin gene is truly sufficient for the development of early-onset familial AD, then a 1% reduction of neprilysin activity per year, leading to 50% reduction of neprilysin activity at the age of 50 and thus about a 1.5-fold elevation of the Aβ levels in the brain, would be sufficient to be causative of late-onset AD in humans.

Obviously, this observation needs to be further confirmed in human brains as well, but the problem is that almost all protein begins to be degraded within 30 min post-mortem and it is practically impossible to obtain samples prior to protein degradation in clinical situations. Besides, data would need to be taken chronologically prior to the disease for scientific evaluation. Proteomics using post- mortem human tissue must therefore be carefully interpreted. For reasons that are not understood, mRNAs seem to be much more stable than proteins under such conditions. A better option at present would be to confirm the chronology using animals that show aging-associated Aβ amyloidosis, such as polar bears, non-human primates, and dogs (Tekirian et al. 1998; Kanemaru et al. 1998; Satou et al. 1997). The immunofluorescence observations indicate that the areas where we see selective aging-induced reduction of neprilysin expression correspond to the terminal zones of mossy fibers and perforant path, which suggest that local Aβ concentrations are particularly elevated at the presynaptic locations originally projecting from the entorhinal cortex. It is notable that the entorhinal cortex

is the region where the initial neurodegeneration takes place in AD brains (Gómez-Isla et al. 1996). Indeed, Mèlanie et al. (2002) reported that not only parenchymal Aβ amyloidosis but also amyloid angiopathy correlates inversely with neprilysin levels in control and AD patients. Several reports also describe the possible role of neprilysin in inhibiting Aβ accumulation in the brain (Akiyama et al. 2001; Fukami et al. 2002; Marr et al. 2003; Leissring et al. 2003; Iwata et al. 2004). Other reports have associated neprilysin gene polymorphisms with the incidence of AD, as summarized elsewhere (Iwata et al. 2005), although the aging- dependent decline of neprilysin activity seems to be a natural process (Iwata et al. 2002; Caccamo et al. 2005).

Our findings also indicate that regulation of neprilysin activity in a manner specific to brain regions that are vulnerable to Aβ deposition could provide an effective strategy to reduce Aβ burdens in the brain. The advantages of utilizing neprilysin activity for the purpose of regulating brain Aβ levels were discussed previously (Saido 2000; Saido and Nakahara 2003). A relatively straightforward approach in experimental terms, but not necessarily in clinical terms, is the application of gene therapy. Indeed, using an in vitro paradigm, we demonstrated that overexpression of neprilysin, but not of an inactivated mutant form, in primary cultured neurons caused by the Sindbis virus leads to clearance of both the extracellular and cell-associated $A\beta_{40}$ and $A\beta_{42}$ forms (Hama et al. 2001). We also succeeded in regulating Aβ levels in vivo and in reducing Aβ burdens in APP transgenic mice by expressing neprilysin using adeno-associated virus (Iwata et al. 2004). Similar results were also reported by Hersh's group (Marr et al. 2003).

Selkoe and colleagues demonstrated that transgenic overexpression of neprilysin and IDE results in a reduction of the Aβ pathology in APP transgenic mice (Leissring et al. 2003). For a number of reasons, the effect of IDE is likely to have been mediated by alteration of APP processing by increased insulin levels rather than by the direct proteolysis of Aβ. First, insulin is a better substrate than Aβ for IDE (Farris et al. 2003), and insulin signaling regulates neuronal APP processing and trafficking (Gasparini et al. 2001; Solano et al. 2000). Also, IDE expression failed to reduce Aβ levels in a Drosophila model overexpressing $A\beta_{42}$ (not APP), whereas neprilysin expression significantly decreased Aβ levels and inhibited $A\beta_{42}$-induced neurodegeneration (Finelli et al. 2004). The advantages of neprilysin utilization over IDE utilization for the purpose of reducing Aβ levels in the brain are the following: 1) IDE is primarily a cytsolic protein capable of degrading the APP intracellular domain in vivo far better than any known substrates (Farris et al. 2003) and thus is unlikely to have direct access to Aβ unless the cells are permeabilized; 2) neprilysin degrades extracytoplasmic peptides, particularly at synapses (Fukami et al. 2002; Iwata et al. 2004; Hama et al. 2004; Saito et al. 2005), where Aβ may cause neuronal dysfunction; 3) the in vivo effect of neprilysin on brain Aβ seems to be unexpectedly selective because neprilysin deficiency does not seem to alter the levels of "neprilysin substrate" neuropeptides, such as enkephalin, cholecystokinin, neuropeptide Y, substance P, and somatostatin in the brain (Iwata and Saido, unpublished data); and 4) neprilysin can degrade Aβ oligomers, which can impair neuronal plasticity (Cleary et al. 2005), both in silico (Kanemitsu et al. 2003) and in vivo (Huang et al. 2006), whereas IDE can degrade only Aβ monomers (Morelli et al. 2003).

Regulation of neprilysin activity in the brain

We recently demonstrated that neprilysin regulates synapse-associated Aβ oligomers that impair in vivo neuronal plasticity and cognitive functions (Huang et al. 2006). These observations suggest that up-regulation of neprilysin activity in AD brains is likely to contribute to improvement of cognitive functions. Since the gene therapy approach, which requires surgical procedures, is not yet realistic for clinical application to humans, we have sought a pharmacological means to selectively up-regulate brain neprilysin activity as a new therapeutic candidate (Saito et al. 2003). The rationale for this strategy was based on the fact that at least two cell type-specific ligands capable of up-regulating neprilysin activity have been identified: opioids for monocytes (Wang et al. 1998) and substance P for bone marrow cells (Joshi et al. 2001) as part of a negative feedback mechanism. It is notable that receptors for these ligands are G protein-coupled receptors (GPCRs). We found that somatostatin (SST) regulates the metabolism of Aβ peptide in the brain via the modulation of proteolytic degradation catalyzed by neprilysin. Among various effector candidates, only SST up-regulated neprilysin activity in primary cortical neurons. A genetic deficiency of SST altered hippocampal neprilysin activity/localization and increased the quantity of a hydrophobic 42mer form of Aβ, $A\beta_{42}$, in a manner similar to presenilin gene mutations that cause familial AD (Saito et al., 2005). Due to these results, SST receptor(s) have now emerged as pharmacological target candidates for the prevention and treatment of AD (Iwata et al.2005).

Thus far, five SST receptor subtypes have been identified, all of which are GPCRs (Moller et al. 2003), the most suitable pharmacological target category of proteins in the history of pharmaceutical science. Among the five subtypes, types two and four may serve as primary candidate targets because they are relatively potently expressed in the neocortex and hippocampus (Bruno et al. 1992; Moller et al. 2003). Synthesis of blood brain barrier-permeable agonists that can distinguish between different receptor subtypes, which should not be an impossible task in modern medicinal chemistry (Moller et al. 2003), would make it possible to develop a medical application for our findings. Alternatively, the use of an "anti-dementia" compound such as FK960, which elevates hippocampal SST levels (Doggrell 2004) in brain, may provide another effective approach. One potential benefit of harnessing neprilysin activity by agonizing SST receptor(s), among other Aβ-reducing strategies, is that, if used conservatively, it is unlikely to be accompanied by major adverse side effects. Obviously, optimum combination of this approach with others would generate maximum beneficial effect (Saido and Iwata, 2006).

The expression of SST in the brain is known to decline with age in various mammals, including rodents, apes and humans (Hayashi et al. 1997; Lu et al. 2004). In human brains, SST mRNA is one of approximately 50 transcripts, the expression of which significantly declines after the age of 40, among approximately 11,000 transcripts examined (Lu et al. 2004). This finding indicates that the aging-dependent reduction of SST expression in the brain is a biologically specific and universal process. A prominent decrease in SST also represents a pathological characteristic of AD (Davies et al. 1980). These facts, combined with our observations that SST regulates neuronal neprilysin activity, led us to propose the following scenario for the etiology of sporadic AD development (Hama and Saido 2005). First, the aging-dependent reduction of SST causes

a decrease of neprilysin activity, which then causes the steady-state Aβ levels in brain to increase. Chronic elevation of the Aβ levels may result in further downregulation of SST levels (Davies et al. 1980), oxidative inactivation of neprilysin (Wang et al. 2003), and increased expression of APP and β-secretase, because APP is a stress- responsive protein (Storey and Capprai 1999) and because expression of both APP and β-secretase has been reported to increase in the relatively downstream cascade of AD development (Yasojima et al. 2001b; Li et al. 2004). These events form a vicious cycle leading to a catastrophic accumulation of Aβ in the brain (Funato et al. 1998; Wang et al. 1999; Morishima-Kawashima et al. 2000). If this hypothesis turns out to be true, we will not only be able to understand the etiology of sporadic AD but will also have identified a primary strategic target for the prevention and treatment of AD.

David M. Holtzman

Synaptic activity, amyloid-β and Alzheimer's disease

John R. Cirrito[1,2,4] and *David M. Holtzman*[1,3,4,5]

An unsolved mystery for most neurodegenerative disorders, including Alzheimer's disease (AD), is why the underlying pathology that occurs is region-specific. In other words, why are some brain regions vulnerable and others not? Several interesting findings and events have come together over the last six years that have led us to hypothesize that one of the reasons that the amyloid-β (Aβ) peptide deposits in a region-specific fashion in AD is related to the overall synaptic activity that occurs over many years in areas vulnerable to Aβ deposition. If this assumption is correct, it has important implications for both AD pathogenesis and potentially for future therapies.

John Cirrito began his PhD thesis in the Holtzman lab in 2000. Because of the key role that Aβ appears to play in the pathogenesis of AD, we thought it was critical to better understand its metabolism in the brain. Since Aβ aggregation and deposition occurs in the extracellular space of the brain, we thought it would be very useful to be able to dynamically and specifically measure the concentration of Aβ in the brain extracellular space over time. John accomplished this by developing a microdialysis system to measure Aβ in the interstitial fluid (ISF) of the brain. This was a difficult task, as microdialysis enables one to assess small molecules such as neurotransmitters much more readily than larger molecules such as peptides. Using a large pore size microdialysis probe (e.g., 38 kDa molecular weight cut-off) enabled us to recover Aβ (4.4 kDa) from the ISF. Addition of bovine serum albumin (BSA) to the perfusion buffer was also required to prevent Aβ from sticking to the tubing and collection vials. While albumin-containing buffers are generally very simple to prepare, handling and preparing the BSA just right for this application was a pain- staking process, however necessary, so that the BSA would not clog the small diameter microdialysis probe or membrane pores. Once the technique was optimized, we were able to measure the concentration of Aβ in the ISF of the hippocampus and striatum in mouse models of AD up to every 30 minutes for 24–36 hours in the same mouse (Cirrito et al. 2003, 2005a). Additionally, specially designed cages allowed the mice to be awake and behaving throughout the experiment, allowing us to show that the half-life of total Aβ species in the hippocampal ISF of human amyloid precursor (APP) transgenic mice was very short, 1–2 hours. This finding was interesting given that, once Aβ forms plaques, some of the Aβ within these structures may have a half-life of months or even years. We also showed that, in mice with plaques, there was a prolongation of ISF Aβ clearance, most likely secondary to the fact that there is a dissociable pool of Aβ in plaques that can re-enter the soluble

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phase when ISF Aβ levels decline. The microdialysis technique has also proven very useful in determining whether endogenous and exogenous molecules influence ISF Aβ metabolism in vivo. For example, we found that endogenous apolipoprotein E (an Aβ chaperone) affects the overall level of ISF Aβ and Aβ half-life (DeMattos et al. 2004). In addition, it was shown that an inhibitor of the molecule P- glycoprotein was able to increase ISF Aβ over hours, suggesting that this molecule is involved in Aβ transport out of the brain via the blood-brain barrier (Cirrito et al. 2005b).

In addition to our own findings, several pieces of information convinced us that it would be important to determine whether neuronal or synaptic activity in some way was linked with the concentration of ISF Aβ in vivo. First, in the 1990s, Gouras, Gandy, and colleagues (1997), studying patients who had a temporal lobe removed as part of surgery for temporal lobe epilepsy, found that a large percentage of these individuals who were less than 50 years of age had amyloid plaques in their hippocampus (Gouras et al. 1997). This finding is otherwise very uncommon before the age of 50, unless one has Down's syndrome or an autosomal dominant form of familial AD. While there are many possible reasons for this finding, one is that deposition of Aβ was somehow linked to excessive electrical activity over time. Second, in 2003, Roberto Malinow's lab made several observations with organotypic brain slices cultures, including the fact that drugs that decrease neuronal activity decrease Aβ in cell culture media over 24–48 hours and that drugs that increased neuronal activity had the opposite effect (Kamenetz et al. 2003). Third, findings from Buckner and colleagues demonstrated that the areas of the human brain that develop the most Aβ plaques also have the highest basal rates of metabolic and synaptic activity, as measured by positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), when individuals are not performing a specific mental task, the so-called "default state" (Raichle et al. 2001; Buckner et al. 2005). If activity in brain regions that make up the "default state" network over a lifetime are the most active in the brain, the high level of activity in these areas may make them particularly susceptible to Aβ deposition if there is a positive relationship between synaptic activity and Aβ levels. Fourth, physical and cognitive activity can alter plaque burden later in life. In APP transgenic mice, it was found that physical and cognitive enrichment results in altered Aβ plaque burden (Jankowsky et al. 2003, 2005a; Adlard and Cotman 2004; Lazarov et al. 2005b). One of several possibilities for this effect is that physical and cognitive enrichment alters synaptic activity (and hence Aβ levels) in specific brain regions.

With these facts as a backdrop, we asked whether neuronal or synaptic activity was in some way dynamically linked with extracellular levels of Aβ in the brain in vivo. We utilized one of the most studied APP transgenic mouse models for these experiments, Tg2576 (APPsw) mice (Hsiao et al. 1996). Young mice (three- to four-months old), several months prior to the appearance of plaques, were studied to assess Aβ metabolism without the complexity of plaques being present. We thought a key experiment was to determine whether direct electrical stimulation of a defined anatomical pathway would alter ISF Aβ. Through a collaboration with Bob Sloviter at the University of Arizona and both Kel Yamada and Steve Mennerick at Washington University, John Cirrito worked out a method whereby he could electrically stimulate the perforant pathway to cause a focal seizure in the synaptic terminal zone of this pathway (the hippocampus) while simultaneously monitoring hippocampal EEG activity and ISF Aβ (Fig. 1a). Immediately after the onset of electrical stimulation that elicited a focal

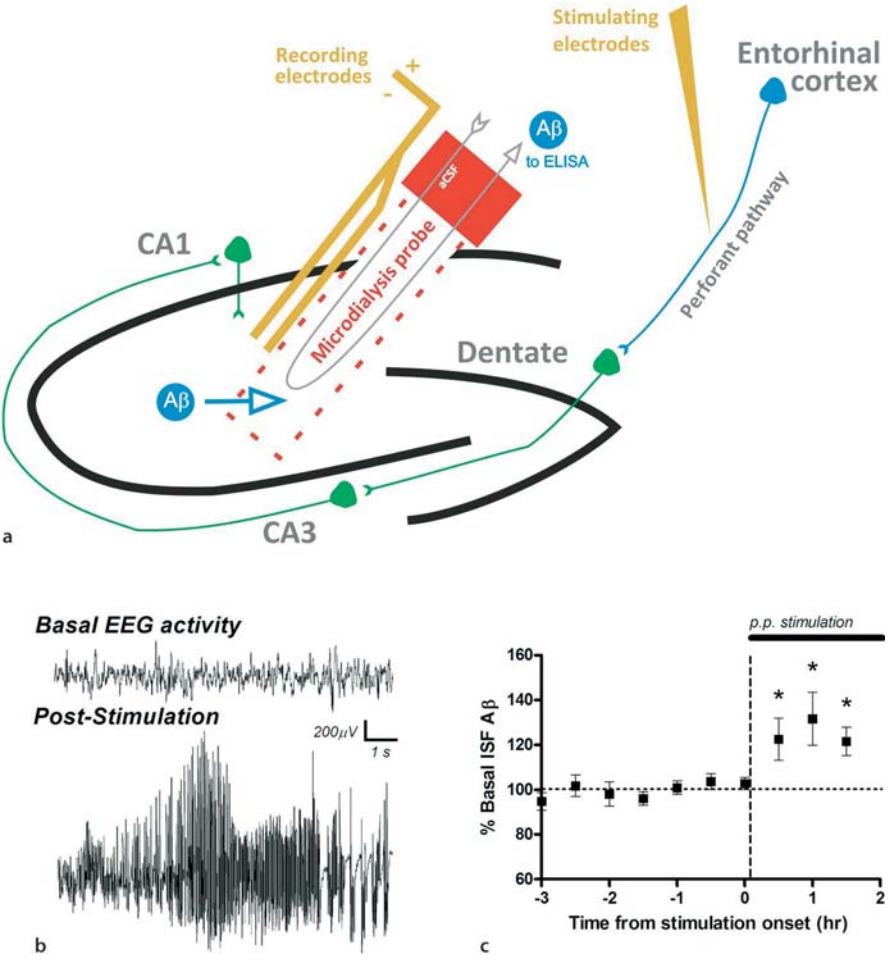

Fig. 1. Electrical stimulation of the perforant pathway increases ISF Aβ levels. (**a**) Diagram of the hippocampus showing an electrode stimulating the perforant pathway and both recording electrodes and a microdialysis probe in the hippocampus. (**b**) Representative traces of basal EEG activity (*top*) and epileptiform discharges during electrical stimulation of the perforant pathway (*bottom*) in three- to five-month-old Tg2576 mice. (**c**) When EEG activity was elevated, ISF Aβ levels increased by 133.3 ± 19.7% ($p = 0.05$; $n = 5$). Modified with permission from Cirrito et al., 2005. p.p., perforant pathway

seizure (Fig. 1b), there was a 30% increase in ISF Aβ (Fig. 1c). We then determined the effects of decreasing neuronal and synaptic activity. Direct administration into the hippocampus by reverse microdialysis of tetrodotoxin, which blocks action potentials but not all synaptic activity, resulted in a very rapid 30% decrease in ISF Aβ (Fig. 2a). Tetanus toxin decreases synaptic activity by blocking synaptic vesicle release. Direct hippocampal infusion of tetanus toxin resulted in an 80% decrease in ISF Aβ by 18 hours after infusion (Fig. 2b). Finally, using brain slices derived from APPsw transgenic

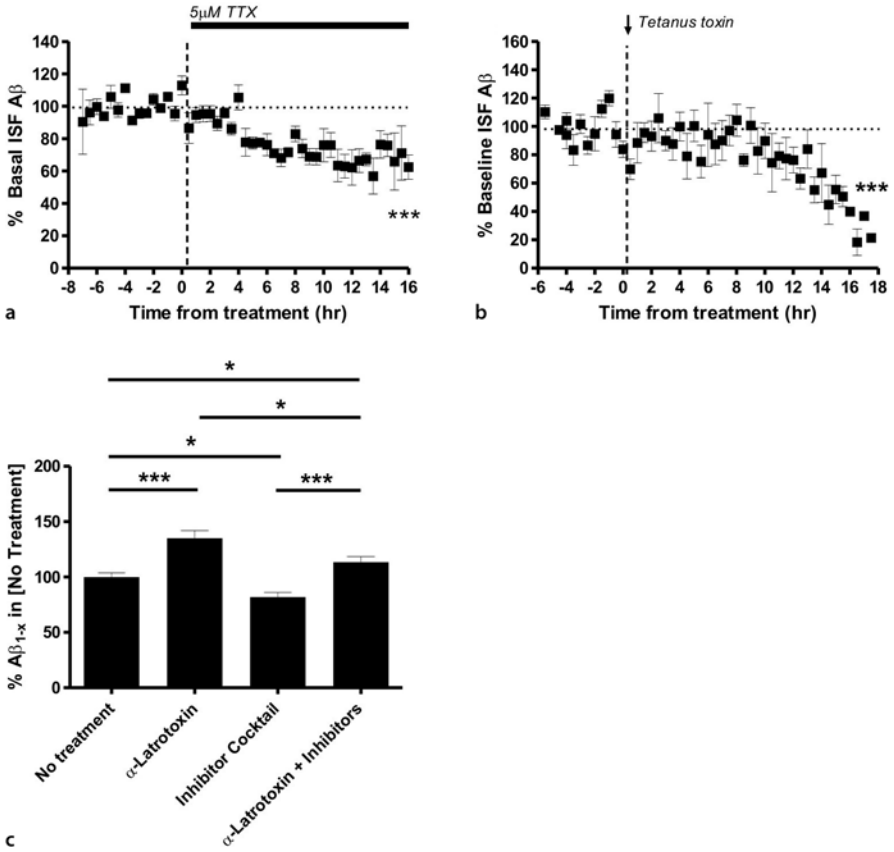

Fig. 2. Synaptic activity and synaptic vesicle release are linked with ISF Aβ levels and neuronal release of Aβ. (**a**) Following local treatment with tetrodotoxin (TTX), ISF $A\beta_{1-x}$ levels declined, reaching 70.4 ± 4.5% of baseline at 16 hours ($p < 0.0001$; $n = 5$). (**b**) 0.2 μg tetanus toxin was injected directly into the hippocampus surrounding the microdialysis probe to inhibit synaptic vesicle release. By 18 hours following treatment, ISF Aβ levels declined significantly compared to baseline ($p = 0.0003$, $n = 4$). The lag time between treatment and an effect on Aβ levels is likely due to the time necessary for the toxin to enter the cell and effectively cleave the synaptic vesicle associated protein VAMP2. (**c**) Acute brain slices were made from four- to five-week-old Tg2576 mice. To determine the affect of synaptic vesicle exocytosis on extracellular Aβ levels in the absence of synaptic activity, Tg2576 brain slices were cultured for two hours in the presence of 0.5 nM α-latrotoxin and/or a cocktail of activity inhibitors including 100 nM TTX, 10 μM NBQX, and 50 μM APV. α-Latrotoxin alone caused a 35±6.9% increase in Aβ levels whereas the inhibitor cocktail lowered Aβ levels by 18.0 ± 4.1% compared to untreated slices. α-Latrotoxin plus the inhibitor cocktail resulted in 13.3% more extracellular Aβ as compared to untreated slices and 38.3 ± 6.2% more Aβ compared to the inhibitor cocktail alone (n = 12–15 per group). Modified with permission from Cirrito et al. 2005

mice, we administered α-latrotoxin, which results in massive synaptic vesicle release without depolarizing the cell. α-Latrotoxin was administered in the absence or presence of blockers of neuronal activity and neurotransmitter receptors to differentiate

between the effects of vesicle exocytosis and neuronal activation. Even in the presence of blockers of neuronal and synaptic activity, there was a rapid 30% increase in Aβ in the media surrounding the brain slices (Fig. 2c). Together, these results strongly argue that synaptic vesicle release is linked, either directly or indirectly, with the release at the synapse of Aβ in vivo. Current evidence suggests that APP/Aβ is not in synaptic vesicles (Ikin et al. 1996; Marquez-Sterling et al. 1997). APP is co-internalized from the plasma membrane with synaptic vesicle integral membrane proteins such as synaptophysin and synaptotagmin, then sorted away from those proteins and incorporated into distinct vesicles (Marquez- Sterling et al. 1997). This suggests that synaptic vesicle membrane recycling and APP endocytosis are linked. APP endocytosis is directly linked to Aβ generation and release (Koo and Squazzo 1994). While synaptic vesicle exocytosis can rapidly modulate extracellular Aβ levels, it may actually be an associated event, such as membrane recycling or another process, that is directly responsible for the rapid modulation of extracellular Aβ levels.

Taken together, these findings suggest that synaptic activity, and specifically synaptic vesicle release, directly results in Aβ release into the brain extracellular space and is likely an important mechanism regulating the ISF level of Aβ in vivo. The implications of these findings are several. First, neurotransmitter and neurotransmitter receptor modulators are likely to directly regulate ISF Aβ levels through modulation of synaptic activity, suggesting that such modulators may provide new drugs to alter ISF Aβ and the downstream process of Aβ aggregation. Second, synaptic activity that occurs within specific vulnerable networks of the brain such as the "default state" network may be very relevant to the onset and amount of Aβ deposition that occurs there and hence the pathogenesis of AD. Overall, further exploration into the relationship between synaptic activity, Aβ levels, and development of AD pathology may lead to new insights into the underpinnings of AD and future therapies.

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Monica Di Luca

Molecular pathogenesis of Alzheimer's disease in human peripheral cells: platelets show it all!

Elena Marcello[1], *Barbara Borroni*[2], *Fabrizio Gardoni*[1], *Alessandro Padovani*[2], and *Monica Di Luca*[1]

Introduction

The complete understanding of the pathological impact in vivo of alterations in expression/level and/or activity of key molecular elements still represents a challenge in the field of Alzheimer's disease (AD).

In fact, although molecular biology and in vitro studies have had a tremendous impact on our knowledge and they represent a crucial advancement in our understanding of the disease, clear-cut data obtained in accessible cells or biological fluids were still lacking until a few years ago.

In this scenario, the ability to measure key pathogenic elements, such as Aβ peptide and Tau, in biological fluids pioneered the field (Olsson et al. 2005) and opened new possibilities in identifying biomarkers of the disease.

Our laboratory focused its attention on the observation that the same pathological pathways could be analyzed in easily accessible peripheral cells of AD patients.

Platelets: an opportunity for molecular pathogenic studies and the search for biomarkers

Importantly, platelets appear immediately to be a reliable peripheral cellular system in which to analyze amyloid protein precursor (APP) metabolism, a key event in AD pathogenesis, since they show numerous alterations typical of neurodegeneration (Fig. 1; Zubenko et al. 1999; de Silva et al. 1998; Bosetti et al. 2002; Ripovi et al. 2000; Zoia et al. 2004) and appear to be the primary source of Aβ in human blood (Chen et al. 1995).

Moreover, among the different peripheral cells expressing APP forms, platelets are particularly interesting because they show concentrations of its isoforms equivalent to those found in brain (Gardella et al. 1990; Bush et al. 1990; Bush and Tanzi 1998).

In the last few years, we have investigated whether a correlation between levels of platelet APP forms and AD could be detected. We reported that patients with sporadic AD showed an alteration of APP forms expression in platelets when compared with age-matched controls and with patients with non-AD dementia (Di Luca et al. 1996).

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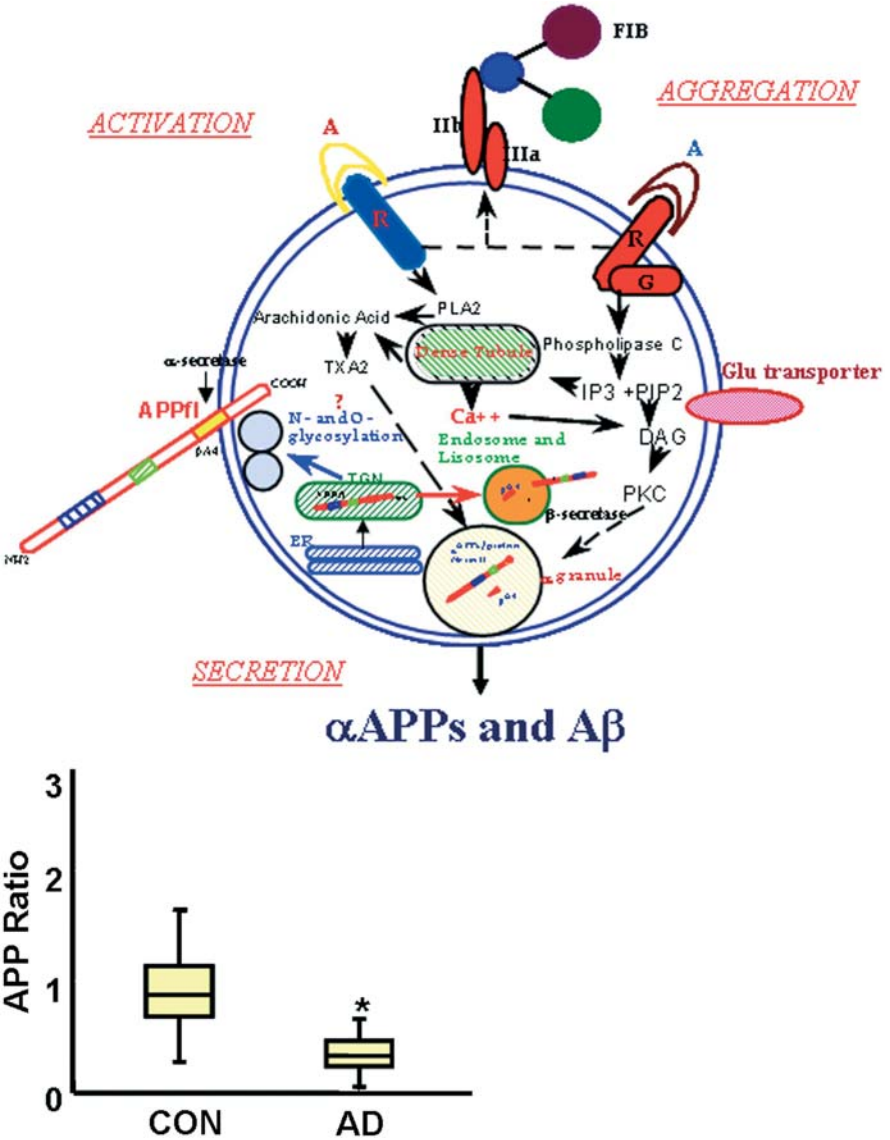

Fig. 1. Platelets contain all the biochemical machinery to process APP. APP forms ratio differs between control (CON) subjects and AD patients, representing a useful biomarker for the disease

This observation has been subsequently confirmed (Rosenberg et al. 1997) and has several implications. APP processing abnormalities, believed to be a very early change in AD in neuronal compartments, occur in extraneuronal tissues, such as platelets, suggesting that AD is a systemic disorder. Further, our data strongly indicate that a differential level of platelet APP forms can be considered a reliable, early and

peripheral marker of AD (Di Luca et al. 1998), since the alteration of platelet APP isoforms in AD patients shows a positive correlation with the progression of clinical symptoms. Furthermore, it was confirmed that the altered ratio of APP forms is specific for AD, in view of the fact that the ratio was unchanged in subjects affected by other kinds of dementia. Finally, the altered APP ratio appears to be a highly specific and sensitive peripheral marker for the diagnosis of sporadic AD (Padovani et al. 2001). In fact, it can distinguish AD subjects from controls with a specificity and sensitivity of about 90%.

Furthermore, platelets contain the same APP processing enzymes found in neurons (Abraham et al. 1999; Colciaghi et al. 2002), BACE and ADAM10. We reported that in AD patients the alteration of APP ratio in platelets is accompanied by a significant modification of the two main enzymatic protagonists involved in APP metabolism (Colciaghi et al. 2004b). We demonstrated an unsettling of the balance between α- and β-secretase activities in AD patients in vivo, with β-secretase activity being predominant, as assessed by measurements of APP fragments, i.e., sAPPα released from activated platelets and the membrane-attached APP C-terminal fragments CTF83 and CTF99 produced by α- and β-secretase activity, respectively (Colciaghi et al. 2002; Zimmermann et al. 2005). It is possible that an alteration of the concerted interplay among amyloid cascade actors occurs in AD with a concomitant decrease in α- and increase in β-secretase activity.

Moreover, we found a marked alteration of APP, BACE and ADAM10 in the very early stages of the disease, where dementia can be barely inferred by neuropsychological assessments (Colciaghi et al. 2004b). Therefore, platelets provide a reliable tool to identify the pathological process even before the onset of clinical dementia and reflect the central pathogenic development.

This finding implies that APP and secretase modification might be considered as a combined testing strategy for early AD diagnosis, favoring the thesis that combining tests should be considered to increase the discriminative power of the analysis. This strategy would include either combining tests related to different pathophysiologic pathways or associating biomarkers linked to the same biologic cascade. We reported that, in the same peripheral system, it is possible to measure the cellular levels of the three molecular identities specifically related to AD pathology that represent key elements in the amyloid cascade (Di Luca et al. 2005).

An ideal biochemical biomarker is required not only for diagnosis but also to map the potential effects of AD therapy on the progression of the disease in morphological, biochemical and functional terms. Pharmacological treatment of AD still represents an unsolved issue. Significant improvements in cognition and global function have been observed upon treatment with Acetylcholinesterase inhibitors (AChEIs; Doody 1999; Farlow 2002; Rogers and Friedhoff 1996), although clinical research has only been able to verify the symptomatic effect of these treatments (Eagger and Harvey 1995; Farlow 2002). Preclinical studies identified unexpected mechanisms of action of these drugs, underscoring the fact that they may tackle alteration in APP processing in systems (Giacobini 2003; Lahiri et al. 2000).

To trace the effect of AChEIs on APP metabolism, we analyzed not only our biochemical biomarker – the APP isoforms ratio – but also the major APP metabolites – sAPPα, CTF83, CTF99 – in platelets of AD patients treated with AChEIs for 30 days. We reported that, ex vivo in AD patients' platelets, a short treatment with a low dose

of AChEIs is capable of rescuing the APP isoforms ratio (Borroni et al. 2001) and of restoring the balance between α- and β-secretase activities (Zimmermann et al. 2005). These results further reinforce the use of platelets as peripheral cells expressing reliable biomarkers for AD. In fact, we showed that the biomarkers of APP metabolism are remarkably influenced by exposure to AChEIs treatment, with their pattern being shifted towards the non-amyloidogenic pathway. This finding implies the possibility of evaluating all steps of APP metabolism dynamically in an intact cell as platelets. Since we demonstrated that alterations of APP metabolites measured in platelets reflect what is occurring in the central nervous system (Colciaghi et al. 2002), the use of this approach of following the effects of AChEIs in patients appears logical. Moreover, these findings suggest a positive interaction of AChEIs treatment with pathogenic mechanisms of AD and might explain the clinical effect reported in the long-term treatment with these compounds (Rogers and Friedhoff 1998; Giacobini 2001).

Trafficking of ADAM10 to membranes: a new target for pharmacological intervention?

At this point, the mandatory question remained: how can AChEIs exert this effect?

We used an in vitro system aimed at studying and elucidating the molecular mechanisms of AChEIs on the key players of APP metabolism. It had been previously described that Gingko biloba Extract EGb 761 was capable of influencing in vitro APP metabolism (Colciaghi et al. 2004b). The main finding of this more recent cellular study is that AChEIs elicits its action through multiple mechanisms involving not only AchE inhibition but also processing and trafficking of two key players of AD pathogenesis, namely, APP and a-secretase. In a differentiated neuroblastoma cell line that expresses both AchE and muscarinic receptors, AChEIs significantly inhibits AchE activity and increases release of sAPPα, not only through a muscarinic receptor pathway but also by directly enhancing trafficking of ADAM10 towards the cellular membrane, where it cleaves APP (Zimmermann et al. 2004).

Hence, the mechanism that regulates APP's and secretase's intracellular localization and trafficking to the neuronal membrane may be central to AD pathogenesis. Particularly important will be those mechanisms regulating the trafficking of ADAM10 as a candidate for a-secretase activity, since the shifting of ADAM10 to a membrane compartment could positively influence its activity, thus shifting APP metabolism towards non-amyloidogenic pathway. These aspects can have important implications for the treatment. Accordingly, we are now interested in the identification of molecular interactors that are responsible for ADAM10 trafficking to neuronal membranes.

Mathias Jucker

The neuronal origin hypothesis of cerebral amyloid angiopathy

Mathias Jucker[1]

With the rapid evolution of mouse genetics 20 years ago, murine models have gained increased attention in the neurobiology of aging. The genetic contribution of age-related traits as well as specific mechanistic hypotheses underlying brain aging and age-related neurodegenerative diseases could be assessed by using genetically selected and genetically manipulated mice. At that time I was a postdoc with Dr. Donald Ingram at the National Institute on Aging, NIH, Baltimore, USA, where I started to examine age-related alterations in the brain among a variety of mouse strains (Jucker and Ingram 1997). At that time, we noticed hippocampal deposits of fibrillary material (inclusion bodies) in the normal aged C57BL/6J mouse brain. At the same time, others reported on identical inclusion bodies in amyloid precursor protein (APP) transgenic mice, then believed to be the first mouse model of Alzheimer's disease (AD). Subsequently we showed that these apparent lesions in this AD mouse model have no relation to the transgene, do not contain Aβ and are a normal, age-related feature of mice with a C57BL/6 background (Jucker et al. 1992).

With this experience, I gained an interest and expertise in assessing brain aging in the mouse brain. I subsequently moved to Basel, Switzerland, and, as an assistant professor at the University of Basel, I had the chance to look at a new APP transgenic mouse model generated by Dr. Matthias Staufenbiel and colleagues at Novartis in Basel (APP23 mice; Stürchler-Pierrat et al. 1997). This mouse model developed extensive age-related cerebral amyloidosis. Together with Michael Calhoun, a PhD student at that time who moved with me from Baltimore to Basel, we noticed significant cerebral amyloid angiopathy (CAA) in these mice, although a neuron-specific promoter had been used to generate this transgenic mouse model.

Neuronal expression of APP causes CAA

To exclude that endogenous mouse APP would contribute to CAA formation in APP23 mice, we then crossed APP23 mice with *App*-null mice generated by Dr. Bernd Sommer, who was also at Novartis. Thus, we had a mouse in hand that only expressed APP in neurons of the CNS. The subsequent observation that this mouse also developed CAA confirmed our hypothesis that CAA in this mouse model is of neuronal origin (Fig. 1; Calhoun et al. 1999). Although we assume that this finding is also true in humans, we cannot exclude an additional contribution of Aβ from the vessel wall itself, e.g., from smooth muscle cells, although experimental proof for the latter is still missing.

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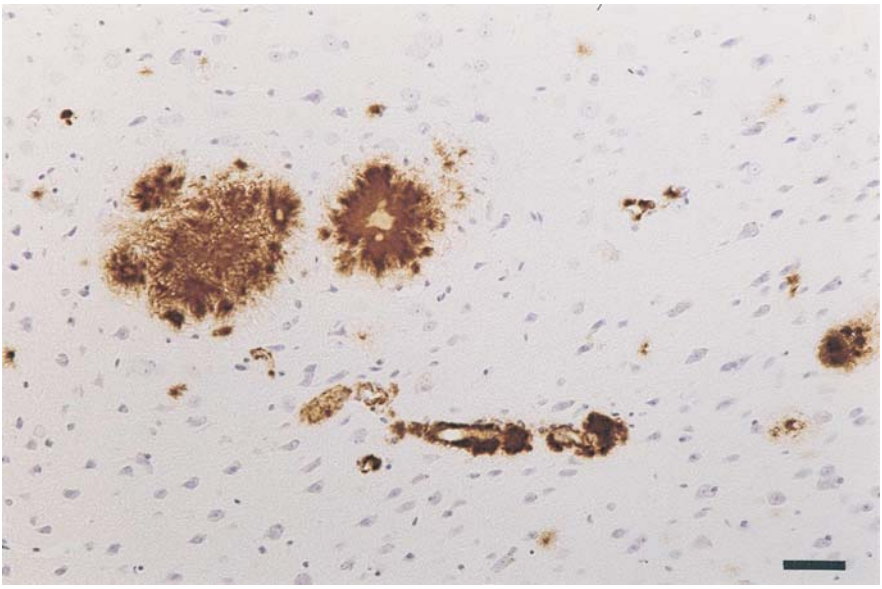

Fig. 1. Amyloid plaques and cerebral amyloid angiopathy (CAA) in APP23 transgenic mice on a *App*-null background. (Reproduced with permission from Calhoun et al. 1999)

First mouse model of CAA proper

It was late at night when Matthias Staufenbiel, Markus Tolnay and I were brainstorming at the Institute of Pathology in Basel about future strategies to generate new mouse models of AD and CAA. We decided on a mouse model for Hereditary Cerebral Hemorrhage with Amyloidosis Dutch-Type (HCHWA-D), again using the neuron-specific Thy-1 promoter cassette. Although this project was realized within months, our disappointment was great when we saw no amyloid at 6, 12 and 18 months of age, and the project lost priority in our laboratory. Then, late in another night (all discoveries were made late at night!), Martin Herzig, at that time a PhD student in my laboratory, found CAA (in the absence of any parenchymal amyloid) in 24-month-old mice. Today the mouse is known as the APP Dutch mouse and is the only mouse model of proper CAA (Herzig et al. 2004).

What have we learned from mouse models of CAA

These mouse models of CAA allowed us to rigorously study the mechanism and impact of CAA. We have subsequently shown that CAA leads to cerebral hemorrhage and that CAA is the cause of the increased cerebral hemorrhage after passive Aβ immunization (Fig. 2; Pfeifer et al. 2002), an observation that is now considered a potential serious complication of Aβ-immunotherapy in humans. By crossing APP Dutch transgenic mice with several other transgenic mouse models, we found that a high ratio of soluble Aβ40/Aβ42 drives amyloid formation in the vasculature, whereas a low Aβ40/Aβ42

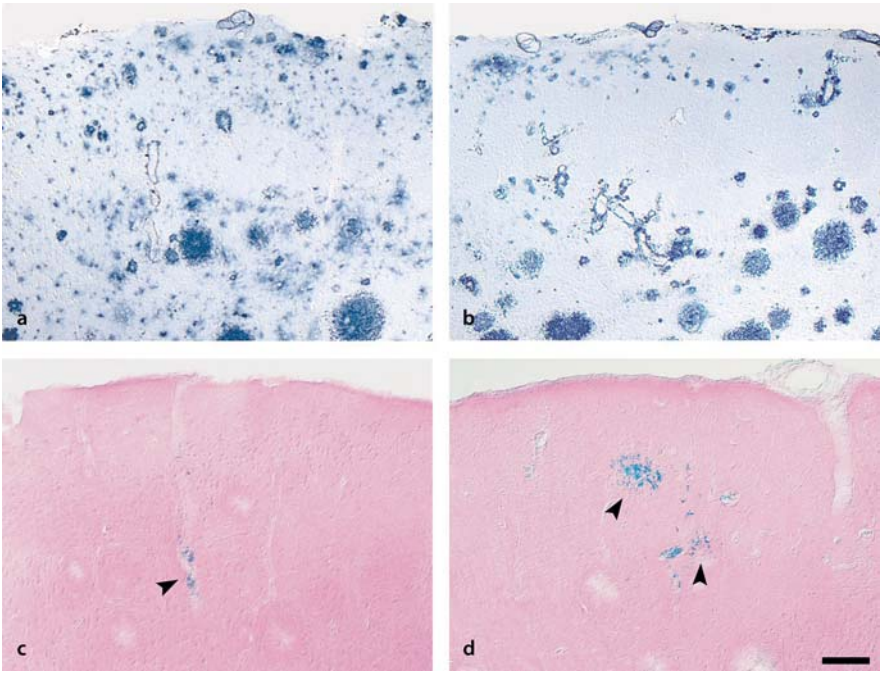

Fig. 2. Amyloid pathology in the neocortex of a control (**a**) and an age-matched immunized APP23 mouse (**b**). Hemosiderin staining reveals an increased number of microhemorrhages (*arrowheads*) in the immunized (**d**) compared to control mice (**c**). (Reproduced with permission from Pfeifer et al. 2002)

ratio leads to parenchymal amyloid. We have further shown that neuronally derived Aβ is transported extracellularly over considerable distances to the vasculature to be cleared via transport into the blood or via perivascular fluid drainage pathway (Meyer-Luehmann et al. 2003; Herzig et al. 2004). Although these and many other findings have begun to highlight the importance of CAA in AD, much more research is necessary to understand its pathogenesis and clinical impact.

Acknowledgements. I want to thank Donald Ingram and Matthias Staufenbiel for their great support. It was the enthusiasm of Donald Ingram that shaped my interest in brain aging, and it was the support of Matthias Staufenbiel that helped me to step into the field of Alzheimer's disease. Over all these years, Konrad Beyreuther greatly supported my work. I also want to thank the many talented students and postdocs in my laboratory for their tremendous contributions and the late-night discoveries.

Jean-François Foncin

A neuropathologist and Alzheimer genetics

Jean-François Foncin[1]

First contact

Early in 1972, a 44-year-old woman proved herself unable to care for her newborn, her ninth child. The general practitioner thought of brain disease, directed her to a neurologist who made a tentative diagnosis of a psychiatric illness and committed her to a psychiatric institution. There, the "spreading" progression of apraxia and disorientation symptoms (speech and memory were difficult to evaluate, with the patient speaking only her native Calabrian dialect and being illiterate) led to a tentative diagnosis of brain tumor. The patient was transferred to the neurosurgery department (Professor Le Beau) at Paris La Salpêtrière hospital; this was before CT scans made noninvasive brain tumor localization possible. Right frontal trephination and ventricular tap were performed on September 5, 1972 (Dr. Redondo). Intracranial pressure was low; the brain was at a distance from the skull; the ventricles were moderately enlarged without displacement. Since a brain tumor was ruled out through this procedure, the rule was to take a cortical biopsy. Using light and electron microscopy, I saw numerous senile plaques (most without amyloid cores), neurofibrillary degeneration (paired helical filaments, PHF), amyloid angiopathy of small vessels, and scarce microspongiosis bubbles, establishing a diagnosis of Alzheimer's disease (AD).

AD in a 44-year-old patient? I remembered that early-onset AD often runs in families, and the neuropathologist did what neither the general practitioner, the neurologist, the psychiatrist nor, of course, the neurosurgeon had done: to explore the family history, I interviewed the husband, an immigrant Calabrian tile-layer. He readily understood the problem. "Ma … la sorella, la stessa malattia … Il padre … morto alla Casa di Riposo di Girifalco … la stessa malattia" (Her sister … the same disease … Her father … died at the Girifalco resting home … the same disease).

I knew I was onto something. I wrote to Girifalco – the seat of the provincial psychiatric hospital – for the father's medical records. Through the courtesy of Dr. Fragola, the medical director of the hospital, I got three summaries of records of patients with the father's surname, all three of whom had died in their fifties with dementia. My teacher and mentor, Dr. J. E. Gruner, the central figure of French neuropathology in the 1960s and '70s and an active amateur genealogist, perceived at once the potential of genealogical methods when applied to this prolific Calabrian family for fundamental research in AD, through the study of the genetics of this condition. There was no hope of obtaining funding from public organizations for research in that direction: "senile dementia" was seen as a manifestation of "brain aging," period. Dr. Gruner

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arranged for funding through a private foundation close to his wife, and a party of four (Dr. Gruner, Dr. Supino-Viterbo – an Italian-born colleague at La Salpêtrière – and my wife as secretary and I) flew to Calabria in May 1973.

Largely due to Dr. Gruner's personal aura. The extended family of the proband and the local authorities were extremely co-operative. We examined anew the proband, now profoundly demented and cared for like a baby by her old mother. We were led around the family and examined affected siblings and cousins. We got free access to the municipal records of the birthplace of the proband and started an unbiased extended family reconstruction up to the late eighteenth century. A trip to the provincial psychiatric hospital (the "famigerata[1] legge Basaglia" had not, at the time, destroyed that irreplaceable wellspring of genetic information) enabled us to inspect its archives, perfectly kept from 1880 on, and to take copies of the records of the father, the grandfather and the great- grandmother of the proband, all of whom had died with dementia in their fifties with a diagnosis of (syphilitic) General Paresis of the Insane (GPI). We were also able to check information given by the family about relatives with "the disease."

A few weeks later, I took the occasion of a congress in Florence to visit a cousin of the proband at a psychiatric hospital in Tuscany. The medical director was a charming gentleman and an admirer of classical French psychiatry. He gave me full permission to examine the patient and to study the records. The patient, then aged 50, was profoundly demented, with loss of verbal communication associated with logorrhoea, and loss of feeding and sphincter control, without focal neurological symptoms. I explained that, as a member of a family with one member affected by histologically proven AD with probable Mendelian transmission, this patient could be of utmost importance for research on that mysterious disease. Neuropathological control of the diagnosis would be crucial: "Oh, I thank you, my dear colleague, for elucidating this very interesting instance of General Paresis of the Insane due to heredo-syphilis." The patient died two years later; an autopsy was not performed (one of her sons died demented in 1989, aged 45). In September of the same year, we presented our first results (Foncin and Supino-Viterbo 1973). We had identified 12 affected persons (alive and dead) in the extended family: 7 males, 5 females, with one affected person for two persons at risk under a Mendelian dominant hypothesis.

Hinc procedes ultra

Our first task was to study the formal genetics of AD and more precisely to quantify the transmission of AD within the kindred I called "family N," from the initial of the proband's birthplace. A friend from my student days, Dr. Denise Salmon, was a pioneer in the applications of computer techniques to human genetics. We started to create, via IBM-format 80-column punch cards, a file on a PDP10 mainframe. Funding, however, became critical. In 1977, I took the opportunity of an INSERM program on brain aging to apply, on slightly disguised grounds (*larvatus prodeo*), for a five-year grant. I got money for CPU time and hard disk allocation and also for field trips to Calabria to extend the data base. Due to the larger number of identified affected persons and to the statistical expertise of Dr. Salmon, we got the first hard results on the transmission of AD within the kindred, with narrow confidence intervals for the segregation ratio

[1] >in<famous

(Foncin et al. 1978). At mid-term, grantees had to present to the committee their interim results to be entitled to the second half of their grant. Elated by our first successes, I threw the mask away and presented extended graphic genealogical trees traced through a computer program developed by M.F. Landre under the guidance of Dr. Salmon, and the corresponding formal genetics, and did not conceal my opinion that this was the way to solve the AD riddle. I was wrong. The correct way was to look for means of delaying the Hayflick phenomenon (the "aging" paradigm); they cut my grant. For some months, I was demoralized and did not work on the subject.

Alzheimer genetics out of limbo

Several factors that I am going to summarize without strictly following the chronology contributed to the rekindling of my interest in the genetics of AD. Professor Macchi, a leading Italian neurologist and neuropathologist and a friend of Dr. Gruner, informed me that one of his pupils, Dr. Caruso, had established a neurology unit within the hospital serving the birthplace of our proband. I flew to Calabria and was met at the airport by Dr. Caruso and his young assistant, Dr. Bruni. Dr. Caruso was too busy with his various clinical and administrative duties to fully take part in the research work, but Dr. Bruni quickly developed a passion for it and soon took full charge of operations in Calabria, after completing her speciality thesis under my direction.

Another factor was the serendipitous realization that a large family with 12 persons affected with early-onset AD that had been described in the US by Feldman et al. (1963) was linked with family N. The authors did not give any indication about the origin of the family they described, but they mentioned an official with a surname I knew from my studies in Calabria. This clue, together with the identity of the clinical picture and of the ultrastructural neuropathology in both families (Krigman et al. 1965), led me to contact Professor Feldman, who generously gave me access to his data. I could thus establish that his patient #1.1 (known by history) was a daughter of the couple #18-17 in my data base; they lived in the first half of the nineteenth century and were ancestors of all the affected people known at the time in Calabria. Professor Feldman had a collaboration with Dr. Polinsky, a neuropharmacologist with an interest in familial AD (FAD; Nee et al. 1983) at NIH-NINCDS, in the section then headed by Dr. Katherine Bick. Dr. Polinsky participated in many ways, first by discovering with Ms. Nee a new American branch of family N and helping through his USPHS grants. Dr. Bick had a working relationship with Professor Amaducci, (Florence), head of an organization (SMID) dedicated to research in dementia, who established in Calabria a branch (SMID-SUD) with its own premises, headed by Dr. Bruni. The first activity of her team was to recruit new patients and to extend the pedigree; notably, she identified a common ancestor of all known affected persons, a woman born in 1715 who died at age 43.

The steps to the gene

In the first years of the 1980s, and particularly after the localization of the "Huntington gene," it became obvious that the future lay in the molecular localization of genes,

called "reverse genetics" at the time, and that large extended families, such as family N were the tools for it. I tried to "sell" the family to molecular genetics laboratories *urbi et orbi* (Foncin and Salmon 1984, 1985; Foncin 1985), and we dedicated to it a full paper (Foncin et al. 1985) that concluded, "Detection of genetic markers constitutes a major objective. We think that familial Alzheimer's disease may constitute an interesting model for the understanding of sporadic Alzheimer's disease and senile dementia of the Alzheimer type." Nevertheless, the French molecular genetics community was uninterested. The only French organization to collaborate was the Institut National de Transfusion Sanguine (INTS, Professor Ch. Salmon), which helped in the collection of blood samples in France and Italy from family N members and from controls in the same population, and undertook immortalization of lymphocytes and DNA extraction. The interests and possibilities of INTS, however, were in immunology and hematology, and results were negative (Clémenceau et al. 1986; Muller et al. 1986), in spite of the *prima facie* evidence for a role of immune phenomena in AD (cf., the Fondation Ipsen 1987 meeting, "Immunology and Alzheimer's disease"). An intriguing result (unpublished) was the negative association of family N AD and thalassaemia. We understood it only later, when we found that family N originated in the mountains, whereas thalassaemia was endemic in the plains, due to its protective effect against malaria.

The same year, I participated in still another meeting to "peddle" "my" family (Foncin et al. 1985a). I met a gentleman, Dr. Peter Saint George-Hyslop perceived at once the opportunities provided by family N. He worked in Dr. Gusella's lab in Boston, closely link with NIH, and he organized a wide- ranging collaboration. All came together, as witnessed by the number of co-authors on the resulting publication (St. George- Hyslop et al. 1987a), which proposed a localization on chromosome 21. For a few months, I became sort of a celebrity in the French genetics and Alzheimer microcosms, with the friends who had cut my grant nine years before saying, "Ah! If we only had known…" I was further invited to present my work and my ideas at the second Fondation Ipsen "Alzheimer meeting" (Foncin et al. 1988).

In fact, the Science paper raised more questions than it answered. First, it postulated in its title that one genetic defect caused one nosological entity, namely FAD. Second, the "magic" lod score significant for linkage was obtained by adding data from several families, and family N (FAD4 in the paper), which contributed a large fraction of the final lod score, nevertheless showed a recombination between the two "positive" markers. Also, the chromosome 21 "FAD locus" was not linked with the amyloid beta protein gene (Tanzi et al., 1987b) or duplicated (St. George Hyslop et al. 1987b), contrary to what had been predicted from the early Alzheimer manifestations in chromosome 21 trisomics.

The quest for refinement of the localization of "the FAD gene" on chromosome 21 was on. P.St. George-Hyslop, now in Toronto, kept asking for new family N informative subjects; the goal, however, seemed ever further off. Finally (St. George Hyslop et al. 1990), the reason became evident: FAD (not to speak of AD in general) is not a single homogeneous disorder, and groups of subjects identical by descent had to be treated separately. Two years later, overwhelming evidence was found for a FAD locus in the middle of the long arm of chromosome 14 (St. George-Hyslop et al. 1992). Remarkably, family N (FAD4) gave a lod score of 5.21 at $\theta = 0.24$, strong evidence from this pedigree alone for linkage at this locus, named AD3. The contrast between this result and the

major contribution of the same pedigree to the 1987 chromosome 21 AD1 locus result remains unexplained.

In the meantime, Dr. Rainero in Professor Bergamini's department (Torino) had clinically diagnosed FAD in a patient originating in Calabria. He asked Dr. Bruni to investigate the family (Bergamini et al. 1989), which we called "family TO" (Tor1.1 in other papers). She found its cradle in a mountain village about 20 km from the one of family N. The phenotype, including age of manifestation and duration of illness, was identical in both families (identity of the neuropathology was later confirmed). Although we could not identify the common ancestor, who presumably lived before the establishment of church records, we felt confident that FAD patients in both families were identical by descent at the FAD locus (Bruni et al. 1990).

This finding proved important for the ultimate identification and cloning of the gene, mutations of which are causative of early onset FAD in a majority of pedigrees, including family N (Sherrington et al. 1995). To cut a long story short, the S192 gene (later called presenilin 1), mutated in FAD, is situated on a fragment characterized by a haplotype common to the N and TO families. Numerous mutations have been identified on that gene, with families N and TO sharing the M146L mutation. In my opinion, insufficient attention has been given to the correlation between the genotype and phenotype of patients carrying a presenilin 1 mutation, many reports being content with the mention that "the patients fulfilled so and so criteria for Alzheimer's disease." We reported (El Hachimi et al. 1996) a French family with a A163G presenilin 1 mutation. The corresponding phenotype was clinically evocative of both AD and Creutzfeldt-Jakob disease; neuropathology (an ultrastructural study and two autopsies) showed plaques and tangles (PHF) but also numerous microspongiosis bubbles. Immunohistochemistry revealed separate βA4 and PrP^{res} plaques. I think that this example shows that there is more than the control of βA4 metabolism in the role of presenilin.

Beyond molecular genetics and FAD?

The initiative now lies with people who explore and explain the role of the presenilin protein and its associates in the pathophysiology of AD and, more generally, in central nervous system physiology. Professor St. George-Hyslop's laboratory is very active in that direction. I feel, however, that formal genetics studies of large AD pedigrees may also produce interesting results. In a study in which Dr. Salmon and Dr. Montesi, a Ph.D. geneticist, played an important role (Bruni et al. 1992), we took as a phenotype index the age of manifestation of AD in family N. We found that the best fit for its repartition was a log-normal one. It was independent of environment and of education: an illiterate farm laborer in Calabria and a university graduate in America shared the same destiny. No influence of any "expression gene" could be detected, inasmuch as correlation of age of manifestation between affected child and affected parent was zero. The wide phenotype variation of AD among people identical by descent at the AD3 locus, as measured by the age of manifestation (SD 6.5 years), is purely stochastic, concordant with the log normal distribution of the best fit.

These results might be extended to "ordinary," late-onset AD. A "Gedankenexperiment" consisting of a 38-year shift to the right of the manifestation of AD observed in family N led, after correction for death from other causes before AD manifestation age,

to an estimation of late-onset AD epidemiology comparable to the observed one. Only one of five carriers of a putative AD mutation would manifest the disease, explaining the apparent "sporadic" character of late-onset AD. A colleague once said, "Common sense teaches us that late-onset AD is sporadic, not genetic." I answered, "Common sense teaches us that the sun revolves around the earth." The null hypothesis, in my opinion, is that AD instances are each caused by one stochastically manifested causal mutation[2] in one gene (multigenic hypothesis). There is little need for elaborate hypotheses involving a complex etiology with, in each instance, many feebly expressed mutations (polygenic hypothesis) combined with environmental influences.

My considered opinion is that the above null hypothesis could be extended to a number of so-called "complex diseases" for which circumstantial evidence points to a genetic factor, whereas all efforts to localise, let alone identify, a causal gene have failed, sometimes after initial apparent success. This situation is nowhere as evident as it is for psychiatric diseases. The present consensus classification of mental "trouble" is not a nosological one, that is, it cannot be congruent with an etiology-compatible classification as needed for phenotype grouping, causing an irremediable heterogeneity within groups. I cannot dwell on that subject; suffice to say that the root of this situation is the incompatibility with a quest for the genetic cause of disease, of the reigning positivist epistemology, which negates the "metaphysic" notion of cause and replaces it with empirical "associations."

All this, however, is no more popular nowadays than in the early 1970s, when we had the idea that pedigree tracing could, indirectly but decisively, contribute to the elucidation of the pathophysiology of Alzheimer's disease.

[2] « Le hasard et la nécessité » Jacques Monod

Genetics, molecular biology, and animal modeling of Alzheimer's disease

P.H. St George-Hyslop[1,2], *P.E. Fraser*[1], *D. Westaway*[1], *J. McLaurin*[1], *E. Rogaeva*[1], *G. Schmitt-Ulms, A. Tandon*[1], *H.T.J Mount*[1], *J. Robertson*[1], and *C. Bergeron*[1]

Our group at the Center for Research in Neurodegenerative Disease, at the University of Toronto, first became interested in several aspects of the biology of Alzheimer's disease (AD) during the early 1980s. The relative homogeneity of the clinical and neuropathological features of (AD) had led to the prevailing assumption that AD was likely to be a single homogeneous disorder. At that time, standard biochemical methods were being applied to dissect the protein composition of both the amyloid plaque and the neurofibrillary tangle (NFT). While these biochemical studies were on the edge of providing important clues to the biochemical pathogenesis of AD, mechanistic insights into the disease remained elusive. One notable exception was the observation that AD clustered in some families and was often inherited as an autosomal dominant trait. This observation alone, however, was insufficient to provide much traction. Indeed, some early attempts to define the chromosomal location of the disease genes using classical blood group antigens as genetic markers had been largely unfruitful. We were aware that individuals with Trisomy 21 had an increased incidence of an AD-like disease after the age of 40 years. Unfortunately, at that time, there were no useful polymorphic markers for genes on chromosome 21. However, we knew that phosphofructokinase-liver type (PFK-L), an enzyme encoded on chromosome 21 and involved in energy metabolism is decreased in AD brain tissue. In 1984, in collaboration with Donald Crapper-MacLachlan, we measured PFK-L activities in the brain of patients with AD but failed to uncover any difference in enzymatic activity. While this finding made variants in PFK-L unlikely to be causal for AD, it did not exclude the possibility that other genes on chromosome 21 might be associated with familial AD.

Fortuitously, in 1983, studies by Ray White, Mark Skolnick and David Botstein led to the discovery that restriction fragment length polymorphisms (RFLPs), arising from nucleotide sequence variations in genomic DNA, could be used as tools to map the chromosomal locations of disease traits. James Gusella, who had been a classmate of P.H. St George-Hyslop and had worked as a postdoctoral fellow of David Botstein, was proposing to use RFLPs to map the gene for Huntington's disease (HD). This approach led St George-Hyslop, in 1985, to initiate, in Gusella's lab, the genetic analysis of families with autosomal dominant familial AD, beginning with RFLP markers on chromosome 21. Gusella was already independently generating these markers to make a genetic map of chromosome 21. The details of the various interactions that followed over the next several years, and which resulted in the collection of several very large families in collaboration with Luigi Amaducci, Amalia Bruni, Jean-Francois Foncin, Peter Frommelt, Linda Nee, Lorenzo Pinessi, Ron Polinsky, Daniel Pollen, Innocenzo

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Rainero, Sandro Sorbi, and many others, are presented in the book "Hannah's Heirs" by Daniel Pollen (1993).

The initial genetic linkage studies conducted in 1985–1987 using RFLP markers from chromosome 21 enhanced the suspicion that there was a genetic susceptibility locus for AD on the long arm of chromosome 21 (St George-Hyslop et al. 1987a), in close proximity to the location of the amyloid precursor protein (APP) gene (Tanzi 1987a) (but see also below). Even more importantly, these studies immediately allowed a direct test of the hypothesis that AD is a single homogeneous disorder (St George-Hyslop et al. 1990). Specifically, the collaborative analysis of a large cohort of pedigrees assembled in conjunction with Jonathan Haines, John Hardy, Alison Goate, and Christine van Broeckhoven led to the clear demonstration that a subset of pedigrees was linked to a region of chromosome 21, at or near the APP gene. However, these genetic linkage analyses also unequivocally demonstrated that a larger subset of pedigrees was not associated with chromosome 21. The resulting conclusion of etiologic heterogeneity in AD, which is now so "mainstream" that it is taken for granted, has had a profound effect on all clinical and basic research studies in AD. Every clinical trial and every basic research study now inspects their results based upon some concept of heterogeneity (e.g., upon age-at-onset, the presence of absence of Apolipoprotein E ε4, etc.).

The discovery of non-allelic heterogeneity also had a very profound effect on the search for AD genes. Until that time, the obligatory assumption was that the disease was homogeneous, and therefore all genetic results had to be considered using a single locus model. This assumption had the confounding effect that positive linkage information from a subset of pedigrees linked to a given locus (e.g., to the APP on chromosome 21) would be obscured by negative genetic results arising from pedigrees with a genetic defect at another chromosomal location. However, upon the discovery of heterogeneity, it became appropriate to subgroup pedigrees according to some other a priori feature (e.g., age at onset) or to analyze single pedigrees individually. Concomitantly, in 1990, Christine van Broeckhoven and Blas Frangione reported that hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D) was due to a missense mutation in the Aβ domain of APP. These two sentinel observations led, in 1991, to a re-investigation of the APP gene for mutations just in the pedigrees showing linkage to chromosome 21. This research eventually culminated in the discovery of missense mutations in the APP gene in a handful of families with early onset familial AD (initially by Goate and Mullan, and by us and others; Karlinsky et al. 1992). Subsequently, three other lines of evidence have suggested that, in addition to these missense mutants, AD might also be associated with other non-coding nucleotide sequence variants in the APP gene. First, strongly positive linkage results for chromosome 21 markers near APP gene had been generated for several of our original large families in 1987. These families turned out to have no missense mutations in the APP gene but to have missense mutations in the PS1 gene as the predominant cause of AD. While this result was initially ascribed to an artifact, it now seems likely that co-segregation of single nucleotide polymorphisms (SNPs) in non-coding regulatory sequences in the APP gene of these families may also have contributed to some of the risk for AD and/or to some of the variation in the AD phenotype in these families. Second, positive genetic association results were generated for SNPs near APP in several sporadic AD:control cohorts. These results are also interpreted to reflect weak disease-associated/disease-

modifying variants in non-coding elements of APP. Finally, a small number of FAD pedigrees have duplication of the entire APP gene, resulting in its misexpression.

The final important effect of the discovery of non-allelic heterogeneity was that it facilitated attempts to clone the other AD genes. This led to the discovery, in 1993, of the association between AD and Apolipoprotein E by Allen Roses, together with members of this group (Saunders et al. 1993), and the discovery in 1995 of mutations in presenilin 1 (Sherrington et al. 1995) and presenilin 2 (Rogaev et al. 1995) by this group.

In addition to the purely genetic experiments, our group also played a key role in showing that the presenilin mutations caused an alteration in APP processing with the increased production of Aβ42 (Citron et al. 1997). This discovery, when coupled with similar observations about the effects of mutations in the APP gene, lent great weight to the focus upon Aβ as a primary player in the pathogenesis of AD that had arisen from the seminal observation in 1984, by George Glenner, that the Aβ fragment of APP accumulated in the brains of patients with Down's syndrome and AD. However, it was the discovery, by our group and by others, that pathogenic mutations in APP, PS1 and PS2 all altered APP processing, and all resulted in Aβ accumulation in the brain (Citron et al. 1997), that conclusively proved that the accumulation of Aβ was causal in the pathogenesis of AD.

Our group has also played a key role in characterizing the biology of the presenilin complex and its role in generating Aβ. In addition to cloning the initial two members of this family, we defined their membrane topology, we showed that the presenilins existed in biologically active high molecular weight complexes, and we isolated three additional components of these complexes. We showed that the loop domain of PS1 interacted with β-catenin (Levesque et al. 1999). We isolated nicastrin (Yu et al. 2000) and showed that it was likely to be the substrate-binding molecule of the presenilin complexes (Chen et al. 2001). We further showed that the presenilin complexes were necessary for γ-secretase activities (Donoviel et al. 1999) and that mutations in the presenilin complexes caused alterations in γ-secretase activity by increasing the production of Aβ42 (Citron et al. 1997). More recently we have identified TMP21 as a novel modulator of γ-secretase activity (Chen et al. 2006). Significantly, TMP21 suppresses γ-secretase-mediated production of Aβ but permits physiological ε-secretase cleavage, raising hope that it may be mimicked therapeutically.

In addition to these studies on the pathogenesis of AD, our group has also provided insights into the biophysics and assembly of Aβ into neurotoxic oligomeric assemblies. Our high-resolution structures of an Aβ amyloid fibril using magnetically aligned preparations of a central Aβ domain provided clues as to the mechanism of amyloid assembly and identified potential targets for controlling aggregation (Serpell et al. 2000a). We unequivocally demonstrated that the structural similarity that defines amyloid fibers exists principally at the level of β-sheet folding of the polypeptides within the protofilament, whereas the different types of amyloid vary in the supramolecular assembly of their protofilaments (Serpell et al. 2000b). We also identified cofactors, such as glycosaminoglycans, chemical chaperones and membrane lipids, that modulate this process. We were one of the first groups to dissect the interactions of Aβ with various lipid membranes (McLaurin et al., 1996, 1998) and the first to demonstrate in situ that Aβ disrupts membrane stability (Yip and McLaurin 2001). These observations suggest that the fibrillogenic properties of Aβ peptide are in part a consequence of membrane

composition, peptide sequence, and mode of assembly within the membrane. Furthermore, our elucidation of the mechanisms by which chemical chaperones control Aβ assembly has led to an interest in naturally occurring small molecules, such as Hsp, as amyloid modulators (Yang et al., 1999).

Finally, we used our knowledge of the genetics of AD to generate robust animal models of AD, such as the TgCRND8 mouse (Janus 2000). This model, based upon a double mutant APP transgene, develops profound amyloid-based neuropathology, synaptic loss, significant microglial and astrocytic inflammation, defects in spatial learning and memory, and increased mortality. This mouse model, which has been shared with many investigators, has been invaluable in the preclinical evaluation of several potential therapeutics.

Since total brain Aβ levels were not perturbed by clinically effective immunization, we inferred the existence in the brain of TgCRND8 mice of sub-varieties of Aβ with differential biological effects (Janus 2000). This deduction, and the suspicion that Aβ oligomer assemblies might be important in the pathogenesis of AD, led us to characterize the functional epitope recognized by Aβ42-directed antibodies (amino acids 4-10) that are therapeutically effective as vaccines (Janus 2000). We showed that these vaccine- induced, anti-Aβ antibodies specifically recognize Aβ oligomers and fibrils in tissue sections but not monomeric and diffuse Aβ deposits, an observation subsequently deployed by others as the TAPIR assay (McLaurin et al. 2002). Finally, we demonstrated that these antibodies have an anti-aggregant effect, disrupting neurotoxic Aβ oligomer and fibril formation (McLaurin et al. 2002). The recognition of the significance of Aβ oligomers arising from this and other studies was the basis for our discovery of a small molecule inhibitor of Aβ aggregation. One such compound, scyllo-cyclohexanehexol, effectively inhibits several Aβ-induced, AD-like phenotypes (including cognitive and memory impairment, cerebral amyloid deposition, gliosis, synaptic loss, and accelerated mortality) in the TgCRND8 model of AD (McLaurin et al. 2006). This compound, which has high oral and CNS bioavailability, will shortly enter therapeutic trials in AD.

In summary, between 1985 and 2006, our group made a series of fundamental discoveries that have been instrumental in forming the present mechanistic understanding of AD and in providing the basis for several different therapeutic targets currently being investigated clinically (e.g., γ-secretase inhibitors, Aβ anti-aggregant compounds, Aβ vaccines). However, there is still great uncertainty about how Aβ accumulation leads to the generation of tau-positive NFTs and ultimately to neuronal death. There is still uncertainty as to the identity of the remaining AD-causing genes (the four known genes account for only about one half of the genetic risk), and there is also little robust knowledge about environmental factors that increase risk for AD. Finally, the hypothesis that AD is initiated by the accumulation of toxic oligomeric species of Aβ remains just that – a hypothesis. Although widely held, this hypothesis will only be fully validated by the observation of prevention or cessation of disease activity by anti-Aβ therapies in human clinical trials. Until that has been achieved, the research to address these gaps must remain fully active.

Christine Van Broeckhoven

The genetic Alzheimer-frontotemporal dementia spectrum

Christine van Broeckhoven[1]

Neurodegenerative brain diseases, including dementias, are common diseases among elderly people, and their population frequency is increasing rapidly because people are living longer due to high quality health care systems. Predictions indicate that in 2030 at least one in four people in Western European countries will be 65 years or older. In this age group, people are at high risk for neurodegenerative dementias such as Alzheimer's disease (AD), with risk increasing with age to as high as 20% for those 85 years. Neurodegenerative brain diseases or cerebral prote(in)opathies have in common the presence of inclusion bodies containing abnormal protein aggregates. These protein depositions in specified brain regions occur within neurons or in the brain parenchyma and are commonly used as pathological hallmarks in morphological diagnosis of demented patients at autopsy. In AD, the major protein aggregates are found in parenchymal senile plaques and intraneuronal neurofibrillary tangles. The major constituents of these pathological protein aggregates have been identified for most neurodegenerative brain diseases, and the cerebral proteopathy is often nicknamed accordingly, e.g., tauopathy in cases of tau aggregates, as in frontotemporal dementia (FTD; Rademakers et al. 2004). In AD, the tauopathy consists of cytoplasmic neurofibrillary tangles and is always linked to the presence of β amyloid (Aβ) in senile plaques. The Aβ can also be found without concurrent tauopathy in patients with cerebral amyloid angiopathy (CAA), such as Dutch amyloidosis or hereditary cerebral hemorrhages with amyloidosis Dutch type (HCHWAD; Maat-Schieman et al. 2005; Van Broeckhoven et al. 1990). These observations suggest that Aβ and tau are part of a cerebral proteopathy spectrum ranging from CAA through AD to FTD, linking these dementia phenotypes to a common molecular pathway of neurodegeneration (Dermaut et al. 2005).

AD is the most common subtype of neurodegenerative dementias, affecting nearly 70% of dementia patients, followed by FTD, which in the age group below 65 years comprises 12–20% of patients (Dermaut and Van Broeckhoven 2002). They are multifactorial diseases with both genetic and environmental factors contributing to expression of disease. Genetic factors are strongest in young patients, and in this group one can observe families in which the disease is inherited from generation to generation as an autosomal dominant trait (Martin et al. 1991; Cruts and Van Broeckhoven 1998a). Since the beginning of the 1980s, these families have been the subjects of molecular genetic studies aiming at identifying the underlying disease genes using the positional cloning

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strategy. For AD, this approach resulted in the identification of three genes coding for the amyloid precursor protein (APP) and the presenilin 1 (PSEN1) and 2 (PSEN2) genes (Goate et al. 1991; Sherrington et al. 1995; Levy-Lahad et al. 1995a) and, for FTD, in the microtubule associated protein tau gene (MAPT; Hutton et al. 1998). Mutations in APP were also identified in Dutch amyloidosis (Van Broeckhoven et al. 1990; Levy et al. 1990; Bakker et al. 1991). Retrospectively, it is interesting that APP and MAPT are coding for the culprit protein underlying the proteopathies of AD, CAA (amyloidosis) and FTD (tauopathy). Mutations in these genes have been identified in families worldwide; however, their overall number and relative contribution to risk of disease are very low. In families segregating these mutations, the patients are at high risk, since most of these mutations are highly penetrant and can be used for molecular diagnostic testing. Nevertheless, the identifications of the inclusion proteins and subsequent mutations in their genes in dementia patients have been milestones in dementia research, since they provided entry points to the underlying biological pathways. It is now generally accepted that, because of the mutation, the protein changes configuration, making it more prone to aggregation and deposition, leading to the formation of the characteristic pathological lesions such as senile plaques in AD and tau aggregates in FTD. The fundamental basis for the origin and formation of the protein aggregates still remains largely unknown. Nevertheless, much research is being conducted aiming at developing novel therapeutic strategies based on prevention, degradation or clearance of these protein aggregates from brain.

Only $\leq$ 1% of AD patients have early-onset (< 65 years) of disease, and around 60% of these patients have a positive family history with at least one first-degree relative demented (Ott et al. 1995; van Duijn et al. 1991). Approximately 10% of familial early-onset patients have autosomal dominant inheritance of AD, with a mean onset age that is characteristic for an individual family (Martin et al. 1991). To date, 190 different mutations in APP (N = 25; 17.6%), PSEN1 (N = 155; 78%) and PSEN2 (N = 10; 4.5%) together explain AD in 404 families worldwide (Cruts and Van Broeckhoven 1998b; AD&FTD Mutation Database http://www.molgen.ua.ac.be/ADMutations). All mutation carriers presented with classical AD with abundant senile plaques and neurofibrillary tangles. In a Dutch population-based study of early-onset AD, mutations in these three genes explained 5% overall, 10% of familial and 20% of autosomal dominant early-onset AD (van Duijn et al. 1991; Cruts et al. 1998), indicating that other genes were yet to be found for early-onset AD. We recently reported a novel locus in a Dutch early-onset AD family at 7q36, but the underlying gene remains to be found (Rademakers et al. 2005). The majority of mutations are missense mutations, except in the case of APP, where duplications have recently been reported in 8–10% of autosomal dominant AD families (Rovelet-Lecrux et al. 2006; Sleegers et al., in press). Mutations have also been identified in the APP proximal promoter that increased APP transcriptional activity nearly two-fold (Theuns et al. 2006; Brouwers et al., in press). Mutations in APP, PSEN1 and PSEN2 consistently elevate the Aβ42/Aβ40 ratio, with Aβ42 having an increased propensity to aggregate and deposit in senile plaques (Suzuki et al. 1994; Scheuner et al. 1996; De Jonghe et al. 1998; Kumar-Singh et al. 2006b), making APP processing central in the etiology of AD. While APP mutations cause AD by a Aβ42-driven gain-of-function, mutations in PSEN have recently been shown to produce a loss-of-function of γ-secretase activity (Bentahir et al. 2006; Kumar-Singh et al. 2006b). PSENs harbor the catalytic site of the γ-secretase complex that cleaves APP just outside the C-terminal site

of Aβ (Marjaux et al. 2004). The loss-of-function hypothesis is in line with our previous genetic studies identifying promoter variants in PSEN1 that decreased expression and consequently increased risk for early-onset AD (van Duijn et al. 1999; Theuns et al. 2000, 2003).

AD mutations have been identified in APP and PSEN that are associated with a very strong CAA component (Hendriks et al. 1992; Dermaut et al. 2001). The Flemish APP692 mutation, Ala692Gly, located within the Aβ sequence close to the α-secretase site, was identified in a Dutch family that presented clinically with either hemorrhages or dementia as first symptoms (Hendriks et al. 1992) and showed pathologically an extensive Aβ load in both cored plaques and vessel walls (Cras et al. 1998; Kumar-Singh et al. 2002). Our studies of Flemish AD indicated that the altered biological properties of Flemish APP and Aβ (De Jonghe et al. 1998) facilitated progressive Aβ deposition in vascular walls, causing strokes and the formation of dense-core senile plaques (Kumar-Singh et al. 2002). Of interest is that we observed the same degree of CAA as in Flemish APP692 in AD patients carrying the PSEN1 Leu282Val mutation and presenting with typical AD without strokes or stroke-like episodes (Dermaut et al. 2001). Together, these data suggest that, like the dense-cored neuritic plaques, CAA might represent a pathogenic lesion that contributes significantly to the progressive neurodegeneration occurring in AD.

A positive family history of dementia is present in 38–50% of FTD patients, and in the majority of FTD families the disease is inherited in an autosomal dominant manner. Genetic studies have identified mutations in MAPT in families linked to 17q21 (Hutton et al. 1998). To date, 40 different MAPT mutations have been identified in 113 dementia families worldwide (Rademakers et al. 2004; AD&FTD Mutation Database: http://www.molgen.ua.ac.be/ADMutations). MAPT mutations are missense mutations mainly affecting microtubule binding domains or splice site mutations enhancing exon 10 splicing and resulting in abnormal preponderance of 4-repeat over 3-repeat tau. It has been estimated that MAPT mutations explain 5 to 20% of FTD in general, and 10 to 43% of familial FTD. Neuropathologically, MAPT mutation carriers are characterized by intraneuronal and/or glial tau-positive inclusions (tauopathy) ranging from AD-like neurofibrillary tangles to ovoid Pick bodies, the pathological hallmark of Pick's disease (Lee et al. 2001). Some mutations in MAPT, like Arg406Trp, cause hereditary tauopathy though presenting clinically with AD (Rademakers et al. 2003). Interestingly, PSEN1 mutations have also been associated with familial FTD (Raux et al. 2000; Tang-Wai et al. 2002; Dermaut et al. 2004). The mutation we reported, Gly183Val, was observed in a familial FTD patient with Pick-type tauopathy in the absence of extracellular -amyloid deposits. The functional details of the pathogenic role of PSEN1 mutations in FTD remain obscure; however, in vitro study of another FTD-related PSEN1 mutation, insArg352 (Tang-Wai et al. 2002), has shown that it behaves as a loss-of-function mutation because of its inability to process APP into Aβ peptides (Amtul et al. 2002).

More recent genetic and clinicopathologic studies, however, demonstrated that the majority of FTD patients could not be explained by MAPT mutations and lacked tau pathology (tau-negative FTD). Surprisingly, several tau-negative FTD families showed conclusive linkage to the same region at 17q21 that contains MAPT (Rademakers et al. 2004). The neuropathology in these families has been described as either "dementia lacking distinctive histopathology" (DLDH; Lendon et al. 1998) or "FTD with tau negative and ubiquitin positive inclusions" (FTDU; Rosso et al. 2001; Rademakers et al.

2002; van der Zee et al. 2006; Mackenzie et al. 2006; Pirici et al. 2006). In one Dutch family, 1083, we reduced the candidate region for FTDU to a 4.8 cM interval (Rademakers et al. 2002). We excluded mutations in MAPT by genomic resequencing of 138.5 kb in 17q21-linked FTDU patients (Cruts et al. 2005) as well as complex genomic rearrangements in the MAPT region using stretched chromosome FISH (Gijselinck et al. 2006). All together, these data suggested that FTDU linked to 17q21 was independent of MAPT and most likely resulted from mutations in another gene within the linked chromosomal region at 17q21. Subsequent screening of candidate genes identified null allele mutations in the gene coding for progranulin (PGRN) that lead to partial loss of PGRN protein (Baker et al. 2006a; Cruts et al. 2006a). These data indicated that PGRN growth factor activity has an important role in neuronal survival in FTD, however, the exact disease mechanism remains to be elucidated. Of interest though is that in our hands mutations in PGRN were a more frequent cause of FTD than MAPT mutations underpinning an important role for PGRN in FTD. (Gijselinck et al. 2006). All together, these data suggest that FTDU linked to 17q21 is independent of MAPT and most likely results from mutations in another gene within the region.

In conclusion, careful genotype-proteotype-phenotype correlative studies, including molecular genetic, biochemical, neuropathological and clinical investigations of inherited early-onset forms of AD and FTD, have been instrumental in defining the complete phenotypic spectrum associated with APP, PSEN and MAPT mutations and have significantly advanced our biological understanding of these diseases (Dermaut et al. 2005). Also, recent findings showed that AD and FTD not only share important clinical and neuropathological features but are also etiologically linked at the molecular genetic level, implying that these disorders are part of a genetically interconnected spectrum of neurodegenerative brain disorders.

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Blas Frangione

Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. The role of amyloid in dementia and stroke

Blas Frangione[1] and *Jorge Ghiso*[1]

The first cerebral amyloid molecule identified was extracted in 1983 from cerebral blood vessels obtained at autopsy from Icelandic patients who died from massive brain hemorrhages due to the deposition of cystatin C fibrils in the vessel walls (Cohen et al. 1983), a condition referred to as hereditary cerebral hemorrhage with amyloidosis (HCHWA). The importance of this study extended beyond the identification of a molecule linked to a cerebral amyloid disease: it described a novel and simple method to extract amyloid from leptomeningeal vessels, a protocol that a year later was used by G. Glenner to isolate Aβ from leptomeninges obtained from AD brains (Glenner and Wong 1984a) and has lately provided the basis to establish the relationship between amino acid substitutions and familial cerebral amyloidosis. Complete amino acid sequence analysis of the deposited cystatin C in the Icelandic cases of HCHWA revealed the first mutation associated with cerebral amyloidosis, the replacement of leucine for glutamine (Ghiso et al. 1986) due to a single A to T transversion at codon 68 (Levy et al. 1989).

In 1985, shortly after the initial cystatin C and Aβ reports, we received three brains from familial cases in Holland exhibiting clinico-pathological features that closely resembled the Icelandic cases, e.g., recurrent episodes of cerebral hemorrhages associated with overwhelming cerebral amyloid angiopathy (CAA), features that suggested the name HCHWA-Dutch type to designate the disease. Notably, amyloid deposits were not recognized by antibodies to cystatin C but were immunoreactive with antibodies to Aβ (Fig. 1a). Whereas amino acid sequence analysis of the extracted leptomeningeal material corroborated its Aβ identity (Fig. 1e), immunohistochemical studies verified the co-existence of CAA with widespread parenchymal pre-amyloid (non-fibrillar, Congo red negative) deposits in the absence of neuritic plaques and neurofibrillary tangles. We postulated that HCHWA-Dutch type is a vascular variant of AD (van Duinen et al. 1987). Further studies revealed a point mutation at codon 693 of the APP gene, a single nucleotide transversion (G for C) resulting in the replacement of glutamate for glutamine at position 22 of the Aβ sequence (Kang et al. 1987; Levy et al. 1990; Prelli et al. 1990) and a tight linkage of the APP gene with the disease detected by restriction fragment length polymorphism (Van Broeckhoven et al. 1990). The studies with the Dutch mutation helped to establish the existence of shorter Aβ species in vascular deposits (Prelli et al. 1988), to ascertain that wild-type and mutant APP can have different phenotypic presentation and to pave the way for the discovery of the many APP nucleotide substitutions to come (described in Goate's Chapter). In addition,

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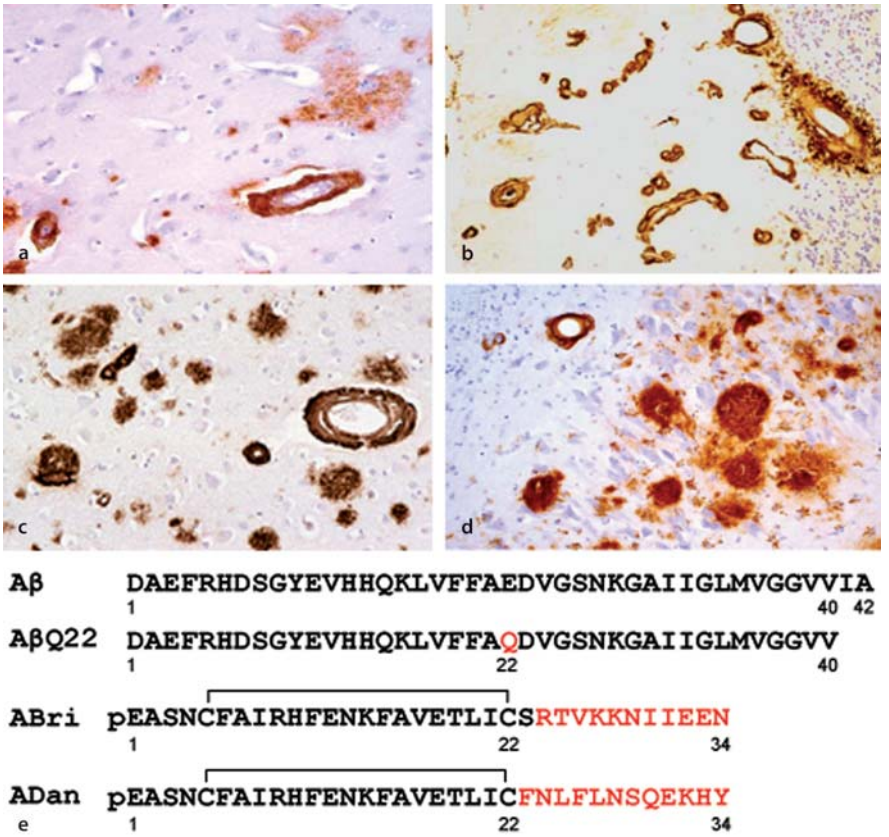

Fig. 1. Immunohistochemical analysis of brain lesions in HCHWA-Dutch type (anti-Aβ antibody 4G8; **panel a**), FDD (anti-ADan antibody 5282; **panel b**), AD (anti-Aβ antibody 4G8; **panel c**), and FBD (anti-ABri antibody 338, **panel d**). The sequence alignment of mutated and wild type Aβ as well as ABri and ADan is shown in **panel e**

Wisniewski et al. (1991) showed that the presence of the Dutch mutation accelerated the rate of amyloid formation by using the methodology applied to our earlier studies, which demonstrated that synthetic Aβ homologues can spontaneously assemble into amyloid-like fibrils in vitro (Castano et al. 1986). Recently, neuronal overexpression of the Dutch mutation in mice has been shown to cause extensive CAA, smooth muscle cell degeneration, hemorrhages and neuroinflammation, making the mouse model the first to develop robust CAA in the absence of parenchymal amyloid, highlighting the key role of neuronally produced Aβ to vascular amyloid pathology and emphasizing the differing roles of Aβ40 and Aβ42 in vascular and parenchymal amyloid pathology (Herzig et al. 2004).

In 1992, two groups independently reported that a soluble form of Aβ, identical in primary structure to the deposited Aβ, is secreted by cells in culture and is normally present in plasma and cerebrospinal fluid (Seubert et al. 1992; Shoji et al. 1992).

The finding of soluble Aβ species in biological fluids prompted the use of synthetic homologues to test 1) their potential as neurotoxic agents, 2) their transport to and from the brain across the blood-brain barrier, 3) the existence of possible carrier molecules that maintained Aβ in solution in biological fluids and 4) their efficacy as therapeutic agents. Employing differentiated neurons in culture and a fragment of Aβ at high concentration, the neurotoxic potential of these peptides was initially established (Yankner et al. 1990). Using in vivo models of brain perfusion, both uptake (Zlokovic et al. 1993) and clearance mechanisms (Shibata et al. 2000) for Aβ at the blood-brain barrier level were unveiled. It was further demonstrated that the AβQ22 Dutch mutant exhibits reduced clearance from the central nervous system into the bloodstream, with about 50% of the molecules being retained by the vessel wall, indicating the vasculotropic nature of the Dutch mutation (Monro et al. 2002) and emphasizing the importance of clearance. In a separated set of experiments, apolipoproteins E and J were identified as major binding partners of Aβ (Wisniewski and Frangione 1992; Ghiso et al. 1993). ApoE4, now recognized as a risk factor for AD (Corder et al. 1993), promotes amyloidogenesis in vitro (Wisniewski et al. 1993; Ma et al. 1994) and in vivo (Holtzman et al. 2000b). Aβ42 or smaller fragments bearing either wild type or modified amino acid sequence have been used as inhibitors of amyloidosis (Soto et al. 1998) or as antigens for vaccination purposes (Schenk et al. 1999; Sigurdsson et al. 2001).

The relationship between amyloid and neurodegeneration has been emphasized by the discovery of two non-Aβ cerebral amyloidosis: familial British and Danish dementias (FBD and FDD, respectively). These disorders share many aspects of AD, including the presence of neurofibrillary tangles, prominent parenchymal pre-amyloid lesions and few amyloid plaques, overwhelming CAA, and a widespread inflammatory response, including complement activation (Rostagno et al. 2003). We carried out the isolation and identification of amyloid deposits in brain tissue samples from the British and Danish kindred (Fig. 1, d and b, respectively). As a result, two new 34-residue amyloid molecules were identified, ABri in FBD (Vidal et al. 1999) and ADan in FDD (Vidal et al. 2000), both related to the same precursor protein BRI2 located on chromosome 13 and bearing specific genetic defects at or near the stop codon that allow the translation of otherwise non-coding 3′ regions (Fig. 1e, lower panel). ABri and ADan, both generated by furin-like proteolytic processing (Kim et al. 1999), represent the first examples of de novo-created amyloid molecules as a result of defects at the stop codon that create elongated precursors. Both molecules share absolutely no homology with Aβ and yet, the pattern of hyperphosphorylated tau immunoreactivity in paired-helical filaments bearing neurons is identical for AD, FBD and FDD (Table 1, Fig. 1), strongly indicating that different amyloid peptides can trigger similar pathological pathways, resulting in neuronal loss and clinical dementia. These disorders also challenge the importance of amyloid plaques in the mechanism of cell toxicity: plaques are absent in FDD and in many brain areas in FBD but both diseases overwhelmingly feature parenchymal pre-amyloid lesions as well as vascular and perivascular deposits, including heavy capillary involvement. Taking into consideration the differences in solubility between amyloid plaques and pre-amyloid deposits, it is possible that the latter reflect richness in oligomers/protofibrils that may trigger neuronal toxicity. We propose that FBD/FDD are suitable models to study early steps in peptide oligomerization/fibrillization, as well as the role of pre-amyloid and vascular/perivascular deposits in the process of

Table 1. Comparison of the clinical phenotypes and the frequency of neuropathological lesions in AD, HCHWA-Dutch type, FBD and FDD

	AD	HCHWA-D	FBD	FDD
CAA	++	+ + +	+ + +	+ + +
Diffuse plaques	++	+	+ + +	+ + +
Mature plaques	+ + +	−	+	−
PHF	+ + +	−	+ + +	+ + +
Clinical phenotype	dementia	stroke-dementia	dementia	dementia

neurodegeneration and dementia (reviewed in Ghiso et al. 2006). New evidence shows that Aβ, ABri, and ADan are able to form morphologically compatible ion-channel-like structures and elicit single ion-channel currents in reconstituted lipid membranes (Quist et al. 2005).

The studies described above suggest the likelihood that the relationship between amyloid and neuronal cell death does not solely depend on the primary structure of the amyloid subunit or in the length of the peptide but on altered conformation/aggregation of the immediate soluble precursors and the presence of factors that promote and maintain altered conformations and inhibit normal catabolic pathways and/or clearance mechanisms. Alternatively, it could be postulated that amyloidogenesis is a secondary event and that the existence of mutations that lead to early onset forms of dementia and/or stroke inherited as autosomal dominant traits simply reflects the role of these variants in rendering accelerated protein polymerization. However, the existence of the BRI2 genetic defects leading to the de novo formation of ABri and ADan provides strong support for a primary role for amyloid in the mechanisms of dementia and cerebral hemorrhages/ischemic strokes.

Christian Haass

Physiological generation of Amyloid β-peptide and its Consequences for Alzheimer's disease research

Christian Haass[1]

Introduction

It is now 100 years since that the first victim with severe dementia associated with neuronal cell death was presented by Alois Alzheimer. At that time such cases were very unusual. Now Alzheimer's disease (AD) is a very common disorder, which affects millions worldwide. In Germany alone, about 1.2 million patients suffer from this devastating disorder. During the last decades, a sharp increase in the numbers of affected persons has been observed and still continues; according to recent statistical predictions, the numbers will become very dramatic in the near future. This increase is due to the enormous increase in our life expectancy. Work on the molecular mechanisms of AD from my laboratory and many others now strongly indicates that AD is an age-dependent syndrome that will affect almost all of us if we live long enough.

Identification of amyloidogenic APP fragments

While writing my PhD thesis with Konrad Beyreuther as a co-adviser and collaborator, I became very much interested in AD and its molecular background. At that time, we did not know very much about amyloid β-peptide (Aβ) generation. The gene encoding β-amyloid precursor protein (APP) had just been cloned (Kang et al. 1987) and the initial evidence suggested that Aβ must be generated by endoproteolysis of APP (Weidemann et al. 1989). However, the common belief was that Aβ could only be produced under pathological conditions, since the sequence of the Aβ peptide was found to end right in the middle of the putative trans-membrane domain of APP. Within that hydrophobic environment, such proteolytic cleavages were thought to be impossible, since proteases require water molecules for their catalytic activity. Consequently, it was thought that APP was only released from damaged neurons and subsequently cleaved by unknown proteases under pathological conditions. This dogma also suggested that we would never have any simple tissue culture systems available to study Aβ generation.

When I joined Dennis Selkoe's laboratory at the Center for Neurologic Diseases at the Harvard Medical School in Boston, I became strongly interested in the cellular trafficking of APP, since Dennis and I started to believe that at least some steps of amyloidogenic APP processing could occur within protease-rich compartments in

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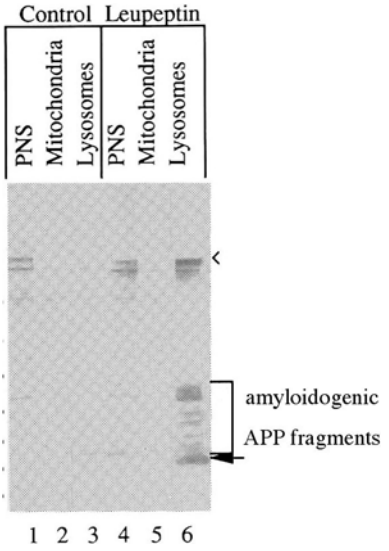

Fig. 1. Original figure (Haass et al. 1992a) demonstrating the identification of amyloidogenic fragments of APP within endosomes/lysosomes. Fractions were immunoblotted with an antibody to the C-terminus of APP. Arrowhead: full-length mature APP; arrow: α-secretase-generated APP C-terminal fragment

living cells. I was therefore investigating whether APP could be targeted to late endosomes. Indeed, we found that APP could be endocytozed from the plasma membrane and targeted to endosomes. This finding led me to isolate endosomes/lysosomes from cells, which I treated with inhibitors of lysosomal proteases to enrich for amyloidogenic APP fragments. Immunoblotting such isolated fractions with antibodies to the C-terminus of APP revealed a very surprising result. I found C-terminal fragments of APP that, based on their molecular mass, could contain the entire Aβ sequence but lack the ectodomain (Haass et al. 1992b; Fig. 1). Such fragments could very well represent amyloidogenic precursors, which upon one an additional intramembranous cut could lead to the generation of Aβ (Haass and Selkoe 1993). In parallel, Todd Golde in Steve Younkin's laboratory made a very similar observation (Golde et al. 1992).

Physiological generation of Aβ

Based on this finding, Dennis and I began to believe that Aβ might be generated in a physiological pathway - at that time, a most provocative idea. But how could one study Aβ generation with the limited technology available then? I thought the best approach would be to stably transfect human cells with an APP cDNA to achieve high expression. Then one should be able to metabolically label these cells for extended time periods to allow collection of sufficient amounts of Aβ. Based on our hypothesis, we expected to find radioactive Aβ in the conditioned media, from which it could be immunoprecipitated. This type of experiment worked immediately at the first shot! A 4-kDa and a 3-kDa peptide were specifically precipitated from the media (Haass et al. 1992b; Fig. 2a).

We then had to prove that Aβ, with its authentic N- and C-terminus as observed in vivo, was produced by our cells. One should remember that sensitive methods such as mass spectrometry were not available at this time. However, together with

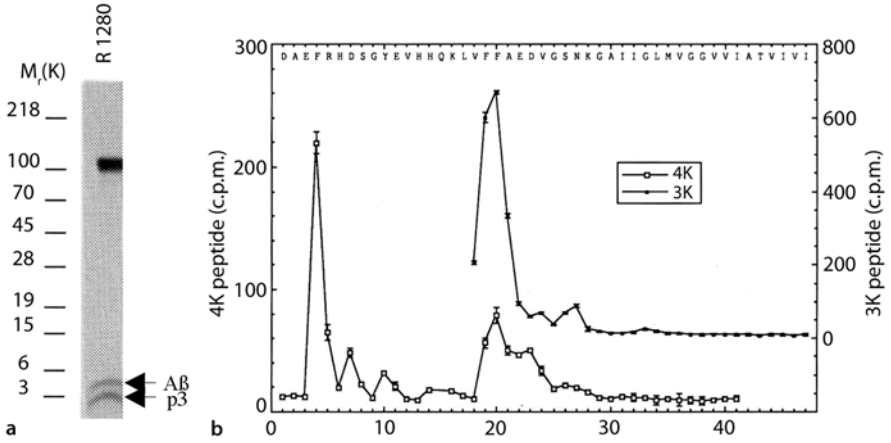

Fig. 2. Original figure (Haass et al. 1992b) demonstrating the identification of soluble Aβ in conditioned media from HEK 293 cells transfected with APP. (**a**) Immunoprecipitation of conditioned media from APP transfected cells labeled with ^{35}S-Met using antibody R1280 against $A\beta_{1-40}$. (**b**) Radisosequencing of Aβ in vivo labeled with ^{3}H-phenylalanine

David Teplow, we developed a radio-sequencing protocol for Aβ. We chose to label cells with ^{3}H-phenylalanine, since this amino acid occurred at positions 4, 19 and 20. Thus automated Edman-degradation should be possible to led to unequivocal results. Moreover, due to the relatively high specific activity of ^{3}H-phenylalanine, we believed it to be sensitive enough to sequence rather limited amounts of Aβ. This approach was so well designed by David Teplow that he was even able to predict the expected counts in the respective cycles. I could not believe my eyes when I saw the counts from our very first radio-sequencing experiment (Fig. 2b). They almost perfectly matched David's predictions and fully proved that indeed Aβ was produced, against all predictions via a completely physiological pathway not involving any membrane damage. Radiosequencing also led to a second surprise. The 3-kDa peptide (p3; (Haass et al. 1993b) was identified as a N-terminally truncated peptide starting at the α-secretase site, which had just been discovered (Esch et al. 1990).

We were not the only ones who found physiological production of Aβ. Similar findings were made in parallel by Younkin, Seubert, and Yankner (Busciglio et al. 1993; Seubert et al. 1992; Shoji et al. 1992).

The consequences for AD research

We immediately knew that this was a milestone experiment in AD research. It not only provided a novel concept for Aβ generation but it also offered immediately the possibility to tackle some of the most important question in AD research. These included:

1. identifying the cellular pathway involved in Aβ generation;
2. identifying the molecular mechanisms of familial AD (FAD) associated mutations;

3. identifying the proteases (secretases) involved in Aβ generation; and
4. screening for inhibitors and modulators of Aβ generation for therapeutic treatment.

Not surprisingly, all these questions have now been successfully addressed and have even resulted in the generation of prototype drugs that are being investigated in human trials. Trafficking pathways of APP have been described, including the definition of the precise cellular localization of β- and γ-secretase activity within endosomes and at the plasma membrane (Haass 2004; Kaether et al. 2002, 2006).

Martin Citron in Dennis Selkoe's laboratory found that the Swedish mutation at the N-terminus of the Aβ domain strongly increases the β-secretase cleavage and consequently Aβ generation (Citron et al. 1992). This finding was not only the very first identification of a FAD-associated mechanism but also provided strong evidence for the amyloid hypothesis (Haass 2004). Moreover, shortly thereafter I was able to demonstrate that the Swedish mutation shifts the cellular site of β-secretase processing to an earlier compartment (from endosomes to the trans-Golgi), which has some deadly consequences (Haass et al. 1995). This shift allows β-secretase to successfully compete with α-secretase and to strongly increase the rate of Aβ generation. Our initial finding of secreted Aβ also allowed the identification of $A\beta_{42}$, which is now known to be the major toxic player and to be increased by APP and presenilin mutations (Citron et al. 1997).

It is also not surprising that β-secretase was identified using the exact same cell line originally generated for the analysis of Aβ production. Martin Citron, at the time at Amgen Inc., was able to identify β-secretase as a membrane-bound aspartyl protease with the help of an expression cloning approach using Aβ secretion as a readout (Vassar et al. 1999). Very recently, we idntified the biological function of β-secretase. Surprisingly, β-secretase is involved in a signaling pathway regulation myelination.

The identification of γ-secretase turned out to be tremendously difficult. However, the demonstration that $A\beta_{42}$ is increased by all presenilin (PS) mutations investigated (Citron et al. 1997), the absolute requirement of PS for Aβ generation (De Strooper et al. 1998), and the identification of the two critical aspartate residues within transmembrane domain 6 and 7 of presenilins (Wolfe et al. 1999) strongly suggested a pivotal role of PS for γ-secretase activity. However, we found that γ-secretase exists as a high molecular weight complex (Capell et al. 1998). Thus additional co-factors had to be identified before it was possible to investigate the nature of γ-secretase (Francis et al. 2002). One of my biggest dreams was fulfilled when Dieter Edbauer and Harald Steiner in my laboratory were able to reconstitute γ-secretase activity and to demonstrate that a complex composed of PS1 or PS2, Aph-1, Pen-2 and Nicastrin is necessary and sufficient to produce Aβ (Edbauer et al. 2003). Once again, we used physiological production of Aβ as a readout for successful reconstitution.

Our work on γ-secretase also led to identification of a completely new family of intramembrane cleaving aspartyl proteases (Haass and Steiner 2002). We called this family the GxGD-type of proteases, based on the importance of this domain for the catalytic activity of PS and consequently for Aβ generation (Steiner et al. 2000). Furthermore, very recently this concept was proven by the identification of the same domain in the signal peptide peptidases and their homologues (Krawitz et al. 2005; Weihofen et al. 2002). Moreover, we could even demonstrate that probably all GxGD proteases use similar multiple intramembrane cleavages to release their products (Fluhrer et al. 2006).

Consequences for therapeutic treatment

Finally, one must emphasize that secretase inhibitors and modulators were screened using physiological Aβ secretion as a readout. This procedure may lead soon to the development of the first successful drugs able to lower the Aβ burden in AD patients, a dream that I thought was absolutely unrealistic when I entered the field in 1990. Thus the finding of physiological secretion of Aβ is strongly influencing AD research until today. Without that finding, we would still be far away from understanding and treating AD.

Acknowledgements. I want to thank my mentors and friends, Dennis Selkoe and Konrad Beyreuther, for their great support throughout these many years. I also want to thank the many talented co-workers in my laboratory for their tremendous contributions.

Falk Fahrenholz

Activation of α-secretase as an approach for treatment of Alzheimer's disease

Falk Fahrenholz[1]

Introduction – The non-amyloidogenic α-secretase pathway

In the non-amyloidogenic pathway, the amyloid precursor protein (APP) is cleaved by an α-secretase within the Aβ sequence, thus precluding Aβ peptide generation. Following α-secretase cleavage, the C-terminal APP fragment undergoes γ-cleavage, leading to the generation of the p3 peptide, which is generally not found in the amyloid plaques characteristic for Alzheimer's disease (AD). The α-secretase cleavage releases the N-terminal ectodomain of APP (APPsα), which has neurotrophic and neuroprotective properties. Therefore, activation of the non-amyloidogenic pathway provides a logical alternative strategy to β- or γ-secretase inhibition for treatment of AD. Although cleavage by α-secretase was the first proteolytic pathway of APP to be characterized in detail, this idea remained almost forgotten as long as the α-secretase had not been identified.

Identification of ADAM10 as an α-secretase in vitro and in cultured cells

The cleavage of APP by α-secretases was first described in 1990 (Sisodia 1992; Esch et al. 1990; Pasternack; et al. 1992). Studies in various cell types showed that the major α-secretase cleavage site is between lysine-16 and leucine-17 in the Aβ domain (Esch et al. 1990). The principal determinants of APP cleavage by α-secretase appear to be the distance of the hydrolyzed bond from the membrane (12 to 13 residues) and a local helical conformation (Sisodia 1992). A reduction in α-secretase-cleaved APP was evident in the cerebrospinal fluid of AD patients (Lannfelt et al. 1995).

Besides APP, many other transmembrane proteins can undergo proteolytic cleavage and release of their extracellular domain. This proteolytic process is often referred to as "extracellular shedding" and affects cell surface molecules such as growth factors, growth factor receptors, ectoenzymes and cell adhesion molecules. In our effort to identify sheddases, we isolated a membrane-bound protease from bovine kidney that was identified, after N-terminal sequencing, as ADAM10, a member of the metalloprotease disintegrin protein family (a disintegrin and metalloprotease). The function of this enzyme was not known at that time.

During our studies with ADAM10, we noticed a striking similarity between the inhibition of ADAM10 by various inhibitors and the results reported for inhibition of

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APP cleavage by a putative α-secretase (Roberts et al. 1994). Purified ADAM10 cleaved Aβ-derived peptides at the α-secretase cleavage site and depended on an α-helical conformation. In contrast, many other peptides spanning the cleavage site in other proteins that are substrates for shedding enzymes were not cleaved by ADAM10.

Overexpression of ADAM10 in several cell lines resulted in an increased α-secretase activity for the different isoforms of APP. The proteolytically activated form of ADAM10 was localized by cell surface biotinylation in the plasma membrane, but the majority of the proenzyme was found in the Golgi. These results support the view that APP is cleaved both at the cell surface and along the secretory pathway. Expression of mutated ADAM10 containing the amino acid substitution E384A in its zinc-binding site significantly decreased the endogenous α-secretase activity. It was possible to stimulate the α-secretase activity of ADAM10 with phorbol esters and to inhibit its activity by hydroxamic acid-based inhibitors for metalloproteinases. Thus, ADAM10 exhibits many properties of a physiologically relevant α-secretase, as expected from various publications since 1990. Our observations were published in 1999 in the *Proceedings of the National Academy of Sciences* (Lammich et al. 1999).

As early as 1996 it was reported that high cholesterol concentrations in the medium of cultured cells inhibit secretion of soluble APP (Bodovitz and Klein 1996). A project was started in my group to investigate whether the α-secretase ADAM10 is a target of the cholesterol effects on APP metabolism and on the cellular mechanisms that might be involved. Treatment of various peripheral and neural cell lines with either the cholesterol-extracting agent methyl-β-cyclodextrin or the hydroxymethyl glutaryl-CoA reductase inhibitor lovastatin resulted in a drastic increase of secreted α-secretase-cleaved soluble APP. This strong stimulatory effect was in the range obtained with phorbol esters and was further increased in cells overexpressing ADAM10. In cells overexpressing APP, the increase of α-secretase activity resulted in a decreased secretion of Aβ peptides. Several mechanisms were elucidated as being the basis of enhanced α-secretase activity: increased membrane fluidity and impaired internalization of APP were responsible for the effect observed with methyl-β-cyclodextrin; treatment with lovastatin resulted in higher expression of the α-secretase ADAM10.

The results were published in the *Proceedings of the National Academy of Sciences* in 2001 (Kojro,et al. 2001). In the same issue of the journal, Fassbender and colleagues reported that treatment of guinea pigs with high doses of simvastatin decreased Aβ production (Fassbender et al. 2001). The two papers were accompanied by a comment by Benjamin Wolozin (2001). He expressed his hope "… to develop medicines that target the brain lipids or lipid compartments that specifically regulate Aβ production. This opens up new therapeutic approaches to Alzheimer's disease." In 2005, it was reported that simvastatin treatment of AD patients affected the brain cholesterol metabolism and favored the non-amyloidogenic pathway of APP processing (Hoglund et al. 2005).

Prevention of amyloidogenesis in an AD mouse model by the α-secretase ADAM10

It had long been hypothesized that upregulation of α-secretase activity might preclude the formation of Aβ and its deposition in plaques. Identification of ADAM10 as an α-secretase allowed this concept to be proven in vivo. For this purpose I initiated a study

between my group at the University of Mainz and the group of Fred van Leuven/Leuven, Belgium.

In a first step, mice were generated that overexpressed either ADAM10 to different extents or the catalytically inactive ADAM10 mutant. Overexpression of the transgenes was under control of the neuron-specific, postnatally active *thy1*-promoter. Neuronal overexpression of ADAM10 had no detrimental effects on ADAM10 single-transgenic mice; these animals exhibited normal behavioral abilities (Schmitt et al. 2004). This finding was promising given that ADAM10 is also involved in the cleavage of membrane proteins other than APP, such as Notch, EGF, and cadherins. We examined whether Notch signalling was affected by analyzing the expression level of *Hes5*, a gene affected downstream of Notch, and we found no significant difference between *Hes5* transcript levels of adult non-transgenic mice and ADAM10-overexpressing mice. Gene profiling studies of transgenic mice recently showed that overexpression of ADAM10 does not lead to an increased expression of genes coding for pro-inflammatory or pro-apoptotic proteins (unpublished data).

In a second step, we generated double-transgenic mice by crossing the ADAM10-overexpressing lines as well as the line expressing mutant ADAM10-dn with mice transgenic for human $APP_{[V717I]}$. We found that even moderate neuronal overexpression of ADAM10 in mice transgenic for human $APP_{[V717I]}$ increased the release of APPsα, and reduced the formation of Aβ40 and Aβ42 by direct competition with the β-secretase BACE1, and prevented Aβ deposition in plaques. Expression of the catalytically inactive ADAM10 led to an enhancement of the number and size of amyloid plaques in the brains of double-transgenic mice (Fig. 1). Most importantly, defects in long-term potentiation and cognition, which are evident in the AD mouse model before the formation of amyloid plaques, were alleviated by modest overexpression of ADAM10. While $APP_{[V717I]}$ transgenic mice showed defects in hippocampus-dependent spatial learning and memory, both learning and memory were nearly fully restored in mice moderately overexpressing ADAM10. [The results of this study were published in Postina et al. 2004, and were the subject of a commentary entitled, "Amyloid at the cutting edge: activation of alpha-secretase prevents amyloidogenesis in an Alzheimer disease mouse model" (Lichtenthaler and Haass 2004).]

Future directions

Now, as there is evidence that the strategy of α-secretase activation may work, the question arises how such an activation may be achieved in humans with beneficial effects for the prevention or treatment of AD. One approach is to stimulate the non-amyloidogenic pathway by activation of G protein-coupled receptors that are localized in brain areas affected by AD. Recently, it has been reported that a newly developed M1 agonist reduced both the β- and tau-pathology in hippocampus and cortex and reversed cognitive deficits of the transgenic mice. The authors described an upregulation of ADAM17, another α-secretase candidate (Caccamo et al. 2006). We discovered that the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) promotes the α-secretase pathway via activating its specific PAC1 receptor. The α-secretase ADAM10 was primarily responsible for this effect (Kojro et al. et al. 2006), but no upregulation of

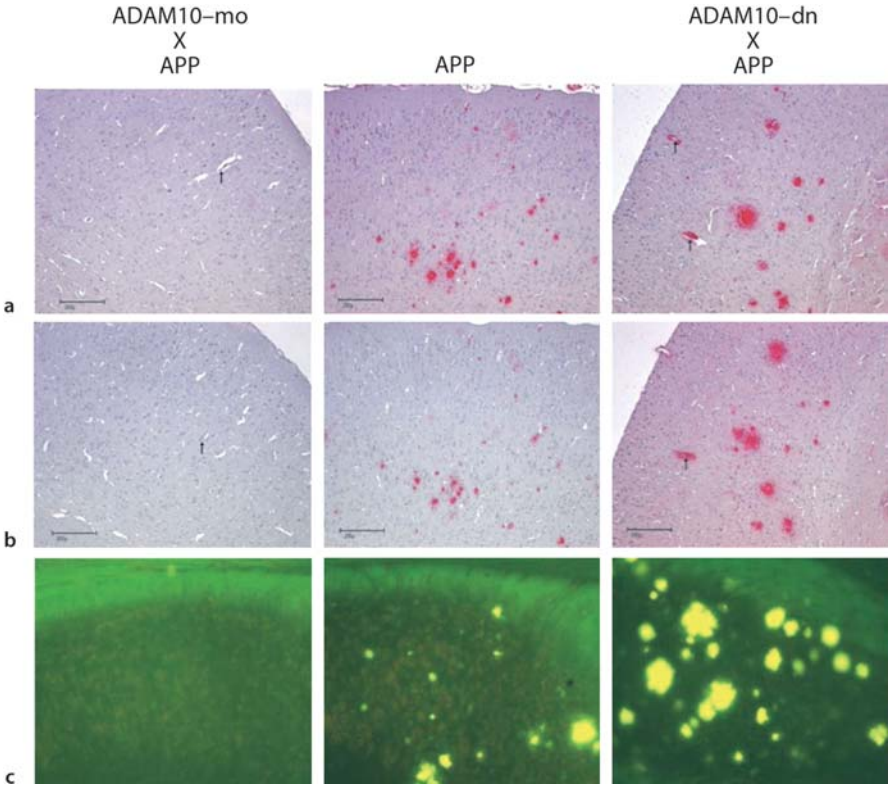

Fig. 1. Prevention of plaque formation by the α-secretase, ADAM10. Plaque formation was analyzed by two different antibodies in the cortex (**a**, **b**), by thioflavin S-staining in the hippocampus (**c**). *Right column*: ADAM10-moderate x $APP_{[V717I]}$ mice; middle column: AD $APP_{[V717I]}$ mice; *left column*: ADAM10-dominant-negative x $APP_{[V717I]}$ mice (from Postina et al. 2004)

the protein was observed. So, the detailed molecular mechanisms by which activation of GPCRs results in enhanced α-secretase activity have not yet been elucidated.

Since enhancing the ADAM10 gene expression appears to be a reasonable approach for the treatment of AD, the human ADAM10 gene was functionally analyzed in my group (Prinzen). In this study we identified retinoic acid as an activator of the α-secretase ADAM10 promoter. A common upregulation of ADAM10 and its two substrates, APP and APLP2, was found that may result in a preferential cleavage of these two substrates, as compared to other substrates of ADAM10, by activation of the retinoid signalling pathway (Endres et al. 2005). Therefore, pharmacological targeting of retinoic receptors by vitamin A and its metabolites may increase the expression of the α-secretase ADAM10, with beneficial effects on AD pathology.

Despite potential problems emerging from α-secretase upregulation, it is encouraging that a variety of currently available medications and endogenous hormones have been shown to increase α-secretase activity at the cellular level (Fig. 2) with only few side effects. The proof of the concept that upregulation of the α-secretase could have

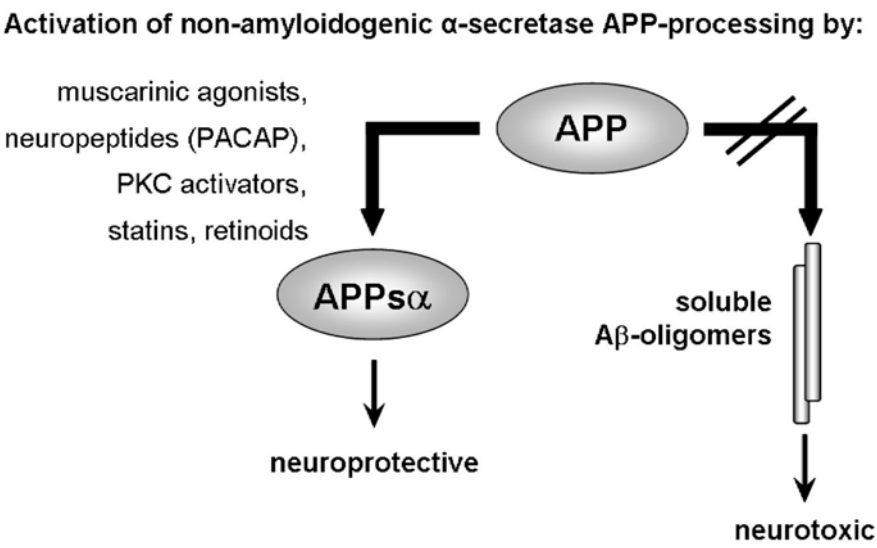

Fig. 2. Alpha-secretase as a target for the therapy of Alzheimer's disease. Activation of the non-amyloidogenic APP-processing prevents the generation of neurotoxic Aβ peptides and increases the release of neurotrophic and neuroprotective APPsα. The non-amyloidogenic pathway can be enhanced, for example, by muscarinic agonists, neuropeptides such as PACAP, PKC activators, statins and retinoids

beneficial effects has been provided by the transgenic mouse model with moderate overexpression of ADAM10. Further studies with medications and dietary regimens that enhance the non-amyloidogenic pathway of APP processing are, therefore, valuable approaches for AD therapy.

Acknowledgements. I want to thank my talented coworkers for their engagement and their valuable contributions.

Takeshi Iwatsubo

Challenges to the enigma of γ-secretase and to Alzheimer's disease

Takeshi Iwatsubo[1]

Our interest in Alzheimer's disease (AD) and in γ-secretase, a mysterious and fascinating machinery for the production of amyloid β peptides (Aβ), was aroused by the *in vitro* demonstration that Aβ ending at position 42 (Aβ42) forms amyloid fibrils much faster than Aβ40 (Jarrett et al. 1993), the latter being the predominant Aβ species produced by cells. Owing much to the groundbreaking invention by Drs. Nobu Suzuki and Asano Asami of the monoclonal antibodies that discriminate the C-terminal clip-site structures of Aβ40 and Aβ42 (Suzuki et al. 1994), we were able to visualize these different Aβ species in the brain tissues of patients with AD and Down's syndrome, showing that Aβ42 deposition, typically as diffuse plaques, is one of the earliest changes in the "Alzheimerization" of human brains (Fig. 1; Iwatsubo et al. 1994).

Important discoveries in the genetics of familial AD consolidated the significance of Aβ42 in AD: mutations in APP (Suzuki et al. 1994) and presenilin (PS) genes enhance the production of Aβ42 by shifting the preferred γ-secretase cleavage site from position 40 to 42, resulting in an increase in Aβ deposition in brains (Duff et al. 1996: Borchelt et al. 1996). Subsequently, a series of insightful studies, i.e., showing APP metabolism in PS1 KO cells (De Strooper et al. 1998), elucidating the role of the two intramembrane aspartates in PS1 (Wolfe et al. 1999), and photocrosslinking of PS1 fragments with transition-state analogue γ-secretase inhibitors (Li et al. 2000), unequivocally demonstrated that PS polypeptide comprises the catalytic center of γ-secretase, which is responsible for the intramembrane proteolysis of APP, Notch and other type I membrane

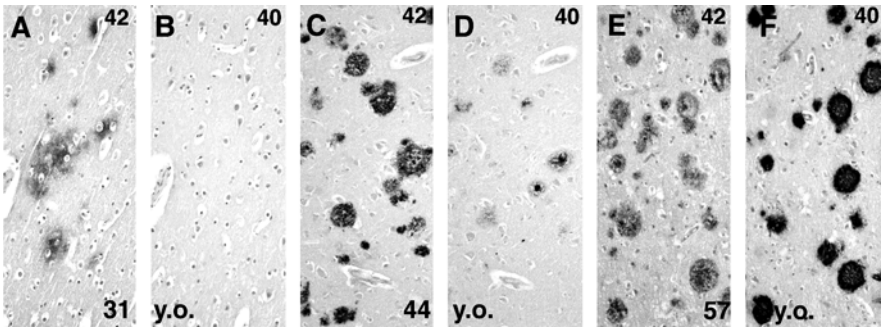

Fig. 1. Deposition of Aβ42 precedes that of Aβ40 in Alzheimerization of human brains. Sections from frontal cortices from patients with Down's syndrome at young [31 y.o. (years old), **A** and **B**], middle (44 y.o., **C** and **D**) and old (57 y.o., E and F) ages were immunostained for Aβ42 (**A**, **C** and **E**) or Aβ40 (**B**, **D** and **F**)

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proteins that undergo ectodomain shedding. These findings highlighted γ-secretase as one of the prime therapeutic targets for the "disease-modifying therapy" of AD. In contrast to the putatively complex mechanism of proteolysis within membrane, various types of small molecule compounds exhibit inhibitory activity to γ-secretase, and the "hit" rates in random screening are exceptionally high (Takahashi et al. 2006). Many of the γ-secretase inhibitors, however, suppress production of Aβ40, Aβ42 and Notch activation to a similar extent, potentially causing failures in lymphocyte maturation or mucosal turnover of digestive tracts as side effects. The unexpected discovery by the Koo group (Weggen et al. 2001), that a subset of non-steroidal anti-inflammatory drugs preferentially inhibit γ_{42}-cleavage of APP, preserving Notch S3 cleavage, provided us with significant implications: 1) NSAIDs or their derivatives can be used as potential Aβ-lowering drugs for AD, and 2) "modulation" of γ-secretase selectively inhibiting γ_{42}-cleavage is feasible by small molecule compounds. Both ideas are being realized in clinics, in the clinical trial of R-flurbiprofen and in the development of γ_{42}- or APP-specific modulator compounds.

Equally intriguing are a number of "basic" questions, e.g., the composition and structure of the γ-secretase complex, as well as the mechanism whereby γ-secretase hydrolyzes the transmembrane segment of substrate proteins. Rigorous protein chemical analysis (Yu et al. 2000), as well as elegant genetic studies in invertebrates (Francis et al. 2002), identified three "cofactor" proteins, i.e., nicastrin, APH-1 and PEN-2, that are essential to the formation and function of γ-secretase complex (Fig. 2). Reconstitution studies in cells strongly suggested that four proteins, i.e., PS, nicastrin, APH-1 and PEN-2, are the minimal set of components that constitute the framework of catalytically "active" γ-secretase complex (Takasugi et al. 2003; Kimberly et al. 2003; Edbauer et al. 2003), although additional modulators or accessory proteins, e.g., TMP21 (Chen et al. 2006), may exist. Genetic and proteomic screening would be powerful methodologies in searching for these additional components. Specific functions of individual co-factor proteins, and structure-function relationships of γ-secretase components, remain to be clarified: what are the relationship between the substrate capturing function of nicastrin (Shah et al. 2005) and the substrate binding site (distinct from the catalytic site; Tian et al. 2002) within PS? How does the nicastrin/APH-1 subcomplex (LaVoie et al. 2003) stabilize the γ-secretase complex? How does PEN-2 elicit the proteolytic activity (Takasugi et al. 2003)? Furthermore, structural analysis of γ-secretase complex would be a rewarding, but painstaking task, considering that γ-secretase is a hetero-tetramer of transmembrane proteins and that their absolute amounts are quite low. An attempt to purify the complex and analyze the proteins directly by X-ray crystallography might be in vain, although recent efforts to visualize the purified complex by single-particle electron microscopic analysis and 3-D reconstruction should provide useful information regarding the structure of the active γ-secretase complex (Lazarov et al. 2006; Ogura et al. 2006). An equally promising approach would be to systematically re-analyze the membrane orientation and water-accessibility of each segment of PS by a mutagenesis-based method, e.g., cysteine scanning. A combination of the molecular/cellular, chemical/biological, proteomic/genetic and structural approaches would surely advance our understanding of the mechanism by which γ-secretase cleaves its substrate within the membrane, leading us to the new horizon of the biology of intramembrane proteolysis, and explaining how single amino acid substitutions of PS lead to overproduction of Aβ42, which eventually leads to AD.

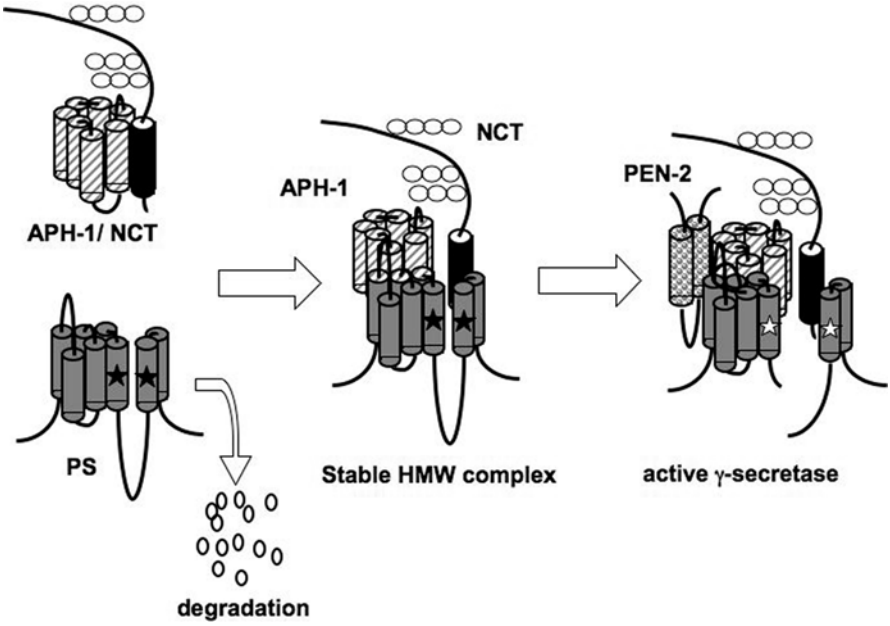

Fig. 2. Schematic depiction of the stepwise assembly and activation of γ-secretase complex. Nascent PS holoprotein is rapidly degraded, while a fraction of PS is stabilized to form a HMW complex by binding to the subcomplex formed by APH-1 and NCT. PEN-2 elicits the final step of maturation of the γ-secretase complex, facilitating endoproteolysis of PS and conferring γ-secretase activity. Tubes represent the putative transmembrane domains (TMD) of each protein, and stars within the 6/7th TMD of PS symbolize active (*white*) and inactive (*black*) aspartate residues involved in γ-secretase activities

What is urgently needed in the immediate future is a method to evaluate and develop anti-Aβ therapies, including secretase inhibitors and immunotherapy, to prevent and cure AD in clinics. Given that β-amyloid deposition, which is believed to play a significant role in the pathogenesis of AD, precede over > 10–20 years to the clinical manifestations of AD, or even those of mild cognitive impairment (MCI), development of diagnostic measures for preclinical diagnosis will be mandatory for an effective application of the "disease-modifying" therapies. Although specific biomarkers in body fluids from AD/MCI patients are currently unavailable, PET amyloid imaging using PIB or other compounds would be the most promising diagnostic method (Klunk et al. 2004a). Moreover, establishment of a combination of surrogate markers, based on more conventional neuroimaging methods like MRI, that correlates well with the clinical and pathological progression of AD and predicts the development of MCI or AD, would be indispensable for clinical trials of disease-modifying drugs for AD. To bring the fruits of basic research back to clinics, large-scale clinical studies to establish a standard method for objective evaluation of AD, typically represented by the ADNI in the USA (Mueller et al. 2005a), should now be conducted on a worldwide scale, so that human beings can get rid of this devastating disease that they have been *encouraged* to bear in exchange for the realization of longevity.

Michael S. Wolfe

Targeting γ-Secretase for Alzheimer's Disease

Michael S. Wolfe[1]

γ-Secretase is responsible for the final proteolysis that produces the amyloid β-peptide (Aβ) from its precursor, amyloid precursor protein (APP), and has been considered a potential therapeutic target for Alzheimer's disease (AD) since the early 1990s, even before anything was known about its character or identity. This protease activity, which takes place within the transmembrane domain of APP, generates heterogeneity at the C-terminus of Aβ peptides, forming longer, minor variants, especially the 42-residue variant (Aβ42), which is highly prone to aggregation and represents the major species of Aβ found deposited in the characteristic cerebral plaques of AD (Hardy and Selkoe 2002). The first hint to the identity of γ-secretase was the discovery that AD-associated missense mutations in the presenilin genes, *presenilin-1* and *presenilin-2*, cause increases in the ratio of Aβ42 to the less aggregation prone 40-residue variant (Citron et al. 1997; Duff et al. 1996; Lemere et al. 1996; Scheuner et al. 1996). Subsequently, two major clues were the finding that knockout of *presenilin-1* dramatically reduces Aβ production at the level of γ-secretase (De Strooper et al. 1998) and the observation that aspartyl protease transition-state mimics can likewise inhibit γ-secretase activity in cultured cells (Wolfe et al. 1999b).

Connecting these clues led to the hypothesis that presenilin might be a novel membrane-embedded aspartyl protease and the discovery that two conserved transmembrane aspartates in the presenilins are indeed critical for γ-secretase activity (Wolfe et al. 1999a, 1999c). Further support for this hypothesis soon followed. First, the aspartyl protease transition-state mimicking inhibitors of γ-secretase were found to directly interact with presenilin-1 (Esler et al. 2000; Li et al. 2000). Second, presenilin consistently came along with γ-secretase activity through biochemical purification steps as part of a high molecular weight complex (Esler et al. 2002; Kimberly et al. 2003; Li et al. 2000). Third, a distantly related presenilin homolog was discovered to be the protease signal peptide peptide (Weihofen et al. 2002). It is now clear that γ-secretase is a complex of four different integral membrane proteins, with presenilin ostensibly being the catalytic component (Edbauer et al. 2003; Fraering et al. 2004b; Kimberly et al. 2003; Takasugi et al. 2003; Fig. 1). During assembly of this complex, presenilin undergoes cleavage into two subunits (Thinakaran et al. 1996) (likely through autoproteolysis; Wolfe et al. 1999c)), each of which contributes one of the key aspartates to the active site (Wolfe et al. 1999c). Because the active site contains water and two aspartates, it is likely sequestered from the hydrophobic lipids (Wolfe et al. 1999a). Indeed, the enzyme apparently contains an initial docking site for substrate that is distinct from the

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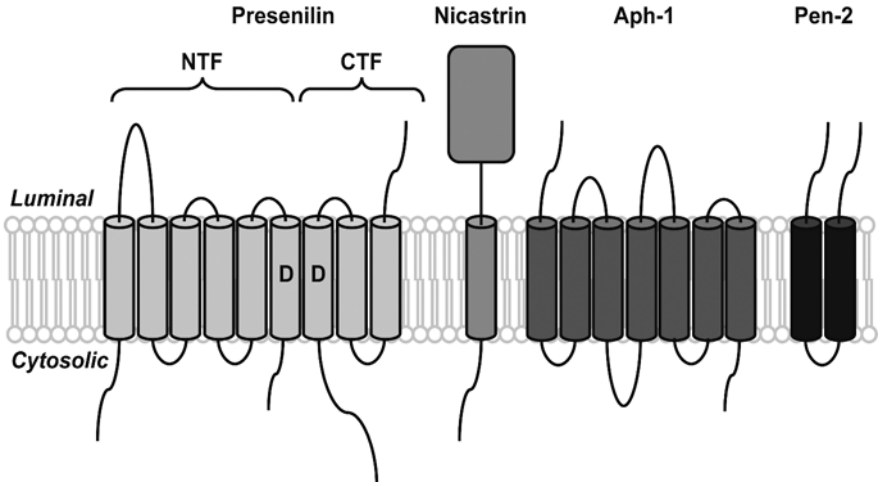

Fig. 1. Components of the γ-secretase complex. γ-Secretase is composed of four different integral membrane proteins: presenilin, nicastrin, Aph-1, and Pen-2. Presenilin undergoes endoproteolysis into an N-terminal fragment (NTF) and a C-terminal fragment (CTF) that remain associated. Two conserved aspartates within adjacent transmembrane domains are essential for both presenilin endoproteolysis and γ-secretase activity

active site (Esler et al. 2002), and evidence suggests that this docking site is also at the interface between the two presenilin subunits (Kornilova et al. 2005). Thus, substrate passes, in whole or in part, between these subunits to access the internal active site.

In parallel with the discoveries connecting presenilin to APP processing and AD were studies revealing a role of presenilin in the Notch signaling pathway of developmental biology (Selkoe and Kopan 2003). This revelation proved critical for identifying other members of the protease complex, two of which were discovered via genetic screens using Notch-deficient phenotypes as a read-out (Francis et al. 2002; Goutte et al. 2002). Notch, like APP, was found to be cleaved within its transmembrane domain, and this proteolysis is necessary for Notch signaling and cell fate determinations (Schroeter et al. 1998). Presenilin is necessary for this transmembrane cleavage (De Strooper et al. 1999), and knockout of presenilin-1 results in a lethal phenotype similar to that seen upon knockout of Notch1 (Shen et al. 1997; Wong et al. 1997). These findings began to raise concerns about γ-secretase as a target for AD: inhibition of this protease, while lowering Aβ production, might cause severe toxicities due to blocking critical cell differentiation events. The remainder of this chapter provides a current assessment of the therapeutic potential of targeting γ-secretase, especially strategies for lowering Aβ without affecting Notch signaling.

Although γ-secretase has in many ways been an attractive target for Alzheimer therapeutics, interference with Notch processing and signaling may lead to toxicities that preclude clinical use of inhibitors of this protease. Knockout of Notch1 or presenilin-1 is lethal in embryonic mice (Shen et al. 1997; Wong et al. 1997), but Notch signaling and γ-secretase activity are crucial in adulthood as well, because Notch plays a critical role in many cell differentiation events (Selkoe and Kopan 2003). Indeed, long-term

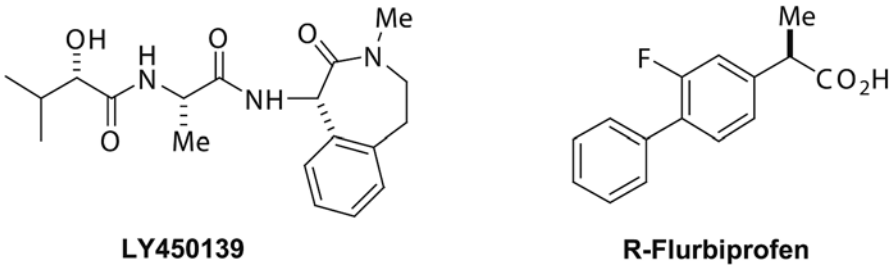

Fig. 2. γ-secretase inhibitor (*left*) and modulator (*right*) currently in clinical trials for the treatment of AD

treatment with γ-secretase inhibitors causes severe gastrointestinal toxicity and interferes with the maturation of B- and T-lympocytes in mice, effects that are indeed due to inhibition of Notch processing and signaling (Searfoss et al. 2003; Wong et al. 2004). Nevertheless, hope remains that a γ-secretase inhibitor might lower Aβ production in the brain enough to prevent Aβ oligomerization and fibril formation while leaving enough Notch signaling intact to avoid toxic effects. Presently, the Eli Lilly compound. LY450139 (Fig. 2), is in Phase II clinical trials in the US, with the dose being cautiously increased to that needed for Aβ lowering. Compounds in this general structural class have not displayed selective inhibition of APP processing with respect to that of Notch (Wong et al. 2004). So far, LY450139 has been shown to lower Aβ in plasma but not in cerebral spinal fluid, while signs of toxicity have been minimal (Siemers et al. 2006).

In contrast, compounds that can modulate the enzyme to alter or block Aβ production with little or no effect on Notch would bypass this potential roadblock to therapeutics. Recent studies suggest that the protease complex contains allosteric binding sites that can alter substrate selectivity and the sites of substrate proteolysis. Certain non-steroidal anti-inflammatory drugs (NSAIDs; e.g., ibuprofen, indomethacin, and sulindac sulfide) can reduce the production of the highly aggregation-prone Aβ42 peptide and increase a 38-residue form of Aβ, a pharmacological property independent of inhibition of cyclooxygenase (Weggen et al. 2001). The alteration of the proteolytic cleavage site is observed with isolated or purified γ-secretase (Fraering et al. 2004b; Weggen et al. 2003), indicating that the compounds can interact directly with the protease complex to exert these effects. Enzyme kinetic studies and displacement experiments suggest the selective NSAIDs can be noncompetitive with respective to APP substrate and to a transition-state analogue inhibitor, suggesting interaction with a site distinct from the active site and the docking site (Beher et al. 2004). The site of cleavage within the Notch transmembrane domain is similarly affected, but this subtle change does not inhibit the release of the intracellular domain and thus does not affect Notch signaling (Okochi et al. 2006). For this reason, these agents may be safer as Alzheimer therapeutics than inhibitors that block the active site or the docking site. Indeed, one compound, R-flurbiprofen or Flurizan (Fig. 2), has recently advanced to Phase III clinical trials in the US. However, the potency of this drug candidate (Eriksen et al. 2003) and other NSAIDs toward Aβ42 lowering raises questions about efficacy.

Another type of allosteric modulator includes the compounds that resemble kinase inhibitors and interact with a nucleotide binding site on the γ-secretase complex. The

discovery that adenosine triphosphate (ATP) can increase Aβ production in membrane preparations prompted the testing of a variety of compounds known to interact with ATP binding sites on other proteins (Netzer et al. 2003). In this focused screen, the Abl kinase inhibitor Gleevec emerged as a selective inhibitor of Aβ production in cells without affecting the proteolysis of Notch. In light of these findings, ATP and other nucleotides were tested for effects on purified γ-secretase preparations and found to selectively increase the proteolytic processing of a purified recombinant APP-based substrate without affecting the proteolysis of a Notch counterpart (Fraering et al. 2005). Furthermore, certain compounds known to interact with ATP binding sites were found to selectively inhibit APP processing vis-à-vis Notch in purified protease preparations. The γ-secretase complex could be pulled down with beads containing immobilized ATP, and the presenilin-1 CTF was specifically photolabeled by 8-azido-ATP. This labeling was not blocked by a transition-state analogue inhibitor or by purified, recombinant APP- and Notch-based substrates; however, the APP-selective inhibitors could prevent photolabeling by 8-azido-ATP. Taken together, these results suggest that the γ-secretase complex contains a nucleotide binding site, to which the presenilin-1 CTF is at least a contributor, and that this site allows allosteric regulation of γ-secretase processing of APP with respect to Notch. Whether this regulation is physiologically important is unclear, but the pharmacological relevance is profound and may lead to new therapeutic candidates for AD. This hope is tempered by the fact that γ-secretase cleaves numerous other type I membrane protein stubs that result from ectodomain shedding (Kopan and Ilagan 2004). Agents selective for APP versus Notch may reveal new long-term toxicities due to blocking proteolysis of these other substrates, toxicities that are masked by the severe Notch-related effects with nonselective inhibitors.

In conclusion, our knowledge of γ-secretase and its role in AD and in biology has increased dramatically in the past ten years. The identification, purification and characterization of the full protease complex leave structural biology as the next frontier toward an intimate understanding of how this enzyme carries out hydrolysis within the boundaries of the hydrophobic environment of the lipid bilayer. Meanwhile, the discovery that the protease complex can be modulated to block or alter Aβ production without affecting Notch proteolysis or signaling suggests that the toxicities associated with nonselective inhibitors can be overcome. Ultimately, these paths should intersect, allowing structure-based design of selective γ-secretase modulators for the treatment of AD.

Bart De Strooper

The Presenilin/gamma-secretase complex and its potential as a drug target in Alzheimer's Disease

Bart De Strooper[1]

A brief history

In 1995, the Presenilin 1 (*Psen1*) gene on chromosome 14 (14q24.3) was identified by positional cloning (Sherrington et al. 1995). Mutations in the closely related Presenilin 2 (*Psen2*) gene on chromosome 1 (1q42.2) can cause FAD as well (Levy-Lahad et al. 1995a; Rogaev et al. 1995). From studies in transgenic mice (Borchelt et al. 1996, 1997; Duff et al., 1996) and human fibroblasts (Scheuner et al. 1996), PS mutations appeared to increase the relative Aβ42/Aβ40 levels. The direct demonstration that Presenilins are involved in γ-Secretase cleavage of APP came from studies in *Psen1*-deficient neurons (De Strooper et al. 1998) and *Psen1&2*-deficient embryonic stem cells (Herreman et al. 2000; Zhang et al. 2000). Overall these findings implied that mutations in the substrate (APP) or in the proteolytic machinery (Presenilin) resulted in changes in Aβ generation. This finding provided strong support for the "amyloid cascade hypothesis."

Presenilin was shown to be the catalytic subunit (Wolfe et al. 1999) of a multimeric complex consisting of Nicastrin (Yu et al. 2000), Pen2 (Francis et al. 2002) and Aph1 (Goutte et al. 2002). These four proteins are necessary and sufficient for γ-Secretase processing of APP (Edbauer et al. 2003; Kimberly et al. 2003; Takasugi et al. 2003). Nicastrin is responsible for the recognition and binding of the γ-Secretase substrates (Shah et al. 2005). The four proteins assemble into a globular complex with a cylindrical internal chamber and two pores that could allow release of proteolytic fragments from the interior of the complex (Lazarov et al. 2006). Recent evidence indicates that several different complexes co-exist in the same cell line (Hébert et al. 2004; Shirotani et al. 2004) and have different biological functions (Serneels et al. 2005).

The proteolytic function of Presenilin

Genetic deficiency of Presenilin in C. elegans (Levitan and Greenwald 1995), as was later confirmed in mice (Shen et al. 1997; Wong et al. 1997) and fly (Struhl and Greenwald 1999; Ye et al. 1999), causes essentially Notch signalling deficiencies. Notch is an important regulator of cell differentiation and is involved in embryogenesis, neurite outgrowth, and T cell differentiation, but also in cancer. The mechanism by which Presenilin regulates Notch signalling was unravelled in 1999, by showing that Presenilin/γ-Secretase cleaves Notch (De Strooper et al. 1999; Struhl and Greenwald 1999) and releases its cytoplasmic domain, which activates the transcriptional regulator CSL in the

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nucleus (Jarriault et al. 1995). Presenilins are thus molecular switches between proteolysis and signal transduction (Annaert and De Strooper 1999). These observations, together with studies of the cholesterol biosynthetic pathway and bacterial signalling pathways, have led to the concept of "regulated intramembrane proteolysis" (Brown et al. 2000), a signalling mechanism conserved from bacteria to human. The crucial role of γ-Secretase in the Notch pathway is still a major concern when contemplating γ-Secretase inhibitors for the treatment of Alzheimer's Disease (De Strooper et al. 1999).

Over the years it has become clear that Presenilin/γ-Secretase is involved in the proteolysis of many type I integral membrane proteins (Struhl and Adachi 2000). Cleavage by γ-Secretase releases type I transmembrane proteins from the membrane. The cleavage seems to be regulated indirectly: only when the bulk of the ectodomain has been removed by another protease (sheddase) does the remaining membrane bound fragment become a substrate for this enzyme. Nicastrin acts as the gate-keeper of the complex, binding the free amino terminus of substrates by a similar mechanism as aminopeptidases (Shah et al. 2005). Upon binding, a complicated conformational change in γ-Secretase is postulated to occur that drives the substrate into the catalytic cleft of the complex (De Strooper 2005). Cleavage then releases short peptides in the extracellular environment (Aβ peptides in case of APP; Nβ peptides in the case of Notch; Okochi et al. 2002)) and an intracellular domain of various lengths and importance into the cytoplasm. Ever since the analogy between APP and Notch cleavage was established (Annaert and De Strooper 1999), the possibility that these intracellular domains, like the Notch intracellular domain, could be involved in nuclear signalling mechanisms has been considered by many groups in the field. The evidence supporting these claims, albeit sometimes impressive (Marambaud et al. 2003), is largely based on cell based (in vitro) approaches. Only for Notch has a convincing case been made with a series of in vivo studies. Particularly elegant was the demonstration that deleting the γ-Secretase cleavage site by a knock-in of the murine Notch gene was sufficient to cause a Notch signalling-deficient phenotype in mice (Huppert et al. 2000). An alternative interpretation of the broad substrate specificity of γ-Secretase was provided: γ-Secretase could be responsible for the removal of transmembrane domains of integral membrane proteins after they have performed their function, thus acting as a proteasome of the membrane (Kopan and Ilagan 2004). The release of protein fragments would in that view reflect mainly protein degradation. The Notch intracellular domain would then be only one of a few examples where these fragments have evolved to a role in signalling. The debate on the role of these intracellular fragments is, however, not easily resolved because they could theoretically be involved in many different signalling pathways, not necessarily involving direct gene transcription regulation. Presenilins indeed appear to be associated with N- and E-Cadherin at the cell surface and could be involved in the disassembly of E-Cadherin, β- and α-catenin and cytoskeleton adhesion complexes (Marambaud et al. 2002). Presenilin-generated proteolytic fragments are also invoked in the regulation of receptor tyrosine kinase signalling (Georgakopoulos et al. 2006) . Finally, a role for Presenilins that is independent from their proteolytic activity in the phosphorylation and turn-over of β-catenin (Kang et al. 2002) and in Ca^{2+} regulation (LaFerla 2002) has been proposed. Discussion of these aspects of Presenilin biology is beyond the scope of this small review.

In conclusion, Presenilins apparently have a broad biological role. Nevertheless, it remains unclear to what extent all these functions are equally important in the adult organism and to what extent these functions have to be taken into account when contemplating Presenilins as drug targets.

Do familial Alzheimer's Disease mutations cause loss or gain of function of γ-Secretase?

Ever since the ADcausing mutations in Presenilin were identified, it has been discussed whether they contribute an abnormal gain or loss of function to Presenilin. The facts that Psen knockout in neurons results in loss of Aβ peptide generation (De Strooper et al. 1998) and that all investigated clinical Psen mutations cause a "gain" in the relative amount of Aβ42 peptide versus Aβ40 peptide (Borchelt et al. 1996; Duff et al. 1996; Scheuner et al.,1996) have been taken as an argument for the "gain of abnormal function" hypothesis. However, in principle, such a relative change can be caused either by increased $A\beta_{42}$ or decreased $A\beta_{40}$ generation, or a combination of both changes. Rescue experiments with Psen-deficient cells using wild type Presenilin or Presenilin-containing clinical mutations provided a more definitive answer: most tested mutations caused an overall decrease in γ-Secretase cleavage efficiency of different substrates in the context of a Presenilin-negative background (Bentahir et al. 2006; see also Kumar-Singh et al. 2006a); Schroeter et al. 2003; Song et al. 1999). Rescue experiments with Presenilin-containing clinical mutations in *Psen*-deficient *C. Elegans* had also previously indicated that clinical mutations caused a loss of function in Notch signaling (Baumeister et al. 1997; Levitan et al. 1996). Rescue experiments with Psen1-deficient mice demonstrated, in contrast, that the clinical PS1-A246E mutant was able to partially rescue the Notch signaling-deficient phenotype (Davis et al. 1998; Qian et al. 1998). However, the conclusion of these experiments should likely be reconsidered since a partial rescue could still reflect a loss of function with this mutant. Indeed the loss of function effect of the PS1-A246E mutation on γ-Secretase activity is relatively mild in reconstituted Psen-deficient cells compared to other mutations (Bentahir et al. 2006). A better experiment would be to generate a knockin of this mutation into the endogenous gene and to evaluate to what extent such an affected allele is capable of restoring Notch cleavage and other γ-Secretase functions in a Psen1&2 negative background. This type of experiment has not yet been done in the absence of the Psen2 gene, but the phenotypes of three knockin mice (PS1M146V, PS1I213T, PS1P264L; Guo et al. 1999; Nakano et al. 1999; Siman et al. 2000); Wang et al. 2006) is quite normal, indicating that the loss of function caused by these mutations on Notch signaling is mild at the "physiological level." In contrast, in all cases Aβ42 peptide is increased relative to Aβ40. In patients and animals, the effect of a partial loss of function allele in the context of two or three other healthy alleles (two *Psen2* and one *Psen1*, depending on the case) is quite difficult to predict. Clearance factors, compensatory mechanisms, and additional pathogenetic factors can considerably complicate the picture. It is likely that a FAD mutation in one single *Psen1* allele will not dramatically affect the total Aβ peptide production in brain since the healthy *Psen* alleles will compensate for the partial loss of the diseased Presenilin function. It is also possible that in vivo APP-CTF substrate accumulates as a consequence of the partial loss of function of the FAD-PS1,

which then would lead to a new steady state situation and more substrate again driving Aβ peptide generation. Compared to the normal situation, this could theoretically result in quantitatively similar levels of Aβ peptide but qualitatively higher amounts of the Aβ42 variant. Even a small relative increase of Aβ42 peptide variant could critically affect Aβ amyloid deposition and generation of the putative toxic Aβ oligomer form. Recently, the effect of clinical Presenilin mutations in the context of wild type alleles was investigated in mice and it was shown that, in line with these assumptions, a wild type *Psen1* allele acted protectively against amyloidosis (Wang et al. 2006).

Does the loss of Presenilin function contribute in other ways to AD? Indeed, in conditional targeted mice in which both *Psen1&2* alleles were inactivated, a progressive neurodegenerative disorder was observed in the absence of Aβ deposition (Saura et al. 2004). The hypothesis that Presenilin loss of function contributes to AD is, however, difficult to explain in the context of sporadic AD and especially familial AD with APP mutations. Hypotheses that do not take into account Aβ peptide toxicity do not explain how, for instance, the Swedish APP mutation (Mullan et al. 1992) causes AD. This mutation increases absolute amounts of Aβ peptide but does not affect, as far as we know, Presenilin function.

The amyloid hypothesis has a big advantage in that it accommodates APP mutations, APP gene duplications, Presenilin mutations and the presence of amyloid plaques in genetic as well as in sporadic AD. It is clear that tangles have only recently been incorporated in the hypothesis, downstream of Aβ peptide toxicity. Putting tangles downstream of Aβ is consistent with the genetic mutations in Tau (FTD-17) that cause tangles but not amyloid plaques. Of course, the amyloid hypothesis will evolve over the years to further incorporate new experimental findings. The amyloid hypothesis accounts for many more experimental data than any other theory in the Alzheimer literature and therefore provides a very strong theoretical framework for Alzheimer's research. The only way forward now is to perform the critical experiments in the clinic by treating patients with anti-Aβ peptide therapies, and γ-Secretase modifiers or inhibitors could be one of those therapies.

Martin Citron

Identification of β-secretase

Martin Citron[1]

Historical Background

In the late 1980s, when it was first recognized that the Aβ peptide is a proteolytic cleavage product of the large amyloid precursor protein (Kang et al. 1987), the protease activities that cleave the peptide from the precursor, termed β- and γ-secretases, became highly interesting. Initially, it was unclear whether numerous proteases were involved and it was unknown whether β- and γ-secretases were constitutively active or only became activated in the brains of Alzheimer's disease (AD) patients. Obviously, in the latter case, any isolation effort would have to be based on AD brain material. The finding that Aβ peptides very similar to the plaque constituents are generated by a variety of intact, living cells suggested that Aβ generation per se is not a pathological process (Haass et al. 1992). Moreover, it now seemed reasonable to assume that the enzymatic machinery for Aβ generation may be present in a broad spectrum of cells, including standard cultured peripheral cells. Consequently, a number of groups began to characterize the effects of various manipulations on Aβ production. These manipulations included pharmacological treatments as well as changes to the APP (Amyloid Protein Precursor) substrate (e.g., Haass et al. 1993a). Of particular relevance for the later identification and characterization of β-secretase were mutagenesis studies that defined "good" (Citron et al. 1992) and "bad" (Citron et al. 1995) β-secretase substrates.

But despite rapid progress in understanding the cell biology of APP processing, the isolation of secretase enzymes did not make much progress in the early 1990s. Numerous proteases were proposed as potential secretase enzymes based on various levels of evidence (summarized in Evin et al. 1994). Most of these efforts focused on biochemical purification, and there clearly was a problem with irrelevant enzymes performing artifactual cleavages that obscured the less robustly expressed secretases. Another problem was the low level of validation of the various candidates.

Identification and validation of BACE1 as β-secretase

Like many others, we were interested in the identification of β- and/or γ-secretase as drug targets. We made three strategic decisions that – in hindsight – turned out to be critical to success: 1) we decided to circumvent the intrinsic problems of a biochemical secretase purification approach by using an expression cloning strategy to identify genes that modulate Aβ production, assuming that overexpressing a secretase in cells

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overexpressing APP would lead to increased Aβ production; 2) we chose tissue culture cells (and not post-mortem brain) as a source of cDNA; and 3) we insisted on the most rigorous validation cascade for any potential candidate to avoid pursuit of irrelevant enzymes, an issue that had plagued the field for many years. Our assay system was designed to identify β- and γ-secretase activities, and we were well aware of the intrinsic limitations of our approach in terms of assay sensitivity and in terms of being unable to identify multi-component enzymes (since this would require co-transfection of all the components). But at the time we speculated that both β- and γ-secretase would turn out to be membrane-bound single-chain proteins. Despite considerable efforts, we failed to identify γ-secretase, which can now be explained by its multi-component structure. However, we picked up a signal by a novel transmembrane protease, BACE1, which we ultimately identified and validated as β-secretase. Our initial characterization of BACE1 demonstrated that it is an aspartic protease of the pepsin family. Its most unusual feature is the presence of a transmembrane domain. In in vitro studies with purified enzyme, we demonstrated a relatively low turnover number for peptidic substrates containing the APP wild-type sequence, but a 60-fold increase for peptides with the APP Swedish mutation. Our validation cascade included immunohistochemistry, immunocytochemistry, detection of the protein in human brain, overexpression and antisense inhibition, radiosequencing of BACE-induced metabolites and studies of the purified enzyme with "good" and "bad" β-secretase substrates. All these studies demonstrated that BACE1 exhibited all the known properties of β-secretase (Vassar et al. 1999). β-secretase had finally been definitively identified. The only remaining caveat was that we had not formally demonstrated that reduction of BACE1 activity in brain (as opposed to tissue culture) would reduce brain Aβ.

Reaction to our paper and independent confirmations

Our study was published in *Science* in October 1999, and we were able to present it at very short notice during the same week at the Neuroscience meeting in Miami. The significance of the study was immediately and widely recognized both by the scientific Alzheimer's community and by the news media. Because of the extensive validation that we had provided, there was essentially no discussion regarding whether we had identified the enzyme or not; the data were conclusive. Subsequently four other groups reported isolation of the same enzyme using different approaches. Hypothesizing that β-secretase belongs to the aspartic protease family and using a genomics approach and antisense studies, Yan et al. (1999) isolated β-secretase. In contrast, Sinha et al. (1999) used biochemical affinity purification to identify the enzyme. Hussain et al. (1999) also reported identification of β-secretase, but they did not report why the particular candidate was selected initially. Finally, Lin et al. (2000) reported the characterization of a new enzyme, memapsin 2 with β-secretase properties, which is identical to BACE1. Thus, by the end of 1999, there was no doubt in the field that BACE1 is the major β-secretase enzyme.

In-depth Characterization

The identification and initial characterization of β-secretase triggered numerous lines of investigation that can be summarized by their major themes. Only some publications are cited as examples.

Characterization of BACE1 Several groups reported characterization of purified BACE1 protein. As an example, Haniu et al. (2000) established the disulfide bond connectivity and the glycosylation of BACE1. Other publications focused on expression studies and on the cell biology of BACE1, its processing, subcellular localization, trafficking, etc.

Crystallization of BACE1 The identification of BACE1 triggered a race to crystallize and solve the structure of its ectodomain (containing the protease activity). This task was first accomplished by Hong et al. (2000), who demonstrated that the overall structure of the enzyme is very similar to that of other known aspartic proteases but that there are differences in the active site, which is generally more open and less hydrophobic.

Identification of family members Immediately after the identification of BACE database mining led to the discovery of BACE2, an aspartic protease was discovered that has 64% similarity to BACE1 and also exhibits a C-terminal transmembrane domain (Saunders et al. 1999). It is now accepted that BACE2 is not a major secretase, but its physiological role remains unclear.

Knockout studies The finding that BACE1 knockout mice are deficient in Aβ production, independently reported by three groups, was not unexpected. However, these findings provided ultimate in vivo validation that BACE1 is β-secretase and demonstrated that no compensatory mechanism for β-secretase cleavage exists in mice. (Cai et al. 2001; Luo et al. 2001; Roberds et al. 2001). The more unexpected aspect of the knockout studies was the absence of major problems as a result of β-secretase ablation. BACE knockout mice were found to be healthy and fertile and were normal in terms of gross morphology and anatomy, tissue histology, hematology and clinical chemistry (Luo et al. 2001).

Current Efforts

While major aspects of β-secretase biology have been solved, there are several very active areas of investigation, both in academia and in the pharmaceutical industry. Key research fields are:

Analysis of the role of BACE1 in AD pathogenesis No mutations in BACE1 or its promoter that would cause familial Alzheimer's disease have been reported. But there are several studies reporting increased levels of BACE1 protein in AD brains (for example Holsinger et al. 2002). Future work will show, whether BACE1 upregulation is involved in the pathogenesis of sporadic AD or if BACE1 upregulation is just one of numerous changes observed in post-mortem AD brains.

Regulation of β-secretase activity The regulation of β-secretase activity is not well understood. Several academic groups are pursuing studies to better characterize potential regulators of β-secretase activity in vitro and in vivo. Whether such studies will identify additional targets to modulate the activity of β-secretase in Alzheimer's disease remains to be seen.

Identification of β-secretase substrates other than APP The normal physiological role of BACE1 remains unclear. Our demonstration that wild type APP is not a very good substrate for BACE1 (Vassar et al. 1999) is consistent with the idea that BACE1 may have a physiological function other than Aβ generation. The identification of additional BACE1 substrates may provide guidance about what potential side effects of BACE1 inhibition one may have to consider.

Identification of drug-like β-secretase inhibitors Work on β-secretase inhibitors is currently the most active area of β-secretase research. A number of peer-reviewed publications and even more published patent applications have described molecules with improved properties relative to the large peptidomimetics of the 1990s (for a recent review, see Thompson et al. 2005). Ultimately, these molecules may help to clinically test the amyloid hypothesis and in the process contribute to a better understanding of the physiological role of β-secretase. Our discovery of BACE1 as the major β-secretase enzyme has not provided additional support for the amyloid hypothesis, because no BACE1 (upregulating) mutations that cause AD have been identified yet. Rather, the identification of β-secretase has provided one of the best targets to clinically test the hypothesis, and numerous efforts in inhibitor development are ongoing, which may well be the most important consequence of our findings.

ApoE

Allen D. Roses

Apolipoprotein E and Alzheimer's disease: A brief retrospective

Allen D. Roses[1]

The past 14 years from 1992 to 2006 provide an interesting perspective for the application of genetic methods to Alzheimer's disease (AD) research. In the science of genetics, these years saw the beginning of linkage analyses for the identification of inherited disease mutations as well as susceptibility genes for complex diseases. This was followed in 1999–2001 with maps of single nucleotide polymorphisms (SNPs) across the now-sequenced genome, ushering in the latest era of whole genome association studies of sporadic patients with complex diseases. For our AD studies, clinical collection of patients from families in which there were two or more AD patients started in 1981. The hypothesis was that AD was a complex disease with multiple contributing genetic influences that could possibly be identified by a new method of genetic research: linkage analysis using a growing number of genetic variants distributed across the genome. At that time, few authorities considered AD to be a genetic disease – susceptibility genes were a concept of the 1990s. Therefore, the initial work was carried out alongside genetic studies of inherited muscular dystrophies, hereditary neuropathies, and other genetic neuromuscular diseases. By 1990, the Duke Joseph M Bryan ADRC at Duke University had linked late-onset "sporadic" AD to a region on chromosome 19 spanning millions of base-pairs, or hundreds of genes. The linkage method was then, and is now, hypothesis generating; the initial assumption was that there are genetically determined factors contributing to the disease.

As more information about genes located on chromosome 19 began to accumulate, testing each new gene in the broad linkage region became a one gene:one research fellow investigation. When Warren Strittmatter was recruited to Duke, he continued his earlier studies of amyloid. Examination of CSF amyloid on various gel separations always was accompanied by another prominent unidentified protein band. We hypothesized that the band might be an important factor, so Dr. Strittmatter cut out the bands from the separation gels and investigated proteolytic fragments of the extracted proteins for sequence analyses. In late 1992, after a frustrating five months of experiments, Dr. Strittmatter found two peptide fragments from the extracted band contained amino acid sequences that were identical to sequences of fragments from apolipoprotein E. Dr. Strittmatter came into my office quite upset about wasting his time on sequencing a protein that had no known relationship to AD. However, as soon as he said "APOE," a synaptic connection fired in my head.

From my linkage studies in myotonic muscular dystrophy (DM), which is located on chromosome 19, I was aware that APOE was the first gene localized to chromosome 19. APOE was instrumental in localizing a long-known linkage of DM to the Lutheran blood

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group and a salivary secretor locus to chromosome 19. The entire laboratory, including several post-doctoral fellows, was then focused on proteins within the chromosome 19 linkage region for AD. APOE was located in the middle of this linkage region but was not ever considered to be a candidate gene for AD. We immediately searched the textbooks on cardiology and found references to a new PCR method to measure nucleotide polymorphisms of APOE for the three common alleles, APOE2, APOE3, and APOE4. My problem was that not a single fellow or technician would take time off their own candidate genes to organize this APOE allele assay. I had never run a PCR myself, being from another era of biochemistry.

Several days before this revelation 14 years ago, my daughter Stephanie Margaux Roses was born. Her mother, Ann Saunders, was on maternity leave from her fellowship in a mouse chromosome mapping laboratory – and she knew how to perform PCR. A deal was made: if she came into my laboratory for a couple of weeks and set up the APOE assay, I would take care of Stephie at home. Within two weeks, the assay was running and validated with samples of known APOE genotypes obtained from the reporting laboratory in Texas. Approximately 50 DNA samples from AD patients were run along with 50 controls. The differences were obvious to the eye: there were many more APOE4 alleles in the AD patients. The next weeks allowed multiple confirmations using DNA from several hundred patients and several hundred controls. It was clear that there was an extraordinarily statistical significance for the association between APOE4 and AD (Strittmatter et al. 1993a; Saunders et al. 1993).

Since we had reasonably good historical age-of-onset information on the patients collected over the past decade, an age-of-onset analysis based on each APOE genotype clearly established that APOE4 was associated with a significantly lower age-of-onset distribution than APOE3 or APOE2. The epidemiologic data were important as a disease-specific independent confirmation of the genetic association, rather than simply additional series of AD patients. These data were also published in 1993, the same year as our publications reporting the higher allele frequency of APOE4 in both familial and sporadic cases (Strittmatter et al. 1993a; Saunders et al. 1993; Corder et al. 1993). The laboratory also initiated a series of biologic experiments to accompany the genetic and epidemiologic data, including the binding of apoE protein to amyloid and tau and the association of apoE4 > apoE3 with increased amyloid plaques in AD patients.

Since the AD field has had a greater interest in the amyloid causation hypotheses (see most of the chapters in this book), it was not until after several clinical papers and multiple letters in Lancet confirmed the association of APOE4 and AD that a few AD research laboratories accepted the relationship, but always as a factor secondary to the central dogma of amyloid cascade hypotheses.

In 1995, we began a series of experiments examining expressed brain proteins for pathway analysis using 2-dimensional gel electrophoresis of APOE knock-out and human allele-specific genomic APOE transgenic mice (reviewed in Saunders et al. 2000). We quickly identified and confirmed that multiple glucose metabolic enzymes were coordinately affected (increased or decreased expression]) in APOE KO and transgenic mice. A seminal PET study of glucose utilization was published by Eric Reiman's laboratory in 1996 (shortly after the birth of Maija Diane Roses). This study also noted differences in glucose utilization in AD but showed that APOE4/4 normal subjects (averaging 50 years or about 20 years before the mean age of onset of AD for

that genotype) metabolized glucose less than APOE3/3 subjects of the same age range (or about 30–40 years younger than the mean age of onset for the APOE3/3 genotype; Reiman et al. 1996).

Our protein pathway experiments had been supported by a research collaboration with GlaxoWellcome (GW). I left Duke University in 1997 to set up "genetics" of common diseases within the company, but also with the opportunity to pursue the APOE and AD data. The APOE Team very quickly demonstrated an APOE-specific effect on brain glucose utilization from transgenic and KO mice with several PPARγ agonists that were being developed for treatment of type 2 diabetes mellitus, or had been developed by other companies. By late 1999, however, the focus of GW was on merger with SmithKlineBeecham (SB), and almost a year was lost from these experiments during the process of merger until GSK was formed. SB had marketed a PPARγ agonist and this molecule was chosen over an ex-GW compound that was still in clinical trials at the time of the merger. We were then free to use the ex-GW compound in these experiments, but we had already previously tested the ex-SB compound. (In those ancient days, a company tested competitor compounds but avoided testing their own – no longer the practice!) The data were strong that several PPARγ agonists increased glucose utilization. Rosiglitazone (the ex-SB compound) was on the market and has been used in more than 2 million diabetes mellitus patients. A small Phase IIA clinical proof of concept trial was supported using rosiglitazone in the laboratory of Susan Craft. Using the results of this study, which suggested some clinical improvement in patients who were APOE3/3 and had not inherited an APOE4 allele, a formal Phase IIB trial was begun. This monotherapy trial prospectively designated APOE genotype as a biomarker for rosiglitazone efficacy in the clinical protocol. It was initiated in late 2003 and completed in mid-2005. The results of the study were remarkable from several points of view (Risner et al. 2006).

AD patients who had not been exposed to other AD therapies were recruited into a 24-week monotherapy trial using three doses of rosiglitazone, including two doses that were lower than the therapeutic dose for T2DM. A total of 511 patients were in the intention-to-treat group and, when the clinical parameters (ADAS-cog and multiple other measures) were analyzed, there was no statistically significant effect of the drug at any dose. Following this analysis, the APOE genotype data were assigned. All three doses of rosiglitazone decreased the ADAS-cog (increased function), as well as multiple secondary measures compared to placebo in patients who did not carry an APOE4 allele. The placebo group for the APOE4-positive patients made that arm of the study difficult to interpret but, if only the rosiglitazone-treated patients were analyzed, there appeared to be a dose effect with some improvement at higher doses in the APOE4-positive patients. This finding suggests that APOE genotyping might be used to select dosage for AD patients. These pharmacogenetic analyses were sufficient to design and execute a Phase III registration program.

With the consultation process of the FDA called (Voluntary Genomic Data Submission (VGDS), plans were put forward for APOE genotype-specific trial designs that would not only test patients who carried no APOE4 allele but would also look for the need for larger doses in patients with one or two APOE4 alleles. This registration program, for both adjuvant and monotherapy treatments, was initiated in the summer of 2006.

A great deal of data supporting a novel mechanism of action data has been accumulated during the past three years. The Gladstone Institute is the premier institution for APOE research, dating back to the 1980s with the work of Robert Mahley and Karl Weisgraber. Recently, Yadong Huang's laboratory has concentrated on abnormal mitochondrial function in the presence of proteolytic fragments of apoE4 protein. Two recent papers have reviewed these data (Roses and Saunders 2006). In fact, if the data supporting mitochondrial proliferation had been generated before the existing peroxisome studies, PPARγ agonists would probably be known as "MPARγ agonists!" A strong rationale exists for relating the apoE4 > apoE3 effects on neuronal sprouting and maintenance of connections on the difference in the protein structure of apoE4 compared to apoE3, leading to increased cleavage to produce the apoE1-272 fragment from apoE4. This fragment acts as a slow toxin to mitochondria, diminishing their dynamic movements, speed and distance traveled within the confining architecture of neurons. Mitochondria movement to the base of neuronal spouts is diminished so markedly that, over time, there is a decreased rate of energy-dependent dendritic plasticity, leading to simplified dendritic trees, decreased connectivity, and increased scarring with the accumulation of amyloid and other secondary proteins in the areas vacated by neurites. It is remarkable that the pathological data were referred to by Ramón y Cajal but were demonstrated using rapidly autopsied brain in 1994 (Ramón y Cajal 1906; Einstein et al. 1994). Rosiglitazone rapidly increases mitochondrial proliferation so mitochondria that have not been exposed to the apoE fragment increase effective energy metabolism function. Time will tell whether the functional improvement of symptomatic patients can be maintained by rosiglitazone. Once registered as a medicine, epidemiologic studies can be designed to study disease modification and prevention in at-risk subjects whose expected ages of onset are based on differential APOE genotype-specific risks.

That's where we are, 100 years after Alzheimer's first paper. Planning for the success of the current clinical trials, new discovery projects are in progress using functional assays developed around this novel mechanism of therapy. At this time, the research is mostly limited to GSK and the Gladstone Institut, and our collaborators. Should rosiglitazone achieve registration as a new chemical entity for the treatment of AD, future directions for neurodegenerative disease research may be established.

Judes Poirier

Apolipoprotein E4: From synaptic remodeling to genetic risk factor in both familial and sporadic Alzheimer's disease

Judes Poirier[1,2]

Introduction

The year 1993 was a particularly interesting year in the field of Alzheimer's disease (AD) research. Our group described for the first time the contribution of apolipoprotein E (apoE) and its main receptor, the apoE/apoB (LDL) receptor, during synaptic remodeling and terminal proliferation in response to hippocampal deafferentation and reinnervation (Poirier et al. 1993a). This landmark publication described how the cholesterol is recycled from dead or dying neurons in the deafferented hippocampus toward intact neurons actively rebuilding parallel networks while replacing dead synapses with new functional ones originating from cholinergic and glutamatergic neurons. In this energy-efficient molecular cascade, apoE was found to play a pivotal role in the coordination and recycling of cholesterol from dead/dying cells to reinnervating nearby neurons (Poirier 1994). Subsequent work using apoE knockout and apoE4 knock-in mice confirmed the pivotal role of apoE in response to damage and neurodegeneration in the mature and aging CNS.

Also in 1993, Warren Strittmatter and Alan Roses published their landmark study linking the presence of a particular allele of apoE, referred to as the apoE4, to late onset familial Alzheimer's disease (see Roses: chapter in this book). That pivotal publication had been preceded by a key observation from the Roses team, in collaboration with Gerry Schellenberg's genetic group in Seattle, in which they reported the presence of a particularly strong genetic association between specific markers of chromosome 19 and late-onset familial AD (Schellenberg et al. 1992b). The association was actually positioned closer to the apoC1 locus than the apoE gene. Interestingly, the apoC1/sporadic AD association was also subsequently confirmed and replicated in several ethnic groups around the world (Poduslo et al. 1995; Petit-Turcotte et al., 2001).

The Strittmatter observation did not come as a surprise to us, as we were completing our own genetic screening analysis of the apoE4 allele frequency in clinical cases with sporadic AD in a cohort of French Canadian subjects. This particular study linking apoE4 to sporadic AD represented the logical extension of our previous work on hippocampal apoE levels in humans with AD (Poirier et al. 1990a) and in the lesion rats (Poirier et al. 1990b). It was published in the summer of 1993 (Poirier et al. 1993b). Not only did it confirm the original Strittmatter observation in our age-matched, gender-matched, and ethnic- matched case control association study, but it also confirmed

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the E4 allele gene-dose effect on age-of-onset in our sporadic AD subjects. A flurry of reports was subsequently published confirming the apoE4/AD association in both familial and sporadic AD cohorts all around the world, except in certain African populations.

The strong impact of the apoE4 discovery in familial and sporadic AD was recognized through the first International Parke Davis Prize in Alzheimer's Disease, shared by the Roses (USA) and Poirier (Canada) research teams at the 1996's ICAD meeting, in Osaka. However, from that point on, the two teams took very different scientific directions as to what they believed the role of apoE was in the mature CNS and its role in the pathophysiology of AD.

Our team was (and still is) of the view that apoE4, while representing a major genetic risk factor for common AD, is not toxic or detrimental per se. Instead, we proposed in a comprehensive "ApoE Hypothesis for AD" that the E4 allele affects normal brain lipid homeostasis and compromises compensatory synaptic replacement as the result of poor lipid transport performance and low tissue concentrations (Poirier 1994). Figure 1 illustrates the proposed model and its effects both on the cholesterol transport via the apoE /LDL receptor system and on the cholinergic system, the most important lipid-dependent neurotransmitter system. The Roses team, on the other hand, was of the view that the apoE4 allele results in the gain of toxic or detrimental functions, leading to amyloid deposition and tau hyper-phosphorylation (Strittmatter et al. 1993b, 1994).

Interestingly, these opposite views eventually did polarize the field of apoE neurobiology, with one camp focusing on the interaction of apoE with amyloid and tau metabolisms and the other group choosing to focus on the role of apoE on lipid physiology, synaptic integrity and cholesterol neurobiology.

ApoE4: A case of evolutionary underperformance

The original arguments that we put forward to support the concept that apoE4 acts on synaptic integrity and plasticity through alterations of brain lipid physiology were, and are still, based on several key biochemical observations:

1) ApoE4, with its polymorphisms at amino acid sites 112 and 158, represents the ancestral form of the apoE gene (Finch and Stanford 2004). Homo sapiens only recently acquired the apoE3 and apoE2 allelic variants. All the other mammals and primates examined so far (except humans) are apoE4 carriers. Interestingly, the apoE2 allele, which was shown to confer some protection again AD, also happens to be over-represented in human centenarians (Blanche et al. 2001; Frisoni et al. 2001), clearly pointing toward a role in longevity and successful aging.
2) None of the other mammals carrying apoE4 (rat, mice, guinea pig, rabbit, monkeys, etc.) develop AD as we know it in humans. Conversely, there are reports of humans carrying a double dose of apoE4 who live past their 90s without signs of cognitive deficit or a diagnosis of AD (Mayeux et al. 1993). Moreover, there are several subpopulations of Africans in which the apoE4 allele is present at high frequency but fails to increase the overall risk of dementia; these include people from Nigeria, Kenya and East Africa (Sayi et al. 1997; Hall et al. 2006; Zekraoui et al. 1997).

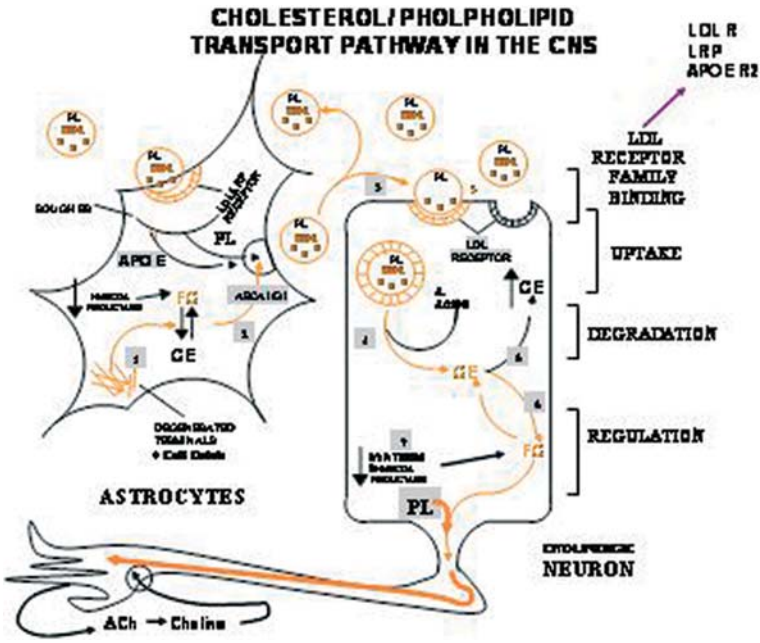

Fig. 1. Schematic representation of hypothesized cholesterol/phospholipid recycling mechanisms in the injured central nervous system, updated from the original hypothesis of Poirier (1994). Degenerating terminals are initially internalized and degraded. The non esterified cholesterol (**1**) is used as free cholesterol (FC) for the assembly of an apoE/cholesterol/lipoprotein complex via the ABCA1/G1 intracellular transport system (**2**) or converted into cholesterol esters (CE) for storage purposes. The newly formed apoE/cholesterol/lipoprotein complexes are then directed (**1**) toward the circulation, presumably through the ependymal cells surrounding the ventricles and/or (**2**) to specific brain cells (such as cholinergic neurons) that require large amount of lipids. ApoE-lipoprotein complexes are apparently internalized by the neuronal LDL receptor pathway (**3**) and the cholesterol released (**4**) for dendritic proliferation and/or synaptogenesis. As a consequence of the internalization process, cholesterol synthesis in neurons [via the HMG CoA Reductase pathway (**7**)] becomes progressively repressed. In cholinergic neurons, phospholipids such as phophatidylcholine and phosphatidylethanolamine are used to generate choline, which is then used by cholinergic neurons as a precursor of acetylcholine. A substantial portion of the choline is recaptured by intact terminals after hydrolysis of acetylcholine by esterases, to be eventually recycled into new acetylcholine molecules. E: ApoE; PL: phospholipids; A.Acids: amino acids; CE: cholesterol ester; FC: free cholesterol; Ach: acetylcholine

These and other observations argue against the notion that apoE4 allele causes a gain in toxic or seriously negative function.

Actually, we have been strong proponents (since 1989) of the notion that humans that are born carriers of the apoE4 allele are unable to maintain effective apoE concentrations in both blood and brain, relative to other isoform carriers. This concept stems from the original observation made by Utermann and colleagues (1980), some 26 years ago, that humans expressing the apoE4/3 and apoE4/4 genotype display the lowest apoE blood levels of all living humans whereas those with an apoE2/2 genotype (centenarian

candidates) belong to a small group of humans with the highest blood concentration of apoE of all humans. We tested this genotype/concentration-dependent concept 1) in autopsy-confirmed brain tissues from AD and age-matched control subjects and 2) in the blood on a large cohort of sporadic AD cases. We and several other teams found an apoE genotype-dependent concentration gradient of soluble apoE in cortical and hippocampal brain tissues of autopsy-confirmed AD case (Beffert et al. 1999) and a similar gradient profile in the blood of 417 probable/possible AD subjects enrolled in a clinical trial. Actually, the genotype- dependent blood gradient in our AD cases was nearly identical to the one reported by Utermann in the early 80s (Poirier 2005).

Over the years, this working concept has received support from several researchers in the field of AD, particularly in the apoE knockout mice literature, where it has been reported that the apoE-deficient animals display:

1. age-related cognitive deficit in the Morris swim maze (Oitzl et al. 1997; Veinbergs et al. 1999; Champagne et al. 2002; Davignon et al. 1982)
2. cholinergic loss with age (Van Uden et al. 2000; Kleifeld et al. 1998)
3. loss of synaptic integrity after 10-12 months of age (Chapman et al. 2000; Veinbergs and Masliah 1999)
4. compromised long-term potentiation (Krzywkowski et al. 1999)
5. no compensatory synaptogenesis or terminal proliferation in response to entorhinal cortex neuron loss (Veinbergs and Masliah 1999; Champagne et al. 2005)
6. tau hyperphosphorylation (Gordon et al. 1996)

The bulk of these observations led us to develop a low throughput screening assay using primary astrocytes from rats and mice to identify potential apoE inducer agents that could be used in vivo for the treatment (and conceivably the prevention) of sporadic AD. Please note that that rodent apoE is technically of the apoE4- type. The working hypothesis driving this program was that apoE4 carriers, which exhibit a high risk of developing AD in their 60s and constitutively express low level of apoE in both blood and brain, would greatly benefit from exposure to a potent apoE inducer drug that enhances the brain's ability to produce and deliver more apoE/lipoprotein complex to neurons, accelerating recovery and thus providing a more effective fight against the progressive loss of synapses associated with normal aging.

These multiple screenings led to the identification of several potent apoE-inducing agents. Among the most interesting candidates are 1) indomethacin (Aleong et al. 2003), a potent anti-inflammatory drug used in the past with some success in a placebo-controlled clinical drug trial in mild to moderate AD (Rogers et al. 1993), 2) estrogen (a problematic hormone used recently with disappointing results in elderly women; Craig et al. 2005) and 3) cholesterol-lowering drug called probucol (Champagne et al. 2003), the first generation of cholesterol- lowering agents that were used mostly in severe familial hypercholesterolemia (Davignon et al. 1982). We also identified two apoE-reducing agents, 1) cortisol [a glucocorticoid hormone associated with stress, known to inhibit synaptogenesis at physiological concentration in vivo and to be markedly up-regulated in MCI subjects (Lupien et al. 1998)], and 2) simvastatin [a second-generation cholesterol-lowering drug that can inhibit apoE secretion at high, non-physiological concentrations (Naidu et al. 2002)].

Of the three apoE inducers identified, we chose to focus on probucol, a relatively safe drug used in the past to treat familial hypercholesterolemia in humans (Davignon

et al. 1982). Probucol causes significant apoE inductions in the rodent hippocampal and cortical areas at doses that mimic the recommended human prescription (Champagne et al. 2003). In a small proof-of-concept human trial, a group of 12 subjects with mild-to-moderate AD, not receiving esterase inhibitors or NMDA antagonist, were administered the standard dose of 1 gr/day of probucol for six months. Lumbar punctures were performed at baseline and one month after initiation of probucol administration. Biochemical assessment of the CSF at month 1 versus baseline revealed a significant reduction in total CSF beta amyloid, a significant CSF apoE induction, but no effect on total Tau concentrations or lipid hydroperoxides (Poirier and Panisset 2002; Poirier 2003). The AD Assessement Scale cognition (ADAS-Cog) and the Disability Assessment of Dementia (DAD) scales indicated a stabilization of the disease for the whole group after six months of treatment and an apoE concentration-dependent improvement of the symptoms in individual cases (Poirier and Panisset 2002). These extremely preliminary data are, at best, suggestive of a benefit. However, the pre- and post-modifications of amyloid and apoE levels in the CSF of these patients represent concrete evidence that it is possible to directly modulate apoE synthesis and secretion in the adult brain with a relatively safe medication. Experiments are underway now to validate

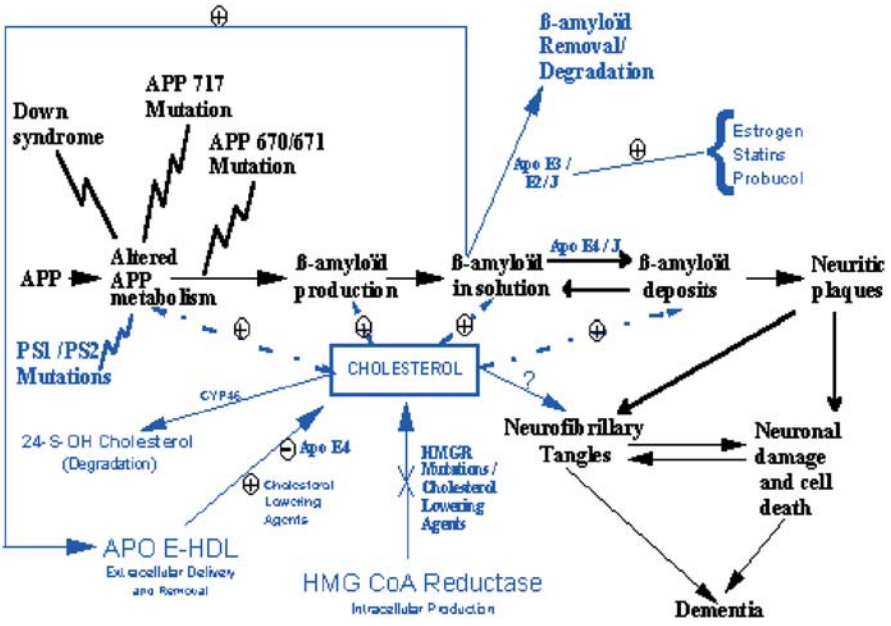

Fig. 2. An ApoE4/amyloid hypothesis of AD. (Pathophysiology updated from the original model presented in Poirier 2003) The sequence of pathogenic events leading to neuronal cell loss and synaptic damage is based on the well-established amyloid cascade hypothesis, which proposes that accumulation of beta amyloid (mono- and multimers) in the brain is the primary influence driving AD pathology. The different modulators of beta amyloid metabolism that were shown to affect lipid homeostasis, such as apoE and apoJ, have been added to the cascade. Finally, the emerging roles of cholesterol and the HMG CoA Reductase (the rate- limiting step in cholesterol synthesis) were added to the cascade in relation to their respective contribution to the pathophysiology of AD

new probucol analogs designed to improve blood-brain barrier penetration and the safety profile.

These human results, combined with those generated in transgenic models of apoE deficiency, apoE4 and apoE3 knock-in and the more recent dual apoE4/amyloid precursor protein over-expressors, led us to propose an integrative model of the apoE/APP neurobiology in the aging brain (Poirier 2003). Figure 2 summarizes the proposed model, in which lipid homeostasis is at the center of AD pathophysiology and in which synaptic damage and the absence of proper compensatory mechanisms in apoE4 carriers explain both the earlier age of onset and the more severe pathology observed in the brains of apoE4/4 subjects.

The recent discovery, in a double-blind, placebo-controlled clinical study in mild cognitively impaired (MCI) elderly subjects, that apoE4 allele testing can be used to predict AD conversion in more than 80% of the subjects "at risk" certainly opens the door to the testing of novel apoE-inducer drugs in the prevention of sporadic AD. While the ethical impact of such an approach remains to be fully ascertained, the use of pharmacogenomic approaches for the prevention of AD in apoE4 carriers represents a sound strategic move for the development of new drugs aimed at preventing the disease in "at risk" subjects.

Tau

Michel Goedert

The Alzheimer tangle – 100 years on.

Michel Goedert, Maria Grazia Spillantini[1], *Bernardino Ghetti*[2], *R. Anthony Crowther*, and *Aaron Klug*

Discovery of the tangle

On 3 November 1906 Alois Alzheimer, head of the Anatomical Laboratory of the Royal Psychiatric Clinic at the University of Munich, described a novel form of dementia at the 37th meeting of the Society of Southwest German Psychiatrists in Tübingen. He published these findings in the short paper of 1907 and the more extensive article of 1911 (Alzheimer 1907, 1911). In 1912, Alzheimer became Professor of Psychiatry at the University of Breslau (now Wroclaw). He died in 1915, aged 51.

The paper published in early 1907 is essentially a transcript of the lecture presented at the meeting in Tübingen. It gives the clinicopathological description of Auguste D., a patient who developed symptoms at age 51 and died aged 56. In her cerebral cortex, Alzheimer saw abundant plaques and tangles using the reduced silver staining method of M. Bielschowsky (1902). The clinical file and histological preparations of Auguste D. were recently recovered (Maurer et al. 1997; Graeber et al. 1998).

In normal brain, the Bielschowsky method visualizes what were named "neurofibrils" towards the end of the 19th century, a network of fine filaments that traverses the nerve cell and corresponds most closely to what we now know as the neuronal cytoskeleton. S. Ramón y Cajal also referred to the "neurofibrillar skeleton" (Ramón y Cajal, 1917). The ability to visualize neurofibrils provided some of the evidence in favor of the Neuron Doctrine, for which Ramón y Cajal was awarded the Nobel Prize in Physiology or Medicine in 1906 (together with C. Golgi).

Alzheimer saw increased silver staining in many nerve cells of the cerebral cortex from Auguste D., which he attributed to an abnormal thickening of neurofibrils and their alignment into bundles (the term "neurofibril" still survives in the expression "neurofibrillary tangle"). Indeed they were found to survive the degeneration of nerve cells (as extracellular or ghost tangles). Alzheimer states that he could also stain these bundles with dyes that did not label normal neurofibrils, thus underscoring their pathological nature.

In April 1907, the American psychiatrist S.C. Fuller, a former collaborator of Alzheimer, reported on neurofibrils in a number of conditions, including three cases of senile dementia (Fuller 1907; he had presented his findings at the June 1906 meeting of the American Medico-Psychological Association). It has been suggested that Fuller may have been the first to describe the tangle (Berrios 1990). However, unlike

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Alzheimer, he did not describe "the abnormal thickening of neurofibrils;" neither did he mention their accumulation inside nerve cells and the ensuing demise of these cells. He observed instead a reduction in neurofibrils in senile dementia and a number of unrelated conditions, probably as a result of the process of nerve cell degeneration. It thus seems clear that Alzheimer described the tangle for the first time. Fuller was of the same opinion, since he referred to the tangle as "the type of intracellular degeneration of neurofibrils to which Alzheimer was the first to call attention" (Fuller 1911).

Plaques were first described by Paul Blocq and Georges Marinesco in the brain of an elderly patient with epilepsy (Blocq and Marinesco 1892). Redlich (1898) then described them in two cases of senile dementia. This observation was followed by Oskar Fischer's description of neuritic plaques in senile dementia and their absence in controls and in cases of progressive paralysis and functional psychosis (Fischer 1907). He concluded that they are a specific feature of senile dementia; they were subsequently often referred to as Fischer's plaques. Like plaques, the clinical characteristics of dementia had been described before Alzheimer, most notably by Jean-Etienne Esquirol (1838).

Emil Kraepelin, Director of the Royal Psychiatric Clinic in Munich, separated the disease from senile dementia and named it after Alzheimer in the second volume of the 8th edition of his textbook of psychiatry (Kraepelin 1910). By this time, additional cases had been described by F. Bonfiglio (1908, one case), U. Sarteschi (1909, one case) and G. Perusini (1909, two cases). The ages of hospital admission of the five cases published by the end of 1909 were 51, 63, 67, 45 and 65 years. The senile period was said to begin at age 60.

It remains unclear why Kraepelin made the distinction between Alzheimer's disease and senile dementia. His introduction of the term *senium praecox* proved highly influential and led to the view that Alzheimer's disease is always a presenile condition. This assumption was overturned later, when it had become apparent that Alzheimer's disease and most cases of senile dementia are similar, both clinically and neuropathologically (for discussion, see Ballenger 2006). It is possible that the early ages of disease onset and the clinical pictures of Auguste D. and Johann F. (Alzheimer's "second patient,", who was hospitalized in Munich from 1907 until his death in 1910 at the age of 59), together with the striking cortical pathology of Auguste D., convinced Kraepelin that Alzheimer's disease was altogether different from senile dementia (Beach 1987; Weber 1997; Möller and Graeber 1998). He strongly believed that observable pathological processes in the brain cause specific psychopathologies. It has also been suggested (albeit without any documentary evidence) that Kraepelin's motivation for distinguishing between Alzheimer's disease and senile dementia may have been in part opportunistic, since this made it easier to name the disease after his collaborator (Amaducci et al. 1986). It would otherwise have been difficult to ignore Fischer, who worked at the Psychiatric Clinic of the German University of Prague, headed by Arnold Pick.

Alzheimer's article of 1911 is devoted to the nosology of the disease. He presents the case of Johann F. (with plaques, but no neurofibrillary pathology, at least by silver staining) and provides additional information on Auguste D. The histological preparations of Johann F. have been recovered (Graeber et al. 1997), and it has been suggested that he may have suffered from a familial form of Alzheimer's disease (Klünemann et al. 2002). Towards the end of the article, Alzheimer also describes the microscopic pathology of two cases with circumscribed lobar atrophy that he had recently examined. Similar cases of what we now know as "frontotemporal lobar degeneration" (FTLD)

had been described clinically and from post-mortem inspection of the brain by Pick (1892). Alzheimer reported on the absence of plaques and the presence of neurofibrillary changes with a characteristic round shape, which distinguished them from the tangles of Alzheimer's disease. They are now known as Pick bodies (despite the fact that they were first described by Alzheimer) and the clinicopathological entity is known as Pick's disease (following a suggestion by A. Gans, a pupil of Pick; Gans,1922).

One hundred years on, much has been learned about the Alzheimer tangle and the Pick body, their molecular composition and relevance for neurodegeneration. We now know that they are closely related at the molecular level, in that they both consist of abnormal filaments made of the microtubule-associated protein tau.

The paired helical filament

In the 1960s, electron microscopy of tissue sections was used to investigate the fine structure of neurofibrillary tangles in the Alzheimer's disease (AD) brain. Bundles of abnormal cytoplasmic filaments were observed in nerve cell bodies and their processes (Kidd 1963, 1964; Terry 1963; Terry et al. 1964). In 1963, Michael Kidd described the characteristic paired helical nature of the majority of filaments. He named the "paired helical filament" (PHF) because it appears to consist of two filaments wound helically around one another, with a longitudinal spacing between crossovers of about 80 nm and a width of 30 nm at the widest point and 15 nm at the narrowest (Fig. 1). There was discussion about the molecular nature of the PHF, with some arguing that it was made of neurofilaments (Terry 1963; Terry et al. 1964), and Kidd himself favoring the view that it was unrelated to the normal cytoskeleton (Kidd 1963, 1964). Also found in the neurofibrillary tangles of AD, as a minority species, is the so-called straight filament

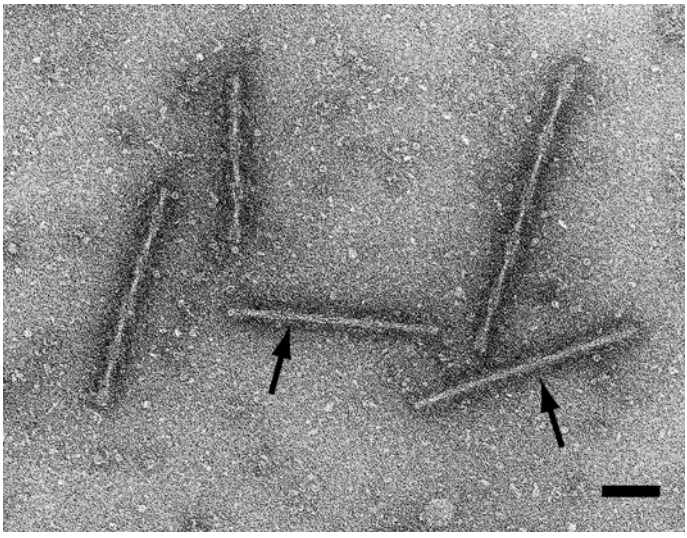

Fig. 1. Electron micrograph of dispersed paired helical filaments and straight filaments (*arrowed*) extracted from the frontal cortex of a patient with Alzheimer's disease. Scale bar, 100 nm

(SF), a filament about 15 nm wide that does not exhibit the modulation in width shown by the PHF (Hirano et al. 1968; Fig. 1).

Molecular composition of the paired helical filament

The molecular composition of the PHF was elucidated in the 1980s. Immunological studies identified several candidate proteins, such as neurofilaments (Miller et al. 1986), vimentin (Yen et al. 1983), microtubule-associated protein 2 (Nukina and Ihara 1983), microtubule-associated protein tau (Brion et al. 1985; Delacourte and Défossez 1986; Grundke-Iqbal et al. 1986b; Kosik et al. 1986; Nukina and Ihara 1986; Wood et al. 1986), Aβ (Masters et al. 1985b) and ubiquitin (Mori et al. 1987; Perry et al. 1987). Such studies suggested that these molecules might share epitopes with the PHF. However, they suffered from the inherent inability to distinguish between molecules that form an integral part of the PHF and material that is merely associated with or adheres to the filaments. This difficulty was compounded by the fact that different proteins possess epitopes in common (Ksiezak-Reding et al. 1987; Nukina et al. 1987). Furthermore, the insolubility of the filamentous material precluded quantitative biochemical purification.

Martin Roth, who was instrumental in establishing that most cases of senile dementia are similar to AD (Roth et al. 1966; Blessed et al. 1968), brought the tangle problem to Aaron Klug at the MRC Laboratory of Molecular Biology, where the following approach was developed. The PHF is biologically inert and defined by its ultrastructural appearance. Solubilization inevitably entails loss of morphology, rendering electron microscopy alone unsuitable for identification of an intrinsic chemical constituent of the PHF. What was required was a label that identified both intact individual filaments in microscopy and at the same time the protein bands obtained by gel electrophoresis from successively purified tangle preparations. The protein bands could then be sequenced and this information used for the isolation of cDNA clones encoding the partial amino acid sequence.

Claude Wischik used proteases to break down the insoluble tangles and he and Tony Crowther used such tangle preparations to study the structural organization of the PHF by 3-D image reconstruction from electron micrographs (Crowther and Wischik 1985). Wischik, Michal Novak and Cesar Milstein produced monoclonal antibodies, one of which decorated individual PHFs isolated from tangle fragments in electron microscopy and also labelled a 12 kDa band extracted from purified PHF preparations. John Walker determined the partial amino acid sequence of this band, which was then used by Michel Goedert to clone and sequence the corresponding cDNAs from a human brain library. The predicted protein of 352 amino acids was unrelated to any sequence known at the time. Its most striking feature was a stretch of three repeats, 31 or 32 amino acids each, in the carboxy-terminal half. By RNA blotting, a major 6 kb and a minor 2 kb band were observed that were similar to the pattern of tau mRNA. The publication in early 1988 of the amino acid sequence of a mouse tau isoform (Lee et al. 1988) firmly established that the 12-kDa fragment was a fragment of human tau. Although there had been several studies reporting the presence of tau-like immunoreactivity in neurofibrillary tangles or PHFs, the first by Jean-Pierre Brion and colleagues (Brion et al. 1985), the three papers published by the Cambridge group in the middle of 1988 provided direct proof that tau protein is present in the PHF (Goedert et al. 1988; Wischik

et al. 1988a,b). In November 1988, Yasuo Ihara and collaborators also reported on the presence of the carboxy-terminal third of tau protein in the PHF (Kondo et al. 1988).

Six isoforms of tau in adult human brain

In the 1988 paper, Goedert et al. also mentioned that they had identified a second form of tau, with sequence variation in the first repeat, and suggested that tau mRNA was undergoing alternative splicing. This second form was identical to the first, with the exception of an additional insert of 31 amino acids in the repeat region. Upon sequencing of genomic clones, the extra repeat was found to be encoded by a separate exon (now known as exon 10), flanked by consensus splice acceptor and donor sequences. This work uncovered the existence of at least two types of tau isoforms in human brain, those with three repeats and those with four repeats (Goedert et al. 1989a). Sequencing of a large number of cDNA clones revealed the existence of additional tau isoforms with 29 and 58 amino acid inserts in the amino-terminal region, in combinations with both three and four repeats. With the isoforms described previously, this gave a total of six human brain tau isoforms ranging from 352 to 441 amino acids in length (Goedert et al. 1989b; Fig. 2). All six isoforms were expressed individually in *E. coli* and their running pattern on SDS-PAGE was compared with that of dephosphorylated tau from fetal and adult human brain. These findings showed that the short three repeat-containing isoform with no inserts is the major fetal form of human tau and that all six isoforms are expressed in adult brain (Goedert and Jakes 1990). They also showed that similar levels of three-repeat and four-repeat containing tau isoforms are expressed in normal adult human brain.

Dispersed filaments are made of full-length, hyperphosphorylated tau protein

The insolubility of the bulk of PHFs and SFs from tangle fractions had been a major impediment to their biochemical purification and molecular characterization. It also precluded the quantitative analysis of tau pathology. In the development of a preparation method involving sarkosyl extraction (Rubenstein et al. 1986), Sharon Greenberg and Peter Davies obtained a fraction consisting of dispersed filaments (Greenberg and Davies 1990). By immunoblotting, three major tau bands of 60, 64 and 68 kDa, apparent molecular masses were observed (a minor fourth band of 72 kDa was described later; Mulot et al., 1994). It was realized that these bands were probably the same as the previously described A68 and SDS-soluble abnormal tau bands (Wolozin et al. 1986; Flament and Delacourte 1989). In 1991, Virginia Lee and colleagues purified dispersed filaments to homogeneity and used protein chemical analysis to demonstrate that they were made solely of tau protein (Lee et al. 1991). Crowther showed that PHFs and SFs represent different assemblies of an identical or closely related structural subunit (Crowther 1991), later shown to adopt a cross-β structure (Berriman et al. 2003). The work on dispersed filaments established that tau is the major component of the PHF and SF and removed any lingering doubt about potential additional components.

Lee and colleagues also showed that tau protein from their purified filament preparations was hyperphosphorylated. Earlier studies by Inge Grundke-Iqbal and

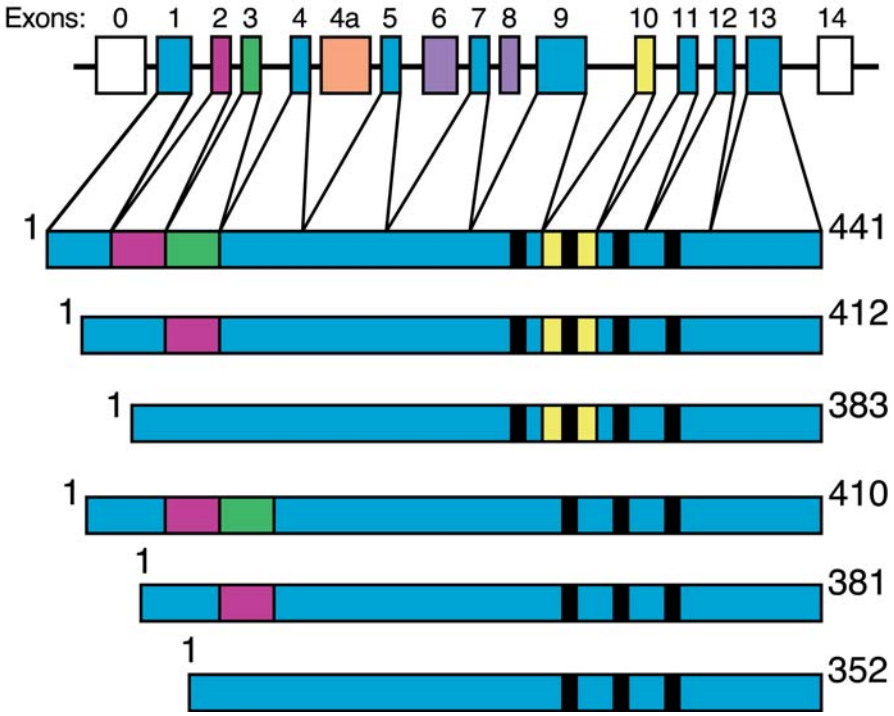

Fig. 2. Schematic representation of the human tau gene and the six tau isoforms (352 to 441 amino acids) that are produced in brain through alternative mRNA splicing. The human tau gene consists of 16 exons (E) and extends over approximately 130 kb. E0, which is part of the promoter, and E14 are non-coding (*in white*). Alternative splicing of E2 (*in red*), E3 (*in green*) and E10 (*in yellow*) gives rise to the six tau isoforms. The constitutively spliced exons (E1, E4, E5, E7, E9, E11, E12, E13) are indicated in blue. E6 and E8 (*in violet*) are not transcribed in human brain. E4a (*in orange*) is only expressed in the peripheral nervous system, where its presence gives rise to the tau isoform known as big tau. Black bars indicate the microtubule-binding repeats, with three isoforms having three repeats each and three isoforms having four repeats each. The exons and introns are not drawn to scale

colleagues and by Ihara and collaborators had already suggested that tau protein is hyperphosphorylated in AD brain (Grundke-Iqbal et al. 1986b; Ihara et al. 1986). In 1992, it was shown that, after dephosphorylation, the PHF-tau bands aligned with the recombinant tau isoform mixture, indicating that PHF-tau consists of all six tau isoforms in a hyperphosphorylated state (Goedert et al. 1992). The relative amounts of the different isoforms recovered were similar to those observed in normal human brain.

Tauopathies and neurodegeneration

By the early 1990s, the presence of tau had also been revealed by immunological studies in the deposits of progressive supranuclear palsy (PSP), corticobasal degeneration

(CBD) and Pick's disease (PiD; Pollock et al. 1986; Lee et al. 2001). Unlike AD, these diseases lack significant Aβ pathology.

Hyperphosphorylation of tau is a feature common to all these diseases. The phosphorylated sites are similar, with only minor differences between diseases. However, differences in the tau isoform composition of the pathological filaments were observed. Thus, the filaments of PSP and CBD are made of tau isoforms with four repeats (Flament et al. 1991; Ksiezak-Reding et al. 1994; Sergeant et al. 1999) whereas filaments of PiD contain mainly three-repeat tau (Delacourte et al. 1996). These studies showed the filaments to be similar in molecular terms to AD filaments. However, they did not provide any direct information about the relevance of tau dysfunction and filament formation for the disease process. What was missing was genetic evidence linking dysfunction of tau protein to neurodegeneration and dementia.

In 1994, Kirk Wilhelmsen and colleagues reported linkage of an autosomal dominantly inherited form of frontotemporal dementia with parkinsonism and amyotrophy [Disinhibition-Dementia-Parkinsonism-Amyotrophy Complex (DDPAC)] to chromosome 17q21.2, the region that contains the tau gene (Wilhelmsen et al. 1994). At about the same time, Bernardino Ghetti contacted Maria Grazia Spillantini, Crowther and Goedert and proposed to join forces in the characterization of the tau pathology in an autosomal dominantly inherited form of presenile dementia that he and Martin Farlow had identified. Tau deposits were extremely abundant and widespread and were present in neurons and glia. In view of this severe phenotype, the disease was named "Multiple System Tauopathy with Presenile Dementia" (MSTD), the first use of the term "tauopathy" (Spillantini et al. 1997). Tau filaments had a twisted ribbon morphology and were made of four-repeat-containing isoforms, in the absence of three-repeat tau. In parallel, Jill Murrell and Ghetti showed that the genetic defect in MSTD mapped to chromosome 17q21-22 (Murrell et al. 1997).

In October 1996, Ghetti, Murrell and Spillantini attended the consensus conference on chromosome 17-linked dementias organized by N. Foster at the University of Michigan, which brought together many of those working in this emerging field. At the time, 13 kindreds were considered to have sufficient evidence of linkage to be included in what was named "Frontotemporal Dementia and Parkinsonism linked to chromosome 17" (FTDP-17; Foster et al. 1997). At the conference, it became clear that a variety of techniques, some suboptimal, had been used to look for tau pathology in many of these kindreds. Spillantini therefore began to collaborate with a number of groups to look systematically for the presence of tau pathology. These findings (Spillantini et al. 1998a,b; Hulette et al. 1999), together with earlier work (Spillantini et al. 1996, 1997), left little doubt that tau deposits are an essential feature of FTDP-17. Aβ and α-synuclein deposits are not generally found in FTDP-17.

The exclusive presence of four-repeat tau in the MSTD filaments naturally led to an examination of the isoform composition of the pool of soluble tau. A striking departure from the 1:1 ratio of three-repeat to four-repeat tau isoforms was observed; there was a marked increase in the level of four-repeat tau and a corresponding reduction in tau isoforms with three repeats, with no apparent change in the total tau level. This explained the exclusive presence of four-repeat tau in MSTD filaments and suggested that increased splicing of exon 10 of the tau gene might be the cause of familial MSTD. Upon DNA sequencing, a guanine (G) to adenine (A) transition at position +3 of the intron following exon 10 was found, which segregated with disease. Following

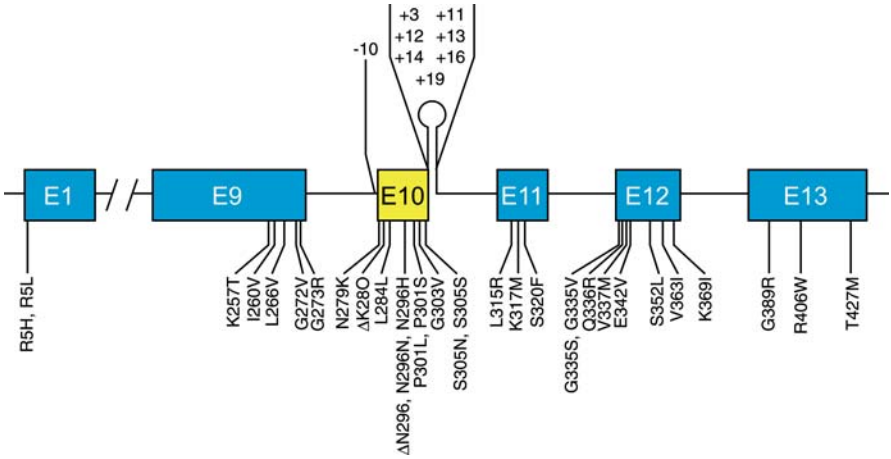

Fig. 3. Mutations in the tau gene in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Thirty-two coding region mutations in exons (E) 1, 9, 10, 11, 12, 13 and eight intronic mutations flanking E10 are shown. The stem-loop in the intron following E10 is indicated schematically. Constitutively spliced exons are shown in *blue*, with alternatively spliced E10 indicated in *yellow*. The exons and introns are not drawn to scale

examination of the nucleotide sequence of the junction between exon 10 and the following intron by Klug, and discussions with Gabriele Varani and Kiyoshi Nagai, it became apparent that the G to A transition at position +3 destabilized a putative stem-loop structure. (Varani went on to determine the three-dimensional structure of a synthetic RNA from the normal exon 10-intron junction; Varani et al. 1999.)

By July 1998, a total of nine mutations in the tau gene had been reported by three groups (Poorkaj et al. 1998; Hutton et al. 1998; Spillantini et al. 1998c). Poorkaj et al. reported exonic mutations (P301L and V337M) in two families with FTDP-17. Hutton et al. reported six different mutations in ten families. Three of these mutations (G272V, P301L and R406W) were in exons. The other three were located in the intron following exon 10 (at positions +13, +14 and +16), where they disrupted a predicted stem-loop. Spillantini et al. reported the +3 mutation in familial MSTD. Later that year, the reported missense mutations were shown to reduce the ability of tau to promote microtubule assembly (Hasegawa et al. 1998; Hong et al. 1998). Some of these mutations also directly promote tau filament assembly (Nacharaju et al. 1999; Goedert et al. 1999).

Since 1998, the number of known mutations in the tau gene has grown steadily (Goedert 2005). At the time of this writing, 40 different mutations, mostly affecting the sequence or splicing of the repeat region, have been reported (Fig. 3). Where investigated, tau deposits are present in either nerve cells or in both nerve cells and glia. As a result of the work on FTDP-17, it is now clear that a dominant pathological pathway leading from normal, soluble tau to abnormal, filamentous tau causes neurodegeneration and dementia.

Acknowledgements. We thank Professor M.B. Graeber and Dr M.M. Weber for helpful discussions about the early history of Alzheimer's disease.

Jean-Pierre Brion

Immunological demonstration of tau protein in neurofibrillary tangles

Jean-Pierre Brion[1]

Summary

Neurofibrillary tangles are one of the neuropathological hallmarks of Alzheimer's disease, described early as part of the pathological criteria of the disease. Ultrastuctural studies in the 1960s showed their unusual features but their molecular composition was not unraveled before the mid-1980s. Initial biochemical studies suggested that they were composed of modified, unidentified brain proteins, although some immunocytochemical studies suggested that they contained polypeptides cross-reactive with antibodies to cytoskeletal proteins. In 1985, we reported that neurofibrillary tangles were systematically immunolabelled by antibodies to the microtubule-associated protein tau and that antibodies raised to neurofibrillary tangles cross-reacted with tau proteins. These results were soon confirmed independently in several laboratories. Many scientists have contributed to this research theme and our contribution to the initial identification of tau proteins in neurofibrillary tangles is summarized below, tentatively in the framework of studies that preceded and followed this observation.

Introduction

Neurofibrillary tangles (NFT) were first described by Aloïs Alzheimer in his seminal paper describing the pathological findings in a demented woman (Alzheimer 1907a), using Bielschowsky silver staining. A subsequent report indicated NFT affinity for amyloid dyes, i.e., Congo red, giving a green birefringence when observed under crossed polarization filters (Divry 1934) and suggesting that they were made of orderly arranged subunits. A new leap in the description of NFT came with the advent of ultrastructural studies of brain tissues from Alzheimer's disease (AD) patients using electron microscopy in the early 1960s. These studies showed that NFT were composed of bundles of filaments. These filaments were described as paired helical filaments (PHF; Kidd 1963; Wisniewski et al. 1976), twisted filaments or tubules (Terry 1963; Terry et al. 1964; Hirano et al. 1968). These filaments were also found in abnormal neurites in senile plaques (Terry et al. 1964; Gonatas et al. 1967).

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Initial biochemical analysis of PHF

Initial studies aimed at purifying PHF pointed to their unusual biochemical properties. Some PHF were observed to be insoluble in denaturing agents such as sodium dodecylsulfate (SDS) and urea (Selkoe et al. 1982b), a property that impeded the analysis of their molecular components. This property was used to prepare fractions enriched in PHF and use them as immunogenic preparations (see below). However, some conflicting results were reported on the solubility of PHF. In purified preparations of PHF, at least a proportion of them were reported to be soluble in SDS by repeated extraction and to contain major unidentified polypeptides (Iqbal et al. 1984).

Initial immunocytochemical analysis of PHF

At about the same time, several groups investigated the antigenic composition of PHF. This approach was taken either by generating antibodies to isolated PHF and studying their cross-reactivity with normal brain proteins or by generating antibodies to normal proteins and studying their cross-reactivity with PHF. The antibodies raised to PHF preparations were found to react strongly with NFT in light microscopy (Ihara et al. 1983) and in electron microscopy (Brion et al. 1985c). This labelling was absorbed by brain homogenates from Alzheimer patients but not by homogenates from control subjects or only with high concentrations of proteins (Brion et al. 1985b). Similarly, an anti-PHF serum was observed to react with none of the polypeptides of normal brain (Grundke-Iqbal et al. 1984). These results suggested that PHF contained highly modified proteins exhibiting antigens mainly present in AD brains.

In view of the filamentous appearance of the PHF, several groups also studied their immunocytochemical cross-reactivity with antibodies to cytoskeletal proteins. Some antisera to brain microtubules were observed to label NFT in light microscopy (Grundke-Iqbal et al. 1979a; Yen et al. 1981; Brion et al. 1985c; Perry et al. 1985), and PHF preparations were observed to contain cross-reacting polypeptides detected by an antiserum to microtubules (Grundke-Iqbal et al. 1985a), but the cross-reacting polypeptides were not identified in these initial studies. Some antibodies to MAP2 (Nukina and Ihara 1983; Kosik et al. 1984) and to vimentin (Yen et al. 1983) also labelled NFT. Early studies also showed an imunolabelling of NFT by some anti-neurofilament antiserum (Ishii et al. 1979; Ihara et al. 1981; Dahl et al. 1982), although the polyspecificity or the bad definition of the antigens was raised as a potential pitfall (Gambetti et al. 1983a). However, well-defined monoclonal antibodies to neurofilament polypeptides (Anderton et al. 1982; Sternberger et al. 1985) and neurofilament antisera (Gambetti et al. 1983a) were found to label NFT in situ and even isolated NFT (Perry et al. 1985; Miller et al. 1986). However, at least some of these neurofilament antibodies were later found to react also with tau proteins (Ksiezak-Reding et al. 1987; Nukina et al. 1987). Thus at that time, despite sound efforts to uncover the molecular composition of NFT and although it was suspected that NFT were made of strongly modified normal polypeptides, their clear identity was unknown. The positive reaction of NFT with antibodies raised to complex mixtures of proteins (e.g., microtubules) did not allow the exact identification of the core component of PHF. In addition, several of these antibodies, even well-defined monoclonal antibodies, labelled a variable proportion

of isolated PHF extracted with SDS, suggesting that some of the normal polypeptides identified in NFT were trapped in NFT rather than being an authentic component of PHF.

Microtubules and PHF

In the early 1980s, Pierre Dustin and Jacqueline Flament-Durand in the Laboratory of Pathology and Electron Microscopy in Brussels were deeply interested in the study of microtubules and their pathology (Dustin 1984), and I joined their laboratory at this time. They had previously made several ultrastructural studies of biopsy specimens from AD patients that convinced them that neurons containing PHF had fewer normal microtubules and contained accumulations of dense bodies (Flament-Durand and Couck 1979; Dustin and Flament-Durand 1982), and they suggested that disturbances of microtubule assembly might be the cause of an abnormal axoplasmic transport in these cells (Dustin and Flament-Durand 1982). Further observation of an accumulation of smooth endoplasmic reticulum also supported this idea (Richard et al. 1989). Although these studies suggested an involvement of microtubules or other filaments in this pathological process, we did not observe a labelling of NFT by anti-tubulin or anti-70 kDa neurofilament antibodies (Brion et al. 1985c). Other groups (Eng et al. 1980; Yen et al. 1981) had also previously reported the absence of tubulin immunoreactivity with well-defined anti-tubulin antibodies in NFT (Nukina and Ihara 1983).

The microtubule-associated protein tau is the main component of PHF

These observations indicated that PHF did not result from the assembly of tubulin; the labelling of NFT by some antiserums to microtubules (Grundke-Iqbal et al. 1979; Yen et al. 1981; Brion et al. 1985c; Perry et al. 1985), suggested however the possibility that PHF might result from the pathological assembly of other microtubule proteins. We thus decided to test for the presence of other proteins associated with microtubules in NFT by generating specific antibodies to some of them. Jacques Nunez was present in the Free University of Brussels in 1983, in the laboratory of Jacques Dumont. He was interested in the developmental study of microtubule-associated proteins and had previously demonstrated that the expression of tau protein isoforms showed a developmental evolution (Mareck et al. 1980). In collaboration with him, we prepared tau and MAP2 proteins from adult rat brain using the microtubule assembly-disassembly method and their property of thermostability. We then generated several antisera against tau and MAP2 proteins using polypeptides extracted from polyacrylamide gels after electrophoretic separation by SDS-PAGE. These antisera were characterized by immunoblotting on purified preparations of microtubule-associated proteins and found to react with their cognate antigens. We then tested these antisera by immunocytochemistry on tissue sections from control subjects and AD patients. The anti-MAP2 sera did not label NFT but, to our surprise, the anti-tau sera strongly immunolabelled NFT and abnormal neurites around senile plaques, giving an immunolabelling indistinguishable from the labelling with our anti-PHF serum (Fig. 1; Brion et al. 1985d).

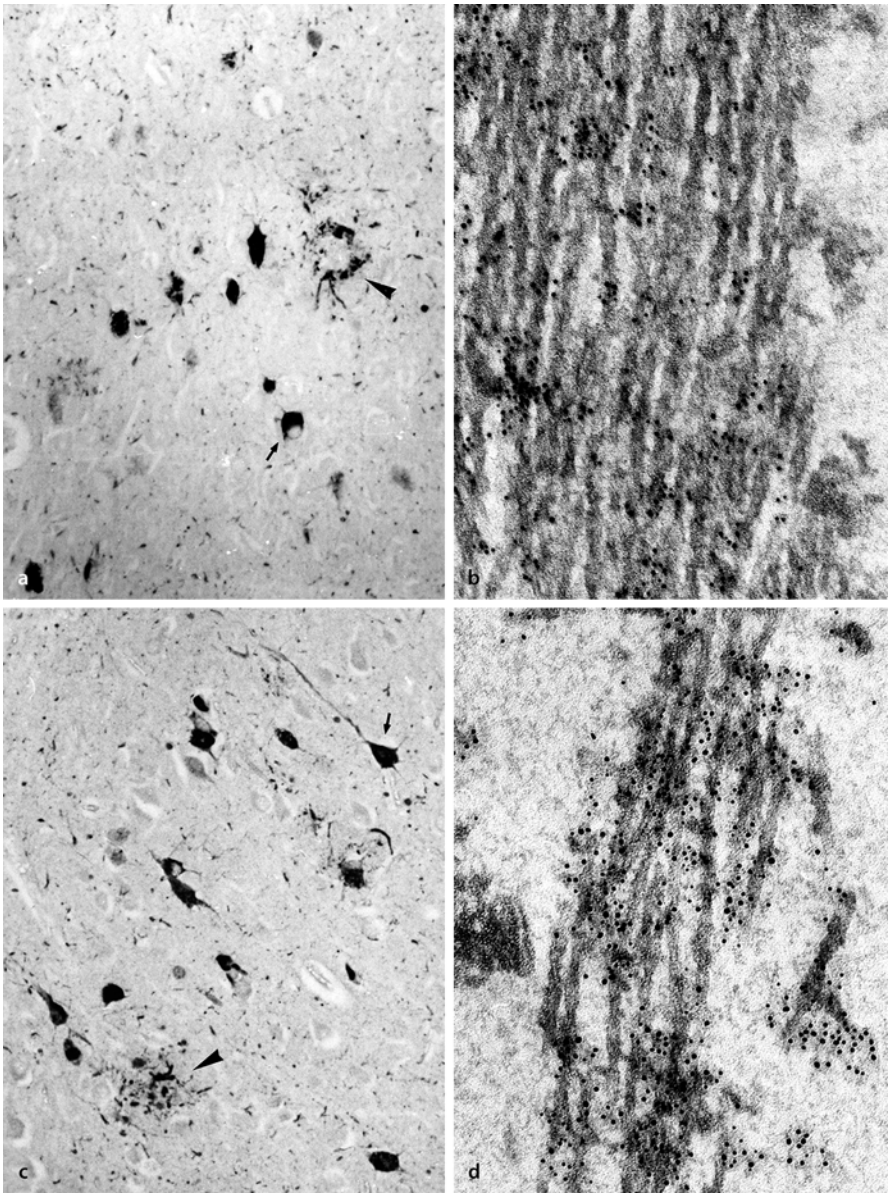

Fig. 1. Original Figure 1 from Brion et al. 1985d, showing the immunolabeling of NFT with anti-tau and anti-PHF antibodies. Immunoabelling of a tissue section of the hippocampus of an AD patient with an antibody to rat tau proteins (**a** and **b**) and an antibody to isolated PHF (**c** and **d**). The antibodies label only NFT in neurons and in dystrophic neurites in senile plaques, as shown in light microscopy (**a** and **c**). The abnormal PHF are also labelled by the antibodies in electron microscopy (immunogold method; **b** and **d**)

We further characterized this immunoreactivity by immunogold labelling in electron microscopy on tissue sections of AD patients: both the anti-tau and the anti-PHF sera also labelled the PHF on ultrathin sections (Fig. 1). Interestingly, the anti-PHF sera were also observed by Western blotting to react with the same set of proteins as the anti-tau sera, confirming that they contained anti-tau antibodies. These results showing that tau was a major component and antigenic determinant of PHF were published (Brion et al. 1985a,b,c,d, 1986) and presented in international meetings (Brion et al. 1985a,b; Flament-Durand and Brion 1985).

Several groups (Delacourte and Defossez 1986; Grundke-Iqbal et al. 1986a; Kosik et al. 1986; Nukina and Ihara 1986; Wood et al. 1986) soon confirmed independently the identification of tau as the molecular component of PHF. The cloning and sequencing of a cDNA encoding the core protein of PHF (Goedert et al. 1988) and the isolation of peptidic fragments from the core of PHF (Wischik et al. 1988b) confirmed that tau proteins were an authentic component of PHF. The immunocytochemical analysis of NFT showed that they were composed of the six tau isoforms (Goedert et al. 1989b). In collaboration with Brian Anderton and its team, we also pursued the immunocytochemical and biochemical analysis of NFT, showing that they were composed of the whole tau proteins (Brion et al. 1991a,b), although some NFT (e.g., "ghosts" tangles) were lacking some of the N- and C-termini of tau proteins (Brion et al. 1991b). The comparison of NFT labeling with anti-PHF/ anti-tau antibodies or silver staining showed that it was a robust method correlated to the clinical data (Duyckaerts et al. 1987). A tau immunoreactivity of fibrillary inclusions observed in other neurodegenerative diseases was soon reported, e.g., in progressive supranuclear palsy (Probst et al. 1988). Several post-translational modifications of tau proteins in NFT were found in other studies. Tau phosphorylation has been largely documented (see below). The detection of ubiquitin in NFT by immunocytochemistry (Perry et al. 1987b) and after isolation (Mori et al. 1987) suggested that these fibrillary lesions were hardly handled by the ubiquitin-dependent degradation system.

Tau proteins are abnormally phosphorylated

The identification of tau as the core component of PHF was soon followed by the finding that the tau proteins in PHF were abnormally phosphorylated (Grundke-Iqbal et al. 1986b; Ihara et al. 1986). The consequences of tau hyperphosphorylation in AD were further shown by studies demonstrating a much-reduced induction of MT assembly in AD brain (Iqbal et al. 1986). We also later observed a reduction of the immunoreactivity for stable microtubules in neurons containing PHF (Hempen and Brion 1996). By comparison with controls, slower migrating tau species were identified in AD tissue homogenates in areas rich in NFT lesions and were shown to be highly phosphorylated tau species (Flament et al. 1989; Hanger et al. 1991). The "A68" polypeptides (Wolozin et al. 1986) identified in Sarkosyl-insoluble preparations of AD brain were found to be modified phosphorylated tau species; they showed an electrophoretic pattern of three main bands (Ksiezak-Reding et al. 1990), contained abundant PHF (Lee et al. 1991) and reacted with antibodies to different tau isoforms (Brion et al. 1991a). However, a physiologically increased phosphorylation of tau is present during brain development (Brion et al. 1994). The mapping of tau phosphorylation sites (serine/threonine residues) by

several groups was accomplished using both specific antibodies and mass spectrometry analysis (Anderton et al. 2001). The existence of highly phosphorylated tau species obviously fuelled the search for protein kinases (and phosphatases) responsible for changes in tau phosphorylation. Many kinases are able to generate phosphorylation sites on tau proteins in vitro; the glycogen synthase kinase-3β was one of the first neuronal kinase shown to generate typical PHF-tau phosphorylation sites (Hanger et al. 1992; Mandelkow et al. 1992; Lovestone et al. 1994) that might also play a role in Aβ amyloid toxicity (Takashima et al. 1993). Other protein kinases also play a role in the abnormal phosphorylation of tau in AD (Buée et al. 2000). The pathogenic role of tau phosphorylation is still a matter of debate; hyperphosphorylated tau species do not bind well to microtubules and this decreased biological activity would thus be responsible for a "loss of function" in affected neurons. Soluble or oligomeric forms of phosphorylated tau could also be toxic by themselves, leading to a "toxic gain of function." The pathological role of PHF themselves is still not well understood; they could mechanically interfere with several cellular processes, e.g., with axoplasmic transport. On the other hand, tau phosphorylation/aggregation might well contribute to a protective answer of neurons submitted to various insults, e.g., by segregation of harmful proteins in the form of inclusions. Thus, despite decades of research on these unusual cellular lesions, many questions remain unanswered; these questions might well be resolved in the forthcoming years by the use of adequate experimental models (e.g., transgenic models). They highlight how much Alzheimer's disease is a fascinating example of a relatively neglected disease that has become a major neurobiological research theme, propelling the interest of the research community in neurodegenerative diseases and the search for the understanding and the treatment of these devastating diseases.

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Inge Grundke-Iqbal and Khalid Iqbal

Bulk isolation of neurofibrillary tangles and discoveries of tau and its abnormal hyperphosphorylation

Khalid Iqbal[1] and *Inge Grundke-Iqbal*[1]

Introduction

Alzheimer's disease (AD) and its two hallmark brain lesions, neurofibrillary tangles and neuritic (senile) plaques, were described by Alois Alzheimer in 1907. However, it was not until over 60 years later that AD caught the attention of neuroscientists. The discoveries that laid the foundation for the exciting research in the AD field were 1) the ultrastructure (Kidd 1963, 1964; Terry 1963, Terry et al. 1964) and counts of tangles and plaques and the clinical-pathological correlation of these lesions to the presence and the degree of dementia (Blessed et al. 1968) and 2) the biochemical isolation of neurofibrillary tangles and plaque amyloid and the discoveries of the abnormally hyperphosphorylated tau as the major protein subunit of paired helical filaments (PHF; Iqbal et al. 1974; Grundke-Iqbal et al. 1979a,b, 1985a,b, 1986a,b), and of Aβ peptide as the major constituent of cerebral vascular and plaque amyloid (Glenner and Wong 1984b; Masters et al. 1985a).

This article describes the work that led to the bulk isolation and polypeptide composition of neurofibrillary tangles, the discoveries of tau as the major protein subunit of PHF and of the abnormal hyperphosphorylation of tau in AD, and some of the major advances made in the field since these discoveries.

Bulk isolation and polypeptide composition of neurofibrillary tangles/paired helical filaments

Because neurofibrillary tangles are seen in the perikarya of affected neurons, we developed a method for the bulk isolation of neuronal perikarya from fresh or frozen autopsied brains as an important initial step for the isolation of tangles/PHF (Iqbal and Tellez-Negel 1972). Employing the protocol that we had developed for subcellular fractionation of neuronal perikarya isolated from Huntington's disease brains, we succeeded in obtaining a tangles/PHF-enriched fraction (Fig. 1a,b) by subcellular fractionation of neurons isolated from AD brain (Iqbal et al. 1974).

In the early 1970s, SDS-PAGE was a relatively new technique that became available to study the protein compositions of complex mixtures and to isolate micro amounts of proteins purified by this technique. SDS-PAGE of the tangles/PHF-enriched preparations revealed the presence of five prominent protein bands, two of the upper three

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of which co-migrated with tubulin (Fig. 1c). We named one of these protein bands neuronal protein (NP) and another, the ~ 50 kDa band, enriched fraction band (EFP). We did so one year before microtubule-associated protein tau was described (Weingarten et al. 1975, Iqbal et al. 1974) and before any biochemical studies on AD were available in the literature.

To confirm that EFP, which we renamed PHF protein (PHFP), was a protein subunit of PHF, we raised rabbit antibodies to this protein, purified by cutting out the protein band from Coomassie blue-stained SDS-PAGE gels. The antiserum to PHFP stained neurofibrillary tangles and dystrophic neuritis of neuritic (senile) plaques in AD brain and produced a reaction line of identity with a brain microtubule-associated protein (MAP) by Ouchterlony double diffusion test (Grundke-Iqbal et al. 1979a,b). Employing the Ouchterlony double diffusion test, we demonstrated that PHFP reacted with a brain microtubule-associated protein (MAP) and not with tubulin or high-molecular weight (HMW) MAPs (Grundke-Iqbal et al. 1979b). We also raised an antiserum to human brain microtubules that reacted with PHFP and labeled neurofibrillary tangles in AD brain (Grundke-Iqbal et al. 1979a). On the basis of these findings, we were confident that we had the PHFP and that it was a MAP other than the HMW MAPs, which essentially left tau as the candidate protein. At this stage, we took a two-pronged approach: (1) to further purify the PHF-enriched fraction and study its protein composition, and (2) to purify the MAP with which the tangles crossreacted.

Discovery of tau as the major protein subunit of PHF

We generated both monoclonal (Wang et al. 1984) and polyclonal (Grundke-Iqbal et al. 1984, 1985a,b) antibodies against PHF purified from AD brains (Iqbal et al. 1984). These antibodies labeled neurofibrillary tangles and plaque neurites in AD brain sections and six protein bands in the 50-kDa–70-kDa area, a typical tau pattern on Western blots of isolated PHF. By immunoabsorption of the polyclonal antibodies to PHF and antisera to microtubules that labeled tangles on tissue sections, with tubulin, HMW MAP, neurofilament triplet, keratin, and fibroblast lysates as a source of tubulin and vimentin, we concluded that PHF polypeptides were MAP of ~ 50 kDA–70 kDa (Grundke-Iqbal et al. 1985a).

While on the one hand our work on purification of tangles/PHF from AD brains, their protein composition and development of their Western blots with antibodies to PHF had led us to MAP of tau-like patterns of molecular sizes, we had also in parallel studies pursued the identification of the tangle-crossreacting antigen in brain microtubules, based on our 1979 observations (Grundke-Iqbal et al. 1979a). We observed that the tangle staining of anti-PHF serum and of anti-microtubule sera could be absorbed only with tau and not with HMW MAP. Since we had established that PHF protein was a normal brain microtubule protein other than tubulin and HMW MAP, we purified tau from bovine brain by five different methods. All of these tau preparations reacted specifically with antibodies to PHF (Grundke-Iqbal et al. 1986a). Employing these purified tau preparations, we affinity-purified antibodies to each of the six brain tau isoforms from the anti-PHF serum and showed that antibodies to each tau isoform reacted with most neurofibrillary tangles on AD brain sections and with all six tau isoforms in normal brain tau as well as in isolated PHF on Western blots (Fig. 2).

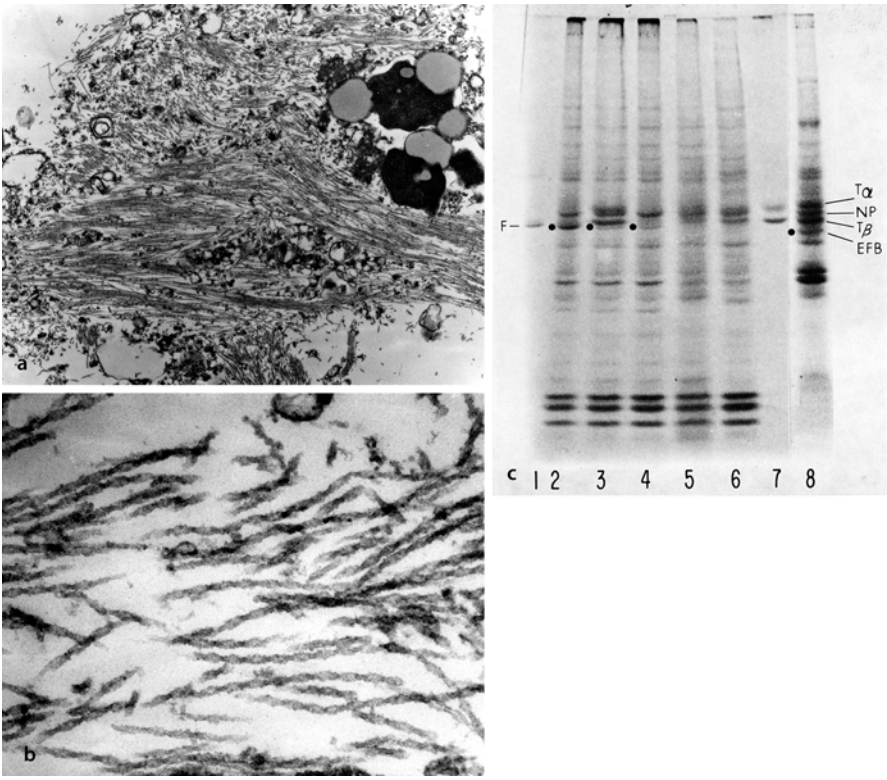
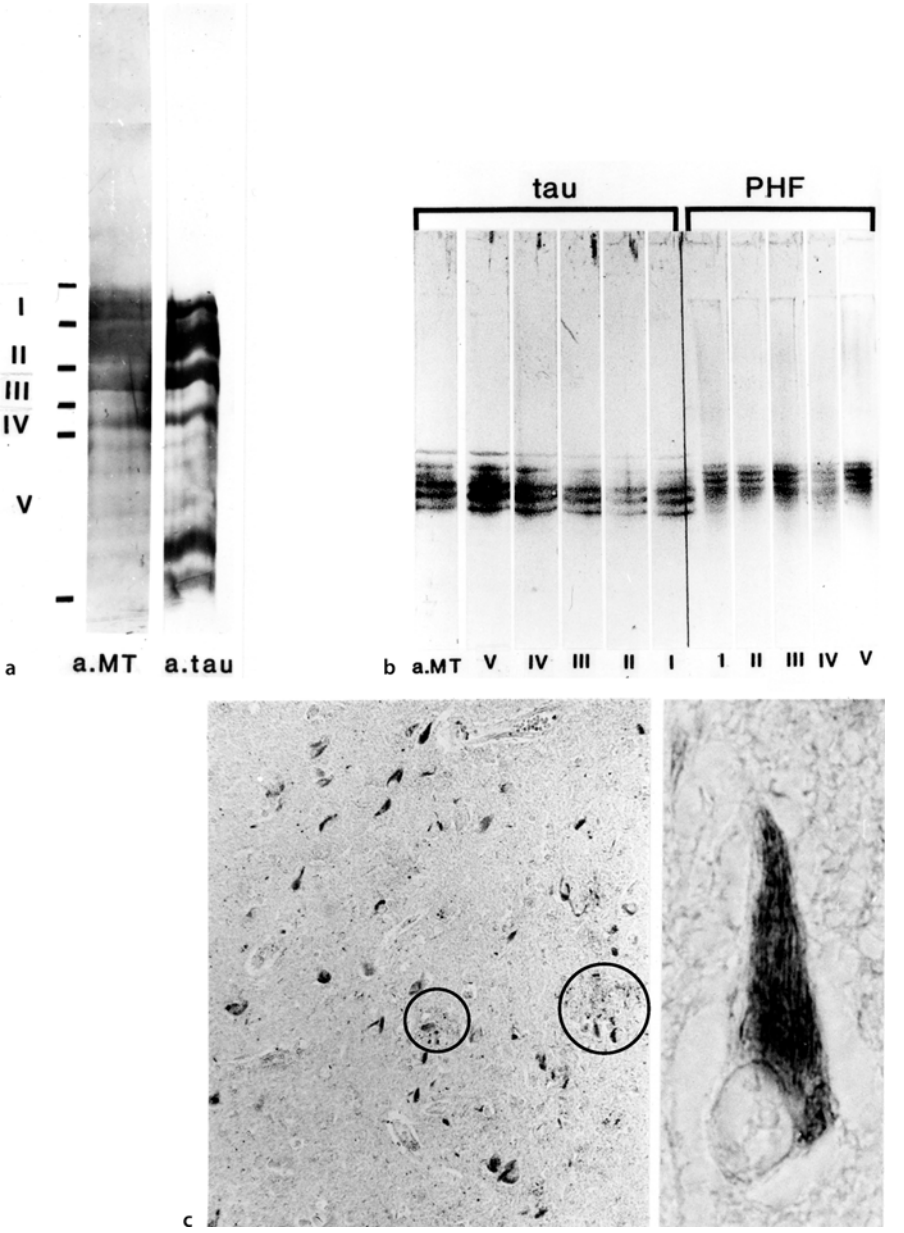


Fig. 1. Bulk isolation and polypeptide composition of neurofibrillary tangles/paired helical filaments from an AD brain. Electron micrographs (**a**) X 9,000; (**b**) X 60,000. (**c**) SDS-PAGE, lane 1, bovine brain filament polypeptide of 51 kDa; lane 2, total AD neuronal proteins; lane 3, same as in lane 2 plus tubulin; lanes 4, 5, 6, neuronal proteins from AD, adult control and young control, respectively; lane 7, tubulin; lane 8, PHF-enriched fraction; Tα and Tβ co-migrate with tubulin; NP sand EFT, a neuronal protein a new enriched protein (position indicated by a dot). (Reproduced with permission from Iqbal et al. 1974)

Employing PHF purified from AD brains, we also demonstrated colocalization of the PHF polypeptides with the six brain tau isoforms by SDS-PAGE (Grundke-Iqbal et al. 1986a). We were most excited that our systematic approach of the previous ~ 12 years had finally resulted in the identification of tau as a major protein subunit of PHF.

The immunohistochemical staining of PHF/neurofibrillary tangles with antibodies to neurofilaments (Ishii et al. 1979), vimentin (Yen et al. 1983), HMW-MAP (Kosik et al. 1984), somatostatin (Roberts et al. 1985), and tau (Brion et al. 1985a; Ihara et al. 1986; Delacourte and Defossez 1986; Kosik et al. 1986; Wood et al. 1986) was observed by several labs. However, immunohistochemical crossreactivity between two proteins does not necessarily allow one to assume any precursor-product relationship. The size of an antigenic site detected by an antibody is relatively small, and identical or closely related antigenic sites comprising a few amino acid residues have been found on molecules that are otherwise unrelated.



Discovery of the abnormal hyperphosphorylation of tau

In 1985, the generation of monoclonal antibody Tau-1 to tau was reported (Binder et al. 1985). When we tested Tau-1, we found that, although it reacted very nicely and specifically with all six isoforms of normal brain tau on Western blots, it immunostained

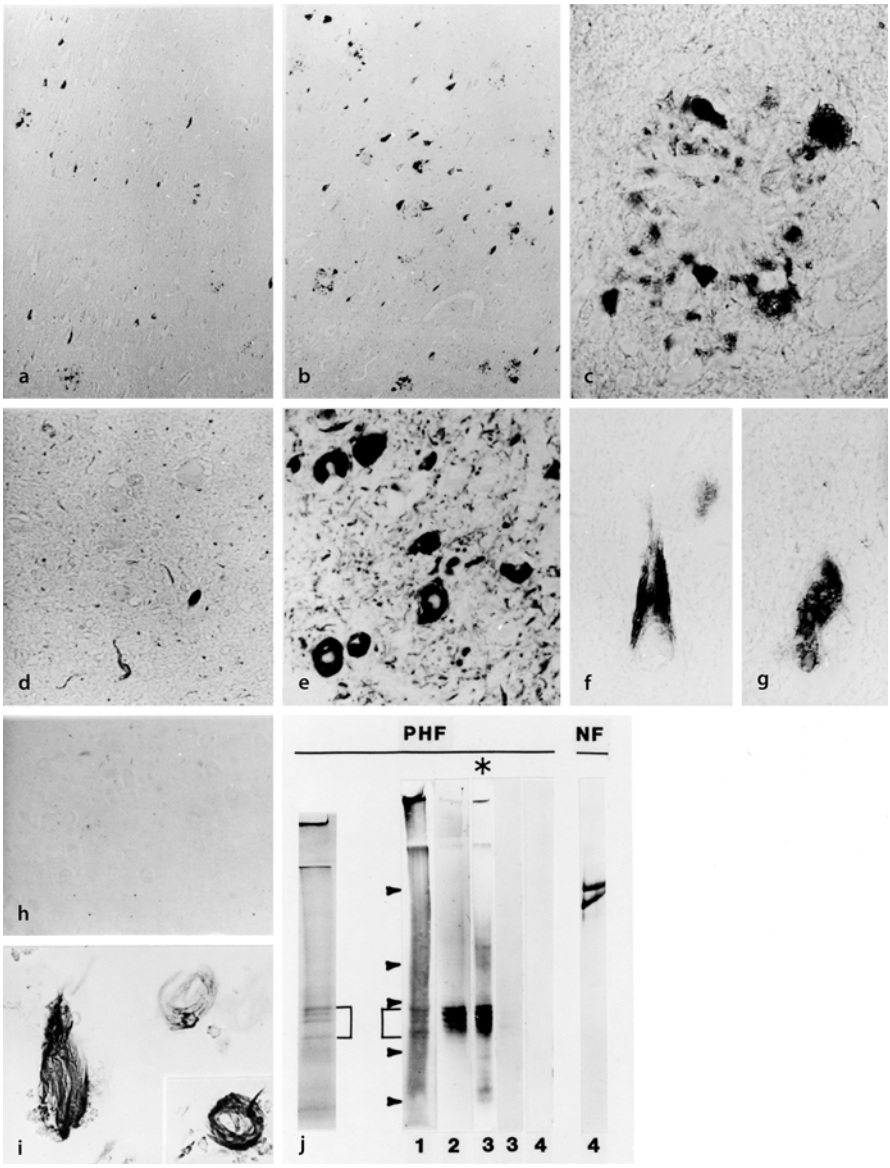
Fig. 2. Labeling of tau and PHF polypeptides on Western blots and of tangles and plaque neurites on tissue sections with antibodies purified by immunoaffinity from five different molecular species of tau. For **a**, tau (Method 3) was resolved by SDS-PAGE and transferred to nitrocellulose paper. Strips were cut from the sides and developed with anti-MT (PHF) serum (a. MT) and monoclonal antibody to tau (a, tau). The remaining blot was saved for affinity isolation of anti-tau antibodies from the anti-MT(PHF) serum. Roman numerals (I–V) indicate the areas of tau species from which the antibodies were purified. In **b**, immunoblots of tau and PHF polypeptides with affinity-purified antibodies from tau polypeptides of areas I–V are shown. Differences in the staining intensities of the different antibodies are due to small individual variations in the amounts of the samples applied to the gel. **c** shows immunocytochemical staining of tangles (some of the tangles *marked with arrows*) and neurites of plaques (*marked with circles*) in paraffin sections of Alzheimer hippocampus with antibodies eluted from tau area I (*left panel*); the background staining might correspond to the normal distribution of tau. The *right panel* shows at high magnification a neuron with the fibrils of its tangle darkly stained by the antibody. Original magnifications: *left panel*, X 130; *right panel*, X 1500. Identical staining was obtained with antibodies eluted from the other four tau areas; plaque amyloid was not stained with any of these five antibodies. (Reproduced with permission from Grundke-Iqbal et al. 1986a)

only a small number of neurofibrillary tangles in AD brain sections compared to our anti-PHF serum and immunoaffinity-purified tau antibodies. We wanted to know the reason for this discrepancy. At that time it was not known if Tau-1 was phosphodependent. We undertook this study and found that Tau-1 labeled practically all tangles and plaque neurites when tissue was first pretreated with alkaline phosphatase. This finding suggested that Tau-1 was phosphodependent and recognized only dephosphorylated tau at the epitope recognized by this antibody, and that tau in tangles/PHF was in an abnormally phosphorylated state (Grundke-Iqbal et al. 1986b). Furthermore, we found that Tau-1 could label PHF polypeptides on Western blots only when they were first dephosphorylated, either in a test tube prior to SDS-PAGE or on the nitrocellulose membrane used for the Western blots (Fig. 3). Because tau, a phosphoprotein, was phosphorylated in PHF/AD brain differently than that from normal brain tau, we coined the term "the abnormally phosphorylated tau" (Grundke-Iqbal et al. 1986b).

We collected several AD and control brains between two and five hours postmortem and processed them for up to two cycles of in vitro assembly of microtubules. We observed that we could assemble microtubules from control aged brains but not from AD brains. We could, however, assemble microtubules from both AD and control brains by DEAE-Dextran, a polycation that mimics tau in promoting microtubule assembly. We traced the in vitro microtubule assembly defect in AD brain to the presence of abnormal hyperphosphorylated tau in the cytosol (Iqbal et al. 1986). This was the first study demonstrating a functional impairment of the abnormal hyperphosphorylation of tau in AD brain and opened a whole new major area of research and drug development.

Subsequent major findings

Our studies on the isolation and protein composition of PHF (Iqbal et al. 1974) and the discoveries of tau as the major protein subunit of PHF (Grundke-Iqbal et al. 1986a), its abnormal hyperphosphorylation (Grundke-Iqbal et al. 1986b), and its inhibition



of microtubules in AD brain (Iqbal et al. 1986) have led to several discoveries, which include identification of (1) the normal level of normal-like tau and of several-fold increase in abnormally hyperphosphorylated tau in AD brain (Khatoon et al. 1992); (2) the decrease in the activities of PP-2A and PP-1 in AD brain as a cause of the abnormal hyperphosphorylation of tau (Gong et al. 1993, 1995); (3) the sequestration of normal tau, MAP1, and MAP2 by the abnormally hyperphosphorylated tau as a molecular mechanism of neurofibrillary degeneration (Alonso et al. 1994, 1996, 1997); (4) the

Fig. 3. Immunocytochemical staining and Western blots showing the abnormal hyperphosphorylation of tau. (**a–c**, **f**, **g**) Sections of Alzheimer hippocampus and (**d**,**e**) temporal cortex; (**h**) section of hippocampus of an 80-year-old non-AD individual; (**i**) tangle-enriched preparation that had been washed twice with 2%(wt/vol) $NaDodSO_4$ in a boiling water bath. (**b**, **c**, **e–h**) Sections were dephosphorylated with alkaline phosphatase prior to immunolabeling with mAb Tau-1; (**a** and **d**) nondephosphorylated controls; adjacent sections and corresponding areas to **b** and **e** treated identically except that the alkaline phosphatase was substituted with buffer. Numbers of immunostained tangles, plaques, and neuropil threads are very much increased in the dephosphorylated tissue sections in **b** and **e** as compared to the control treated sections in **a** and **d**. (**c**) Staining of plaque neurites but not of central core amyloid; (**f**) a neuron with immunolabeled tangle extending into the apical dendrite; (**g**) a neuron with granulovacuolar inclusions; (**h**) no staining is seen in the non-Alzheimer hippocampus even after dephosphorylation. (**a**, **b**) X 75; (**c**, **f**, **g**, and **i**) X 750; (**d**, **e**) X 300; (**h**) X 150. (**j**) Western blots of PHF polypeptides with (*lane 1*) antiserum to isolated PHF, 1 : 1,000 dilution; (*lane 2*) PHF-reactive anti-microtubule serum, 1 : 3,000 dilution; (*lane 3*) mAb Tau-1 at 0.1 µg/ml on dephosphorylated (*) and nondephosphorylated blots and (lane 4) blots of PHF and neurofilament (NF) polypeptides with mAb to NF, SMI 34, 1 : 10,000 dilution. (#) The dephosphorylation of PHF polypeptides on the paper blots was carried out with alkaline phosphatase (43 µg/ml) before incubation with antibody. Arrowheads indicate positions of Mt markers from top to bottom: myosin (200,000), phosphorylase *b* (92,500), bovine serum albumin (68,000), ovalbumin (43,000), α-chymotrypsinogen (25,700). Not shown in this figure, even at a 10-fold increase in the antibody concentration mAb SMI 34 did not label PHF polypeptides. The background smear and the low M_t bands in lane 1 most probably represent oligomers and breakdown products, respectively, of the PHF polypeptides (3–5); similar immunostaining pattern is obtained with mAb to PHF (5). The far left lane shows the Coomassie blue-stained polypeptide pattern of isolated PHF (5-030T acrylamide gradient). (Reproduced with permission fro Grundke-Iqbal et al. 1986b)

promotion of the self-assembly of tau into tangles of PHF by its hyperphosphorylation (Alonso et al. 2001); (5) the intraneuronal localization of Aβ in both tangle-bearing and non-tangle-bearing neurons in AD brain (Grundke-Iqbal et al. 1989); (6) the abnormal glycosylation of tau in PHF (Wang et al. 1996); and (7) the identification of subgroups of AD based on CSF markers (Iqbal et al. 2005).

Acknowledgements. We would like to share the credit for our discoveries with all collaborators, which included a large number of senior scientists and, in our own research group, several graduate students, postdoctoral fellows, and young scientists. We acknowledge Robert D. Terry, at whose initiative and with whose support we entered the AD field. Henry M. Wisniewski carried out the morphological evaluation of the PHF preparations for our first study and stood with us during the PHF solubility controversy in the early 1980s. Sabiha Khatoon's discovery of normal level of normal-like tau and of the several-fold increase in abnormally hyperphosphorylated tau in AD not only laid the foundation of subsequent studies on the molecular mechanisms of neurofibrillary degeneration but also stimulated research on studies on the CSF levels of tau and phosphotaus as potential diagnostic biomarkers. Cheng-Xin Gong's discovery of the decrease in PP-2A/PP-1 activities in AD brain identified a cause of abnormal hyperphosphorylation of tau and demonstrated the involvement of these phosphatases in neurofibrillary degeneration. Alejandra del C. Alonso discovered the sequestration of normal tau, MAP1, and MAP2 by the abnormally hyperphosphorylated tau as a key likely step in the molecular mechanism of neurofibrillary degeneration and demonstrated that abnormal hyperphosphorylation alone is sufficient to cause self-assembly of tau into tangles of PHF. Jian-Zhi Wang found that neurofibrillary tangles/PHF could be dissociated by PP-2A or PP-2B, releasing dephosphorylated tau, which was biologically

active in promoting microtubule assembly. She also discovered that, unlike normal brain tau, tau in PHF was glycosylated and that the generation of AD-like abnormally hyperphosphorylated tau, which results in its self-assembly into PHF, requires catalysis by cdk5 and GSK-3β, or one of these kinases along with PKA and CaMKII. Jin-Jing Pei demonstrated the association of GSK-3β and cdk5 with neurofibrillary pathology from early Braak stages. Toshihisa Tanaka generated the first cell culture model of neurofibrillary pathology. Fei Liu discovered that O-GlcNAcylation and phosphorylation of tau reciprocally regulate each other and that, due to decreased brain glucose metabolism, there is most likely a global decrease in O-GlcNAcylation in AD brain. Hitoshi Tanimukai and Ichiro Tsujio discovered a decrease in the mRNAs of PP-2A inhibitors and translocation of the N-terminal half of the inhibitor-2 of PP-2A from neuronal nucleus to the cytoplasm in AD brains. Last but not least, our two research assistants, Tanweer Zaidi and Yunn Chyn Tung, helped develop the technique for the bulk isolation and solubilization of PHF, and the generation of antibodies and the identification of tau as the major component of PHF, respectively.

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André Delacourte

The natural and molecular history of Alzheimer's disease: Tau is part of the story

André Delacourte[1]

Summary

Alzheimer's disease (AD) is a very common brain pathology of the elderly, with an etiology that is far more complicated than was thought in the 1990s. In particular, the complexity comes from the coexistence of two degenerating processes, tau aggregation and Aβ deposition, that affect polymodal association brain areas, a feature never observed in non-human primates and one that is difficult to model. Genetic studies have shown that AβPP plays a central role in familial and sporadic AD, but the role of tau has been understated for a long time. The first evidence of this came from the demonstration of the concept of pathological tau proteins in AD and their full biochemical characterization as a major triplet (tau 60, 64, 68) plus tau 72. This concept was extended to most neurodegenerative diseases with dementia, since pathological tau proteins present a disease-specific bar code: a major upper doublet for parkinsonian diseases with dementia (PSP, CBD, Guadeloupe), a lower doublet for Pick's disease, and a single band for myotonic dystrophy. This bar code results from the aggregation of specific tau isoforms and was merged with tau mutations in familial frontotemporal diseases as a more global concept of tauopathy linked to most dementing neurodegenerative disorders .

To apprehend the role of tau in AD, which is 99% sporadic, we have developed a spatio-temporal analysis of tauopathy in many brain areas of hundreds of non-demented and demented patients. This prospective and multidisciplinary study showed us that tauopathy always progresses in the brain along a very precise and invariable pathway, from the entorhinal to the hippocampal formation to polymodal association areas, ending in primary regions and in many subcortical areas. The cognitive impairment follows exactly the progression of the affected brain regions. In strict parallel, neocortical Aβ deposits increase in quantity and heterogeneity, suggesting a direct link between both neurodegenerative processes. Altogether, our molecular studies suggest that AD is a tauopathy fueled by AβPP dysfunction. Restoring AβPP loss of function seems to be the most efficient and pragmatic therapeutic approach.

Introduction

First of all, scientists never forget that Alzheimer's disease (AD) is a devastating disease, not only for the patient but also for the family, but from a scientific point of view, AD is

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an exciting field of research. At present, we know that this disease is more complicated than expected, with numerous risk factors. Therefore, finding the right area of research for the scientist working in the Alzheimer field is quite a challenge.

AD is a very complicated disease at the physiopathological level, as was observed by Aloïs Alzheimer himself, who discovered this organic dementing disease with two types of lesions: tangles inside neurons and plaques outside, in the vicinity of degenerating neurons. Aloïs Alzheimer was probably aware of the importance of intraneuronal lesions, since he also discovered the specific lesions of the fronto-temporal dementia characterized by Arnold Pick, namely Pick bodies of Pick disease. But we should not forget the other early pioneers: Beljahow (1889), Marinesco (Blocq and Marinesco 1982), Redlich (1898), and Leri (1906).

One century after the princeps paper of Aloïs Alzheimer, the question of the role of plaques versus tangles is still a matter of debate. Which lesion is the cause, which one is a consequence, and more importantly, which one will lead to a treatment?

The complexity of the approach comes from the fact that, on the one hand, the disease is exclusively present in the brain but that, on the other hand, the brain is inaccessible to molecular investigations and well protected behind the blood-brain barrier, the skull and by our cultural, social or religious rules.

Also, AD is one of the rare diseases that is totally specific to the human species. In very old non-human primates, such as the baboon or the rhesus monkey, the presence of tangles is strictly limited to the entorhinal or the hippocampal formation. The basic NIA neuropathological criteria of AD, namely plaques and tangles in the association neocortex, have never been found in other non-human species (Hartig et al. 2000; Schultz et al. 2000).

Aggregated and hyperphosphorylated tau proteins: a powerful marker of neurofibrillary degeneration

Tau proteins are the basic component of neurofibrillary degeneration (NFD), as observed using histological (Brion et al. 1985c) and biochemical means. Using Western blots, we were able to detect and quantify abnormal tau species in AD brains, as they are aggregated, hyperphosphorylated and abnormally phosphorylated (Delacourte and Defossez 1986), in good agreement with the pioneer work of Brion et al. (1985c) and Grundke-Iqbal et al. (1986b). In addition, we were able to develop the concept of pathological tau proteins in AD and, later on, in many other neurodegenerative disorders with dementia. First we detected two abnormal bands in neocortical areas of AD patients (Tau 64 and 68; Flament et al. 1989) (MW are those given in the literature these days) and then a third one using more specific antibodies (Tau 60; Fig. 1; Delacourte et al. 1990). These pathological Tau bands were specifically detected by an anti-PHF absorbed with normal tau proteins. The antibody Alz-50 of Peter Davies, which detects NFD and a group of pathological proteins named A68 so well, was in fact those abnormal tau proteins Tau 64 and 68 (Flament and Delacourte 1990). Our results were corroborated by Lee et al. (1991). At last, using 2D gels and our knowledge that tau proteins contain six isoforms, as shown by Goedert et al. (1992), we demonstrated the presence of a minor and fourth abnormal tau protein at 72 kDa among the bulk of aggregated tau proteins, corresponding to the largest tau isoform (Fig. 1; Sergeant et al. 1997). With a direct

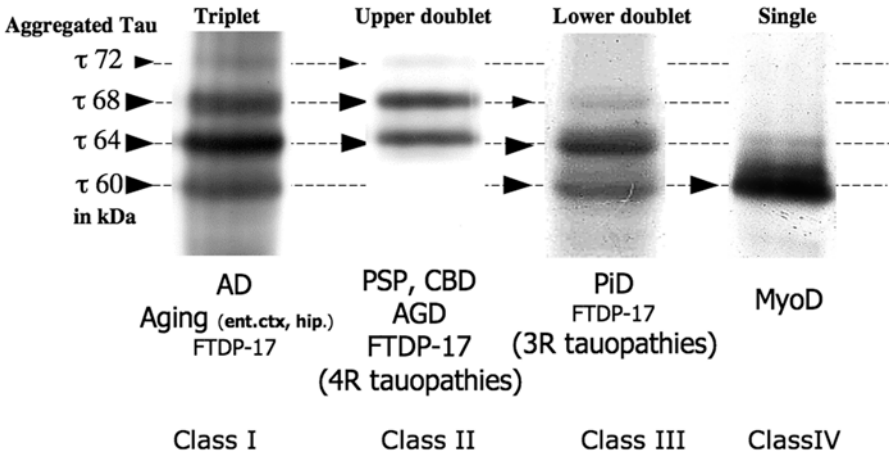


Fig. 1. The bar code of tauopathies. Western blot immunostaining of aggregated tau proteins from brain homogenates of patients affected by Alzheimer's disease (AD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), argyrophilic grain disease (AGD), Pick disease (PiD), dystrophic myopathy (MyoD) and frontotemporal dementia linked to chromosome 17 (FTDP-17). Aggregated tau proteins are specifically stained by AD2, a monoclonal antibody against a phosphorylation site on Ser 396 and 404 of tau. Note that the electrophoretic profile is different for each group of diseases. This is due to the aggregation of specific sets of tau isoforms: all six isoforms for AD, also observed in the entorhinal and hippocampal (ent.ctx, hip.)cortex of aged controls, as well as a few rare diseases including some FTDP-17 with mutations outside exon 10 area; the three isoforms with 4 repeats for PSP, CBD, AGD and most FTDP-17 (4R tauopathies); three repeats for PiD and a few FTDP-17 (3R tauopathies). In MyoD type I and II, the shortest tau isoform is involved in tau aggregates

approach, and to cope with the problem of dephosphorylation during post-mortem delays, we demonstrated that tau are abnormally phosphorylated, since post-mortem tau from AD patients (under the influence of post-mortem dephosphorylation) are more acidic that native tau from post-operative (not dephosphorylated) human brain biopsies (Sergeant et al. 1995).

Interestingly enough, using the same approach, we demonstrated that these tau aggregates were different in other neurodegenerative dementing disorders and that there is a code-bar of tauopathies (Fig. 1). In progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), we observed a specific characteristic upper doublet (Tau 64 and 69), due to the aggregation of tau isoforms with 4 repeats (4R tauopathy; Flament et al. 1991; Buee Scherrer et al. 1996; Sergeant et al. 1999), whereas in Pick's disease, there is a lower doublet (Tau 60 and 64), resulting from the aggregation of 3R isoforms Buee Scherrer et al. 1996; Delacourte et al. 1998; recently confirmed by Dickson and co-workers de Silva et al. 2006). Other diseases have other tau profiles, such as a single band in myotonic dystrophy (DM1; Vermersch et al. 1996; Sergeant et al. 2001) and soluble tauopathy in dementia lacking distinctive histology (DLDH; Vermersch et al. 1995). For DLDH, a heterogeneous group, Zhukareva et al. (2000) clearly showed that a subgroup has a dramatic decrease of normal tau proteins levels.

All these specific biochemical signatures and different sets of tau isoforms aggregated in specific subsets of neuronal populations began to demonstrate that tangles are not such a unique and late answer to different types of neuronal insults. Indeed, many dementia disorders result from a defect of tau proteins, and tau mutations are causal in familial frontotemporal dementia (FTD; Goedert and Spillantini 2000). Our concept of pathological tau proteins was a basis for a more global concept of tauopathy, adapted first for familial diseases but also true for sporadic diseases. Therefore, the question was to determine the contribution of tau pathology to AD etiology.

The spatio-temporal biochemical pathway of tau pathology in aging and sporadic AD

Tau pathology spreading in cortical areas is invariable and hierarchical

A prospective and multidisciplinary study of more than 200 cases, including 70 non-demented patients, was undertaken. We gathered clinical and neuropathological data and, in parallel, studied the presence of NFD at the biochemical level, using the triplet of abnormal tau proteins as a marker. In Alzheimer brains, we observed that tau pathology always extended along 10 stages, corresponding to 10 brain areas that are successively affected. Paired helical filaments (PHF)-tau pathology was systematically found to be present in variable amounts in the entorhinal and hippocampal regions of non-demented patients aged over 75 years. When tau pathology was found in other brain areas, it was always along a stereotyped, sequential, hierarchical pathway (Fig. 2). The progression was categorized into 10 stages according to the brain regions affected: transentorhinal cortex (S1), entorhinal cortex (S2), hippocampus (S3), anterior temporal cortex (S4), inferior temporal cortex (S5), mid temporal cortex (S6), polymodal association areas (prefrontal, parietal inferior, temporal superior) (S7), unimodal areas (S8), primary motor (S9a) or sensory (S9b, S9c) areas, and all neocortical areas (S10; Delacourte et al. 1999).

The mechanism of progression of tau pathology

Determining the mechanism of the spread of tauopathy is likely to open relevant therapeutic avenues in the neuroprotection domain. From the study of AD, we observe that this spreading is not diffuse but, on the contrary, along precise neuron-to-neuron connections, from the limbic structures toward the neocortical association areas. Interestingly enough, we observe a similar mechanism of spreading in other sporadic tauopathies, such as PSP. Neurodegeneration in PSP is observed first in the brain stem, then in the striatum, the primary motor frontal neocortical area (Broadmann area 4), the unimodal frontal areas and at last spreading into all neocortical and limbic areas (Sergeant et al. 1999). In other words, the basic mechanism of tau spreading in sporadic tauopathies likely starts in a specific vulnerable neuronal population (layer II of the entorhinal formation in AD; occulomotor nuclei for PSP). Then, this local tauopathy destabilizes the connected neuronal populations that had a cross-talk of neurotrophic factors with the primary set of vulnerable neurons, and this degenerating process will

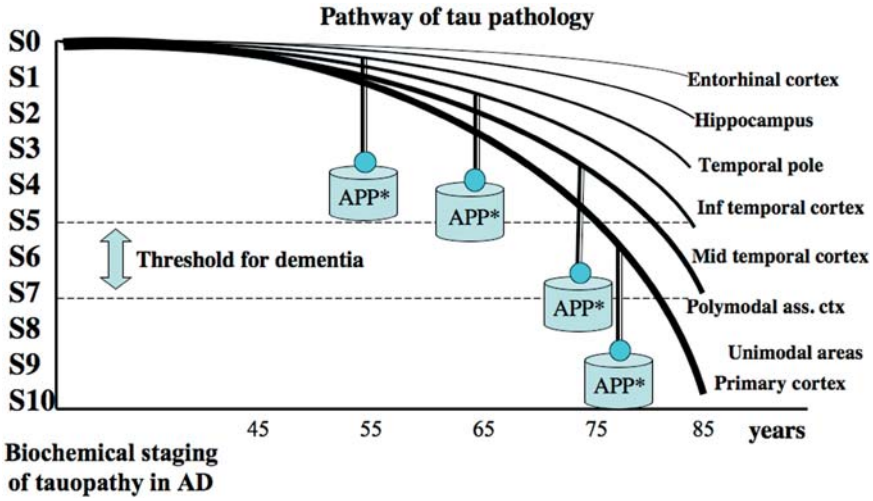


Fig. 2. Pathway of tau pathology in aging and in AD. First, neurofibrillary tangles (i.e., tau pathology) are age-related but not age-dependent brain lesions. They appear in the entorhinal cortex of 20% of people with an average age of 25 years. The ratio increases at 50% at the age of 50 years to affect all people at the age of 70 years or older, as shown by Braak and Braak 1997 and in our study. This vulnerability varies dramatically among individuals. A few nonagenarians in our study were very mildly affected. Therefore, the entorhinal formation is a vulnerable area that is always affected by tau pathology at old age (stages 1 and 2 of tau pathology). Second, tauopathy in aging tends to spread from the affected vulnerable area to other connected neuronal populations, along a neuron-to-neuron propagation that resembles a chain reaction or a domino effect. This spreading can be observed up to the temporal pole (stage 4 of tau pathology) without Aβ deposition. Third, the extension of tauopathy toward polymodal association areas is systematically observed in the presence of Aβx-42 deposits (amyloid stage of 1 to 4), as if these aggregates, directly (neurotoxicity) or indirectly (markers of AβPP dysfunction) were fueling tau spreading. This step represents the beginning of incipient AD. Fourth, after neocortical extension of tauopathy, when neuroplasticity will no longer be able to compensate for the progressing neurodegenerative process, clinical impairment and dementia will appear. The cognitive impairment observed in AD is well explained by the brain areas that are successively affected by tau pathology, from mild cognitive impairment (stages 3 to 6) to the different AD stages, from stage 6 to stage 10 of tau pathology. The amyloid burden will also increase (stages 5 to 10), paralleling tau staging. Fifth, tau pathology will continue its conquest of the brain, through primary regions and subcortical areas, to kill the patient, directly or indirectly

extend, with a domino effect, to other neuronal populations through a neuron-to-neuron propagation phenomenon (Delacourte 2000). Understanding this mechanism of propagation better will certainly open up therapeutic strategies for AD as well as for other sporadic tauopathies and synucleopathies (Deramecourt et al. 2006).

The relationship between tauopathy and amyloidosis in aging and sporadic AD

It is not surprising that tau pathology is well correlated to cognitive impairment, since it shows the neurodegeneration process and its extent. However, we do not know the

factors that generate tauopathy and its extension in brain areas. AβPP dysfunction is the best candidate, as revealed by genetic studies. Therefore, we quantified all AβPP metabolic products to locate a possible relationship with the different stages of tau pathology. AβPP holoproteins, AβPP-CTFs and Aβ species were analyzed in the different brain areas of all our non-demented and demented patients.

First, Aβ species were studied. Insoluble Aβ-42 and -40 species were fully solubilized and quantified, and we were able to propose a biochemical staging of amyloidosis on a scale from 0 to 10 for the quantification of either Aβ40 or Aβx-42 aggregates (Deramecourt et al. 2006; Delacourte et al. 2002a).

Surprisingly, we observed a parallel and synergistic effect of AβPP dysfunction (as visualized by Aβ deposition) on the neuron-to-neuron propagation of tau pathology. Indeed, tau pathology can be found in the hippocampal area without Aβ deposits, as mentioned by Braak and Braak (1997b). In contrast, the extension of tau pathology in polymodal association areas was systematically found in the presence of Aβ deposits (Aβ stages 4 to 10), as if these Aβ species, directly or indirectly, were necessary to stimulate the progression of tau pathology (Fig. 2). Altogether, our results clearly demonstrated that amyloid deposits do not precede tau pathology in sporadic AD, as claimed in the amyloid cascade hypothesis based upon familial cases (Hardy and Higgins 1992b; Hardy and Selkoe 2002). Also, a systematic analysis of tauopathy, amyloidosis and synucleopathy in sporadic Lewy body disease (LBD) revealed a similar pattern. Indeed, the extension of synucleopathy in neocortical areas is observed in the presence of amyloid deposits (Deramecourt et al. 2006). Interestingly enough, our proteomic analysis of the first Aβ42 deposits that appear in the aging human brain and in incipient AD are not the full length Aβ1-42, but N-truncated species. In other words, the first Aβ species that initiate amyloidosis are not physiological species, but pathological species. This finding was observed at the biochemical and immunohistochemical levels, in the brain of patients affected by AD but also LBD (Deramecourt et al. 2006). This discovery could improve dramatically the vaccination approach (Sergeant et al. 2003).

Relationship between Tau pathology and AβPP dysmetabolism

The parallelism and synergy between tau and Aβ aggregation led us to search an AβPP molecular event linking the two degenerating processes. AβPP is a ubiquitous protein found in all cell types of all species, suggesting a basic and important role that remains to be identified. A neurotrophic activity for AβPP and secreted s-AβPP is often mentioned (Turner et al. 2003). Therefore, a loss of function of AβPP rather than a gain of toxic function of Aβ could also be a reasonable hypothesis to explain the stimulation of tau pathology and neurodegeneration (Fig. 2). Complementary to this study of Aβ species, we found no obvious modification of AβPP holoprotein, but all AβPP-CTFs were found to be significantly diminished during the course of AD and well correlated with the progression of tau pathology (Sergeant et al. 2002). An important role of gamma stub, also named AICD (AβPP intracellular domain), as a possible transcription factor could explain its involvement in the disease if these fragments are lacking (Cao and Sudhof 2001; von Rotz et al. 2004; Pardossi-Piquard et al. 2005).

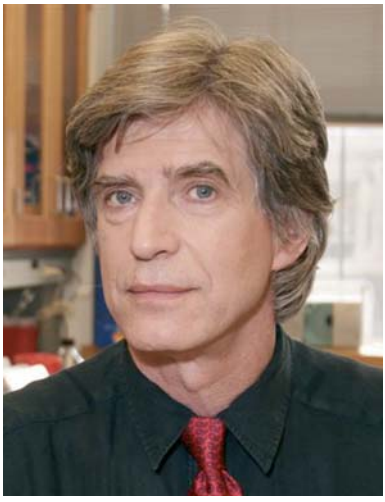
In fact these observations directly lead to other therapeutic strategies concentrated around the concept of a loss of function of AβPP stimulating tau pathology, in good agreement with other teams who contend that Aβ may be a planet, but AβPP is central (Neve and Robakis 1998; Neve 2001; Lee et al. 2004c). From our study on tau and Aβ in the human brain, the stimulation of the non-amyloidogenic pathway seems to be the more powerful and less risky way to decrease Aβ production and simultaneously to stimulate the production of sAPPalpha, a neurotrophic factor, and AICD, a possible transcription factor, which should delay tau pathology (Delacourte 2006).

Conclusion

Altogether, many converging studies show that AD is not a pure pathology of Aβ; neither is it a pure tauopathy. We propose the following definition: AD is a tauopathy fueled by AβPP dysfunction (Fig. 2). The natural and molecular history of sporadic AD shows that both AβPP and tau are equally involved in the etiopathogenesis (Fig. 2). Both are also therapeutic targets and the good news is that βAPPists and tauoists must work together. From observations of the human brain, relevant animal models are most likely those that will demonstrate a synergy between AβPP and tau lesions. Some interesting models have already been described (Gotz et al. 2001; Lewis et al. 2001). Another one with a severe neuronal loss is also interesting to understand the loss of function of AβPP as well as the role of intracellular Aβ deposition (Casas et al. 2004).

At last, one can see that most dementing neurodegenerative disorders are tauopathies, that most demented patients have a tau pathology in neocortical areas, and that many different types of tau dysfunction lead to dementia: mutations on tau gene in FTDP-17 (frontotemporal dementia with Parkinsonism linked to chromosome 17; Spillantini et al. 1998c); the haplotype H1H1, which is a risk factor for PSP and CBD (Baker et al. 1999); the abnormal tau splicing in DM1 (Sergeant et al. 2001); tau-less DLDH (Zhukareva et al. 2001); and the vulnerability of specific brain areas to tauopathy, as observed in the entorhinal cortex and hippocampus for AD (Delacourte et al. 2002b) or in the brain stem nuclei for PSP and CBD (Sergeant et al. 1999; Dickson 1999; Caparros-Lefebvre et al. 2002). In conclusion, tau is a key player in most dementing neurodegenerative disorders.

Virginia M.-Y. Lee



John Q. Trojanowski

Tau focused drug discovery for Alzheimer's disease and related Neurodegenerative Tauopathies

Virginia M.-Y. Lee[1] and *John Q. Trojanowski*[1]

Alzheimer's disease (AD), like most neurodegenerative disorders, results from the aggregation of misfolded proteins that deposit as fibrillar amyloid lesions in the central nervous system (CNS), where they are thought to be toxic and compromise brain function (reviewed in Forman et al. 2004; Skovronsky et al. 2006). For example, neurofibrillary tangles (NFTs) and senile plaques (SPs) were first recognized by Alois Alzheimer at the beginning of the 20th century as the diagnostic hallmark lesions of AD; it is now known that NFTs are formed by abnormal tau filaments in neurons whereas SPs are composed of extracellular deposits of fibrillar Aβ (Forman et al. 2004; Skovronsky et al. 2006). Although the discovery of pathogenic mutations in the genes encoding tau and the Aβ precursor protein in familial neurodegenerative disorders definitively implicated these proteins in disease pathogenesis, the mechanisms whereby brain degeneration results from NFTs and SPs still are not entirely clear. Since this special issue celebrates the centennial of Alois Alzheimer's seminal description of AD and highlights the many remarkable advances in understanding this disorder following Alzheimer's initial report, we provide a brief perspective from our vantage point in the AD research field on how insights into the pathobiology of NTFs can be translated into new disease-modifying therapies for AD and related neurodegenerative tauopathies.

In the 1980s, there was considerable controversy about the role of tau in the formation of paired helical filaments (PHFs) and NFTs in AD, and our contribution to the resolution of this controversy about the building blocks of PHFs (known at that time as A68 proteins) came when we showed in 1991 that abnormally phosphorylated CNS tau proteins (now known as PHFtau) form the PHFs in AD NFTs (Lee et al. 1991). Shortly thereafter, we also demonstrated that PHFtau was unable to bind to and stabilize microtubules (MTs) because it was hyperphosphorylated relative to normal tau (Bramblett et al. 1993). These observations led us to hypothesize that the conversion of normal brain tau into PHFtau disrupts intraneuronal transport due to the depolymerization of MTs as well as to the occlusion of axons and dendrites by aggregated PHFs. Figure 1 schematically summarizes key aspects of our hypothesis of PHFtau-mediated AD neurodegeneration. Briefly, our hypothesis predicted that the conversion of tau into PHFtau 1) disrupts MT-dependent neuronal transport mechanisms, 2) physically "blocks" intraneuronal transport due to accumulations of PHFs within affected neu-

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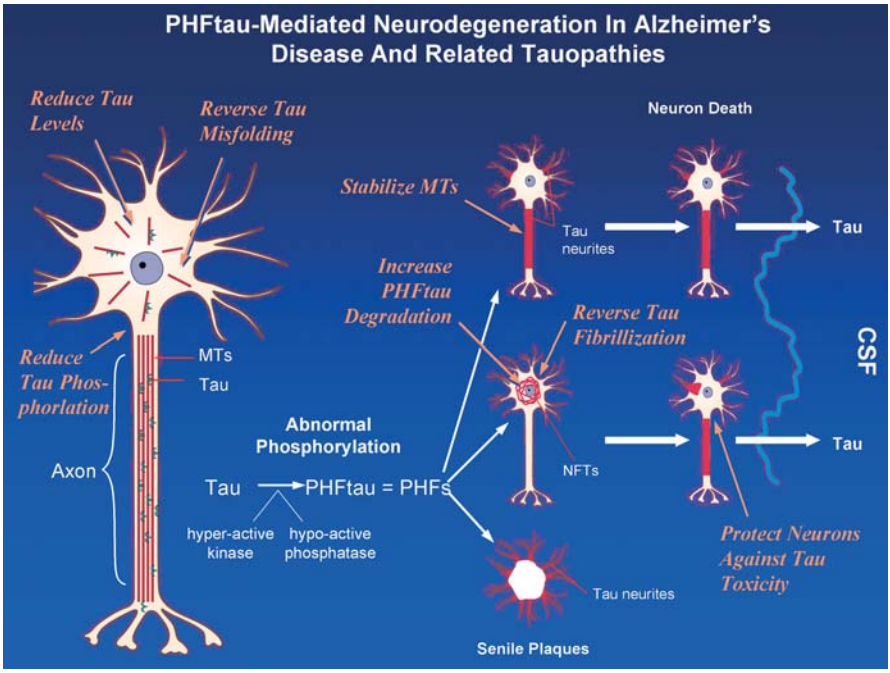

Fig. 1. The misfolding, fibrillization and sequestration of tau into filamentous PHFtau inclusions (e.g., NFTs and dystrophic tau neurites) are schematically depicted here. As described in greater detail in the text, these events compromise the survival of neurons by depleting levels of functional tau below a critical point, which results in the depolymerization of MTs and disruption of axonal transport. The release of tau from dying neurons may account for elevated levels of tau in cerebral spinal fluid (CSF), which is a biomarker of AD. The brown arrows and text indicate points of potential therapeutic intervention to ameliorate tau pathologies and their neurodegenerative consequences

rons and their processes, and 3) as a consequence of these events, neurons fail to export proteins from the cell body to distal processes and to retrieve substances (e.g., trophic factors) internalized at axon terminals, which compromises neuronal function and viability. Accordingly, by analogy with a railway transportation system, tau functions like the cross ties on railroad tracks (MTs) upon which trains (molecular motors) convey cargoes (organelles, proteins) to destinations on the railway network (sites in the perikarya and processes of neurons), so that the loss of a critical number of cross ties (loss of tau function when it is converted into PHFtau) disrupts the railroad tracks, leading to derailment of trains and the failure to deliver cargoes to their assigned destinations (impaired axonal transport) with deleterious effects on the railway network and the communities it serves (the dysfunction and death of affected neurons).

We proposed that these events would be sufficient to culminate in neuronal dysfunction and degeneration, leading to the onset/progression of AD. Indeed, we suggested in 1994 that MT-stabilizing compounds, such as the FDA-approved anti-cancer drug paclitaxel (Taxol), could be used for the treatment of AD by offsetting the loss of

tau function following its conversion into PHFtau (Lee et al. 1994). The subsequent discovery that tau gene mutations are pathogenic for hereditary frontotemporal dementia (FTD) with parkinsonism linked to chromosome 17 or FTDP-17 added further support to our hypothesis (Forman et al. 2004; Skovronsky et al. 2006), and there are data linking *tau* gene mutations to impaired binding of tau to MTs (Hong et al. 1998). Moreover, many predictions of this hypothesis have been validated experimentally in studies of tau transgenic animal models of AD-like neurodegeneration (Lee et al. 2005), including evidence that AD-like tau pathologies retard fast axonal transport (Ishihara et al. 1999).

Thus, evidence from diverse lines of research support the view that brain degeneration in AD could be a consequence of impaired intraneuronal transport, resulting from loss of function defects in tau and/or from toxic gains of functions by pathologically altered tau proteins, including their propensity to misfold, fibrillize and form AD NFTs in neurons (Forman et al. 2004; Skovronsky et al. 2006), and we recently provided proof of the concept that MT-stabilizing drugs may have therapeutic potential for the treatment of AD and other neurodegenerative diseases with prominent tau pathologies (Zhang et al. 2005). These studies were based on data summarized above that linked tau abnormalities to mechanisms underlying AD as well as to other neurodegenerative tauopathies, including rare forms of hereditary FTDP-17 caused by *tau* gene mutations (Forman et al. 2004; Skovronsky et al. 2006). As in AD, the neuropathological hallmarks of these other neurodegenerative tauopathies are inclusions (mainly, but not exclusively found in neurons) that are formed by accumulations of pathological tau filaments with properties similar to those of amyloid fibrils and AD PHFs, including the excessive phosphorylation of pathologically fibrillized tau. Thus, the AD-like tau pathologies in these other tauopathies also result in a loss of normal tau function. As a consequence thereof, axonal transport presumably is impaired in these other tauopathies, thereby leading to the dying back of axons as well as to the degeneration of neurons, just like in AD.

However, other strategies to develop novel therapies that target tau abnormalities in AD already are an increasing focus of AD drug discovery research (Fillit and Refolo 2005). For example, efforts are under way to develop novel therapies that target tau abnormalities are being investigated using high throughput screening (HTS) to identify drugs in large compound libraries that block the fibrillization and aggregation of tau or reduce tau protein levels (Dickey et al. 2005; Pickhardt et al. 2005). Additionally, preliminary data from HTS efforts that target inhibition of tau phosphorylation also appear promising, and proof of concept studies using LiCl to ameliorate tau pathology by inhibiting glycogen synthase kinase-3 (GSK-3) in a mouse model of a neurodegenerative tauopathy suggest that this is a fruitful avenue for drug discovery (Noble et al. 2005).

In conclusion, based on the data reviewed in this personal perspective on AD research on NFTs and PHFtau, we infer that MT-stabilizing drugs are worthy of further investigation for their therapeutic potential in the treatment of patients with neurodegenerative tauopathies, including AD. Indeed, many other tau-focused targets for the discovery of drugs to treat AD and related tauopathies are emerging from basic research on mechanisms of neurodegeneration, creating a sense of optimism that advances in understanding AD and related tauopathies will culminate in the discovery of more effective therapies for these disorders in the near future.

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Yasuo Ihara

Ubiquitin is a component of paired helical filaments in Alzheimer's disease

Yasuo Ihara[1]

Background

In 1982, I came to a conclusion that direct identification of the components of paired helical filaments (PHF) was extremely difficult because of their unusual insolubility in various detergents and denaturants (Selkoe et al. 1982b). Thus, I took an indirect immunochemical approach to identification of PHF components. Although we raised excellent polyclonal antibodies to PHF that revealed for the first time the presence of extensive neuropil threads in addition to neurofibrillary tangles (NFT) in the cortex affected by Alzheimer's disease (AD; Ihara et al. 1983, 1988), we saw no distinct bands. Instead, there was a smear on the blot of AD cortical homogenates (Ihara et al. 1983). I thought that monoclonal antibodies raised against purified PHF might serve to unambiguously identify these components. Thus, I started the PHF monoclonal antibody project as early as 1983, when I returned to University Hospital, as an assistant professor, from Dr. Dennis Selkoe's lab. Every day I was extremely busy working in the outpatient clinic and in the lab, and I always worked on Saturday and often even on Sunday. However, I found it very difficult, for unknown reasons, to raise significantly high titers of PHF antibodies in BALB/C mice. I knew that Dennis experienced the same difficulty.

In addition to this project, I did not give up further characterizing polyclonal antibodies to PHF. Nobuyuki Nukina, a senior resident in neurology, took an entirely different approach. Following a report on the purification of so-called SAF (scrapie-associated filaments), he prepared Sarkosyl-insoluble fractions from AD brains and digested a PHF-enriched fraction with proteinase K (Diringer et al. 1983). Unexpectedly, the digested material provided a ladder pattern of anti-PHF-reactive bands. The smallest and strongest band was at about 10 kDa (Nukina and Ihara 1985). This was the first time I saw a distinct anti-PHF-reactive band. Soon after, using a neonatal (three-month-old) Down syndrome brain, Nobuyuki went further and found a distinctly labeled (although somewhat broad) band at about 50 kDa on the blot of the soluble fraction. He quickly confirmed that this strong reactivity was not confined to Down syndrome brain but was a feature of fetal or neonatal brain. Thus, fetal brains contain a high level of anti-PHF-reactive protein that may be present in only trace amounts in adult or aged brains. Soon we found that anti-PHF antibodies intensely label tau on the blot, and we concluded that 1) tau is one of the components of PHF (Nukina and Ihara 1986) and 2) tau in PHF is phosphorylated (Ihara et al. 1986). Both conclusions were based on immunochemical observations.

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Collaboration with Hiroshi Mori in establishing DF2

In October 1984, after two years of constant exhaustion as the person in charge of the neurology outpatient clinic, I moved to the Tokyo Metropolitan Institute of Gerontology (director: Dr. Kazutomo Imahori) as the head of the Second Laboratory, Department of Physiology. I was almost free from clinical practice and was devoted to lab work every day, in efforts to identify the components of PHF. Dennis and I frequently discussed our progress by letter and over the phone. He wrote that he had read a paper suggesting that, where it proved difficult to obtain mouse monoclonal antibodies, it might be worthwhile to immunize a Lewis rat and to make mouse/rat hybrid hybridoma cells producing rat monoclonal antibodies of interest (Pike et al. 1982). As I had already spent more than one year in efforts to raise PHF mouse monoclonal antibodies without success, I was inclined to try the Lewis rat (I was surprised by the large size of the animal!), and I refocused the monoclonal antibody project in the Institute. I had the feeling that tau was not the only component of PHF, because several monoclonal antibodies to neurofilaments were reported to stain NFT in tissue section (Anderton et al. 1982). Neurofilaments might be another component of PHF. Two Lewis rats were immunized with purified PHF and, after four weeks, were given booster injections every one or two weeks. In contrast to mice, both Lewis rats gave increasingly strong antisera, which are still kept in a deep freezer in my lab.

In the meantime, while working at the Department of Neurology (1978–81), I always saw doctoral student Hiroshi Mori working in the Department of Neurochemistry, next to our department, whenever I went to the lab on weekends. We soon began to chat about many things, and I heard that he purified neurofilaments for the first time and determined the subunit structure (Mori and Kurokawa 1980). I also learned that he was eager to apply the same subcellular fractionation protocol to PHF purification. This surprised me, as it was exceptional for PhD students in Japan to be interested in the mechanism of AD. During my stay in Boston (1981–82), he moved to Osaka and subsequently to Fukui. He seemed to be unhappy with the position in Fukui, as facilities were quite limited. I called him to confirm his continued willingness to work on PHF and proposed a collaboration for the monoclonal antibody project.

Following the final PHF injection in early March 1985, I took a cage containing Lewis rats to Fukui, where it was snowing. Hiroshi struggled at fusion between mouse myeloma cells and rat splenocytes from huge spleens. As a first step, he screened the culture media using ELISA in which purified PHF were immobilized on the plate and found 43 positive clones. He sent me culture media from these clones for a second immunostaining screening using nondenatured NFT smeared on glass slides (Ihara et al. 1983). I initially identified 23 positive media that labeled NFT, but these hybridoma clones were rapidly lost, presumably because of the instability of mouse/rat hybrid cells. Only two clones were left in our hands. These produced antibodies with similar specificities. Subsequently, one clone was used exclusively, and Hiroshi named the monoclonal antibody that was produced DF2 (Dementia Filament 2).

Before 1986, a number of antibodies had been claimed to stain NFT, and thus there was much confusion about the true PHF components. I set one criterion. If a particular antibody really bound PHF, it should immunolabel SDS-treated (stripped) NFT. In this context, DF2 genuinely recognized the PHF framework (Mori et al. 1987). Consistent with this observation, DF2 intensely immunolabeled an extensive smear on the blot of

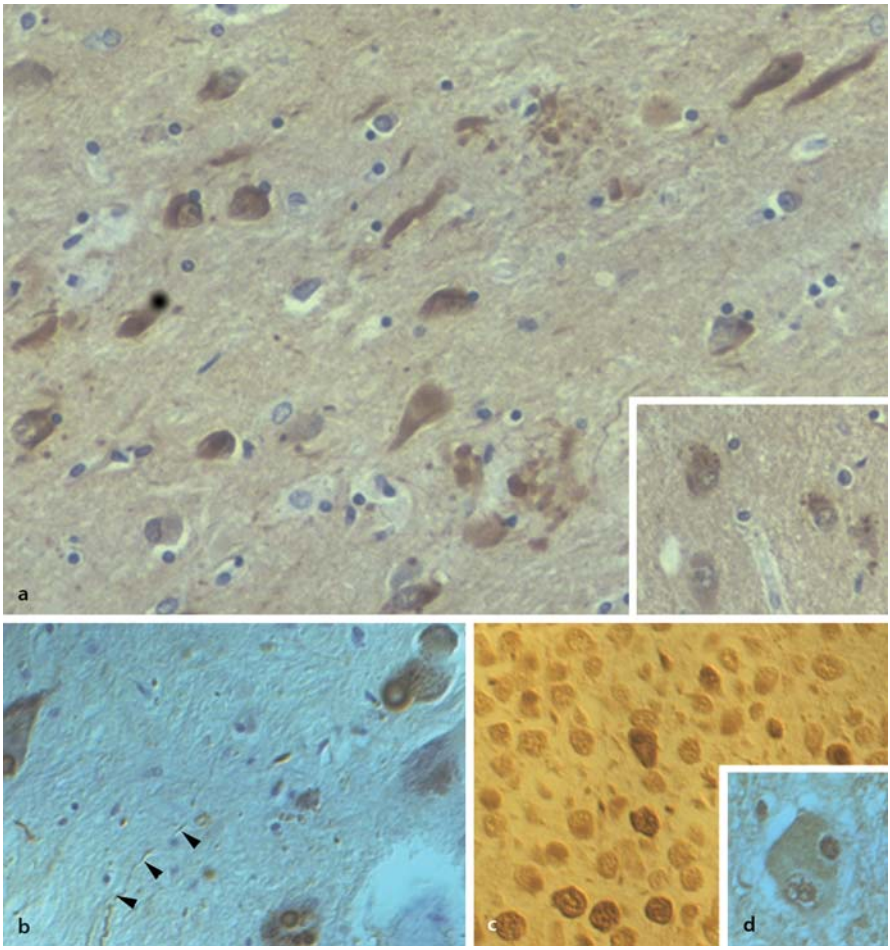

Fig. 1. (**a**) DF2 staining of NFT and dystrophic neurites in AD hippocampus. Inset: Granulovacuolar changes are also intensely labeled. Original, 200×. (**b**) DF2 staining of Lewy bodies and Lewy neurites (*arrowheads*). Substantia nigra. Original, 400×; Nomarski optics. (**c**) DF2 staining of Pick bodies to varying extents. A number of neuronal perikarya and nuclei in the granule cell layer of the hippocampus are also intensely labeled. Original, 200×, Nomarski optics. (**d**) DF2 staining of Lewy-like bodies in the anterior horn cell of the spinal cord affected by amyotrophic lateral sclerosis

AD cortical homogenates, similar to that seen on the blot with anti-tau antibodies. It was puzzling that DF2 immunostaining gave a high background on fixed AD or control brain sections (Fig. 1a). As I believed that PHF was made of unusual components, I was quite unhappy with this finding, which suggested that PHF components were universal in the brain.

Identification of ubiquitin in PHF

Using Western blotting, I soon found that DF2 immunolabeled both an extensive smear on the blot of AD homogenates and a low molecular weight (about 5 kDa) protein in the soluble fractions from AD and control brains. It was not difficult for me to purify this abundant small protein to homogeneity by ammonium sulfate fractionation, gel filtration and reverse-phase HPLC. In December 1985, we noticed that the purified DF2-reactive protein matched ubiquitin perfectly up to amino acid 34. Hiroshi (who had already joined me at the Institute) and I were greatly puzzled with this result. Why ubiquitin? Anyway, to confirm our original finding, we asked Dr AL Haas for his polyclonal antibody to ubiquitin. According to the literature, his antibody seemed to be the most widely used among ubiquitin/proteasome investigators. He generously provided aliquots of the antibody, which strongly stained isolated the NFT as well as the NFT on the tissue section. Interestingly, the polyclonal antibody gave much less background staining on the tissue section, compared with DF2. This finding strengthened the possibility that ubiquitin was a component of PHF.

It was several more months, however, before we submitted our manuscript to *Science*, on 17 September 1986. At that time, careful AD investigators became very afraid that immunochemical or immunocytochemical approaches alone could lead to the wrong conclusions about PHF components (see Nukina et al. 1987). Thus, I was anxious to have definitive evidence in addition to the immunochemical data. for the presence of ubiquitin in PHF. Otherwise, I felt that *Science* would not take our manuscript. I had already tried digestion of PHF to release ubiquitin-derived peptides using several specific proteases. But, on HPLC, I saw only a couple of small peaks on an unusually elevated baseline, suggesting that most PHF were not cleaved at all. I told this to Dr. Imahori, a director of the Institute, who kindly arranged a small meeting with Dr Jun Kondo attending. Although young, Dr Kondo was a highly experienced protein chemist. Listening to my ongoing work, he promptly suggested a certain protocol. Purified PHF should be pretreated with formic acid to break down β-pleated sheet and then digested with *Achromobacter lyticus* protease 1 (AP1) in 6 M urea. AP1 is highly specific for Lys-X and still active even in the presence of a denaturant such as urea. This protocol worked excellently and provided for the first time numerous, distinct large HPLC peaks derived from the PHF digest, without an elevated baseline (Mori et al. 1987).

This success in digestion of PHF revealed that the PHF that had been subjected to protein chemical analysis were not sufficiently purified and were indeed heavily contaminated with ferritin. Thus, a single HPLC peak usually contained four or more different peptides, and it would have taken us too much time to extensively analyze all the peaks (Kondo et al. 1988). To facilitate identification of ubiquitin-derived peptides, I thought to take advantage of the precision of reverse-phase HPLC. In this approach, an authentic ubiquitin-peptide map in which free ubiquitin is digested with AP1 is compared with a PHF-peptide map. Peaks in the PHF profile that coelute exactly with authentic ubiquitin peptides (eight peptides, U1-U8, generated by AP1) are further fractionated and subjected to sequencing. With this protocol, we identified two ubiquitin-derived peptides (U3 and U6) out of eight peptides in the PHF digest. Now we had definitive evidence that ubiquitin was a component of PHF, although we did not know the precise significance of this finding. This strategy of constructing detailed AP1

peptide maps to identify particular peptides or modified peptides was used extensively in our subsequent work on PHF and turned out to be very successful (Kondo et al. 1988; Hasegawa et al. 1992).

I reasonably thought that ubiquitin was conjugated with tau in PHF (Kondo et al. 1988). If so, the Gly-76-containing AP1 peptide (U8) would be involved in the covalent conjugation with the ε-amino group of Lys residue in tau through the isopeptide bond. It should be noted that, once the ε-amino group of a particular Lys is involved in the isopeptide bond, AP1 no longer cleave that Lys-X. To test this hypothesis, I raised antibodies to U8 and attempted to identify a reactive HPLC peak that should contain the tau-ubiquitin isopeptide-linked (Y-shaped) peptide. However, the antibodies generated were not sufficiently strong to identify a peak that might contain the tau-ubiquitin peptide. It was not until 1993 that the isopeptide-linked tau-ubiquitin and ubiquitin-ubiquitin peptides were unambiguously identified in PHF (Morishima-Kawashima et al. 1993).

Intracellular abnormal protein aggregates are ubiquitinated

Thanks to collaboration with Dr Shigeki Kuzuhara, presently Professor of Neurology at Mie University Hospital, a great number of brain tissue sections from consecutive autopsies were available in Tokyo Metropolitan Geriatric Hospital. Using these tissue sections, I examined whether DF2 might stain abnormal organelles other than NFT. To my surprise, DF2 strongly immunolabeled Lewy bodies, the major component of which was not known then. Staining was so strong that one could effortlessly recognize Lewy bodies (Fig. 1b; Kuzuhara et al. 1988). It was obvious that the number of ubiquitin-positive bodies in the sections from substantia nigra affected with Parkinson's disease was much greater than the number of Lewy bodies recognized on H-E sections. Further, varying forms of ubiquitin-positive inclusions were seen, probably representing immature or transitional forms of Lewy bodies. Sometimes ubiquitin-positive tortuous neurites were seen; these had never previously been described. These are now known as Lewy neurites. Medical students without much experience would be able to detect Lewy bodies with an accuracy and sensitivity previously shown only by experienced neuropathologists. Just one year later, ubiquitin staining attained such popularity among neuropathologists as to become a recommended procedure when diagnosing diffuse Lewy body disease (Lennox et al. 1989). Thus, (admittedly on an immunocytochemical basis), we concluded that ubiquitin was also a component of Lewy bodies (Kuzuhara et al. 1988).

In the same line of investigation, Dr Shigeo Murayama and I found that DF2 also labels Pick bodies in Pick's disease (Fig. 1c; Murayama et al. 1990b) and labels Lewy-like hyaline inclusion bodies in amyotrophic lateral sclerosis (Fig. 1d; Murayama et al. 1990a). Thus, the original identification of ubiquitin in PHF quickly led us to the view that abnormal intracellular protein aggregates may be ubiquitinated.

E.-M. Mandelkow

Influence of tau on neuronal traffic mechanisms

E.-M. Mandelkow[1], *E. Thies, J. Biernat*, and *E. Mandelkow*

Summary

One of the earliest changes in the brains of Alzheimer's disease (AD) patients is the loss of synapses, concomitant with the abnormal phosphorylation of tau protein and its redistribution into the somatodendritic compartment that is one of the hallmarks of AD. Tau's major physiological function is to stabilize axonal microtubules for their role as tracks for the transport of vesicles and organelles, suggesting that the abnormal changes in tau could be related to the loss of synapses and neuronal degeneration. Experiments with cell models show that tau can indeed act as an inhibitor of transport in neurons, particularly in the anterograde direction. The result is that cell processes of neurons become starved of their nutrients, leading to the decay of synapses followed by the loss of axons and dendrites. In particular, tau can also interfere with the transport of APP, which therefore may offer a link between the two proteins causing abnormal protein aggregates in AD.

Properties of tau protein

Cellular traffic system

An early feature of AD is the loss of synapses in the hippocampus and entorhinal region, which corresponds to a loss of memory (Flood and Coleman 1990; Terry et al. 1991). It may be initiated by factors such as inflammatory cytokines, oxidative stress, loss of growth factors, or the toxic Aβ peptide (Raff et al. 2002; Selkoe 2002). Synapse loss precedes the abnormal protein aggregation in senile plaques, neurofibrillary tangles, and others. One clue for this vulnerability comes from the elongated structure of neurons. Most synapses are distant from the cell body, the site of synthesis, and therefore rely on a functioning transport system. Cells have a traffic system in the form of microtubules and microfilaments along which motor proteins move their cargoes, using the energy of ATP hydrolysis (Hollenbeck and Saxton 2005). The motor proteins fall into three classes, the myosins (for the microfilament tracks) and the kinesins and dyneins (for microtubule tracks; Hirokawa and Takemura 2005). Microtubules are responsible for the long-haul traffic whereas the microfilament system operates more locally. The

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directionality is determined by the polarity of the tracks and the directionality of the motors. The "plus" end of microtubules points towards the periphery, therefore the plus-end directed motors, such as kinesin, carry out anterograde transport whereas the minus-end directed motors, such as dynein, are responsible for retrograde movements towards the cell body. The "ties" for the tracks are provided by additional proteins, such as MAPs in the case of microtubules (review Cassimeris and Spittle 2001). For neurons the most important MAPs are MAP2 (mostly dendritic), tau and MAP1b (mostly axonal). The interaction of MAPs with microtubules is controlled by phosphorylation and involves several protein kinases and phosphatases (Stoothoff and Johnson 2005). Microtubules are able to self-assemble and disassemble from their subunits tubulin, regulated by GTP turnover. Additional control is achieved by MAPs, such as tau, whose detachment can induce microtubule breakdown, and by destabilizers, such as katanin or kinesin-13 (MCAK; Biernat et al. 2002; Baas et al. 2005).

Structural and biochemical features of tau

In AD research, tau has received considerable attention because of its anomalous aggregation in neurofibrillary tangles and neuropil threads, made up of filaments of tau protein [paired helical filaments (PHFs) and straight filaments (SFs); Crowther and Goedert 2000]. In AD, tau is also highly phosphorylated, missorted into the somatodendritic compartment, partly cleaved by proteases, and otherwise modified. AD-tau is detached from microtubules and no longer stabilizes microtubules. The consequences are the destabilization of transport tracks and the aggregation of tau in the cytosol, both of which can disrupt intracellular traffic. Furthermore, AD-tau has a defined pattern of spreading in the brain, from the transentorhinal region to the hippocampus and later throughout the cortex. Thus the pattern of tau aggregation reflects the progression of clinical symptoms from mild cognitive impairment to severe dementia (Braak and Braak 1991).

The tau gene is located on chromosome 17; the protein occurs in the CNS as six main isoforms arising from alternative splicing (352-441 amino acid residues; Fig. 1; Andreadis 2005). The repeat domain (containing three or four pseudo-repeats of $\sim$ 31 residues) and the domains flanking the repeats are responsible for microtubule binding. The repeat domain also forms the core of Alzheimer PHFs (Wille et al. 1992; Novak et al. 1993). Tau has an overall basic and hydrophilic character due to the numerous lysine or arginine and polar residues; the N-terminal domain has an acidic character. This composition makes tau highly soluble, to the point that tau is heat and acid stable without losing its biological function (Lee et al. 1988). A further consequence is that tau is not compactly folded, as most proteins, but rather is a natively unfolded protein (Schweers et al. 1994). Several mutations in the tau gene can cause different forms of neurodegeneration (FTDP-17; Lee et al. 2001), presumably due to a change in protein function (e.g., lower microtubule binding or faster PHF aggregation) or an altered distribution of isoforms (D'Souza and Schellenberg 2005).

In AD, tau is phosphorylated extensively, $\sim$ 4-fold higher than in normal brain and at numerous sites (Khatoon et al. 1992; Morishima-Kawashima et al. 1995). The biological consequences are heterogeneous. Phosphorylation at certain sites can affect microtubule binding and/or PHF aggregation; other sites appear to be functionally neutral (Biernat et al. 1993; Illenberger et al. 1998). Phosphorylation at the KXGS

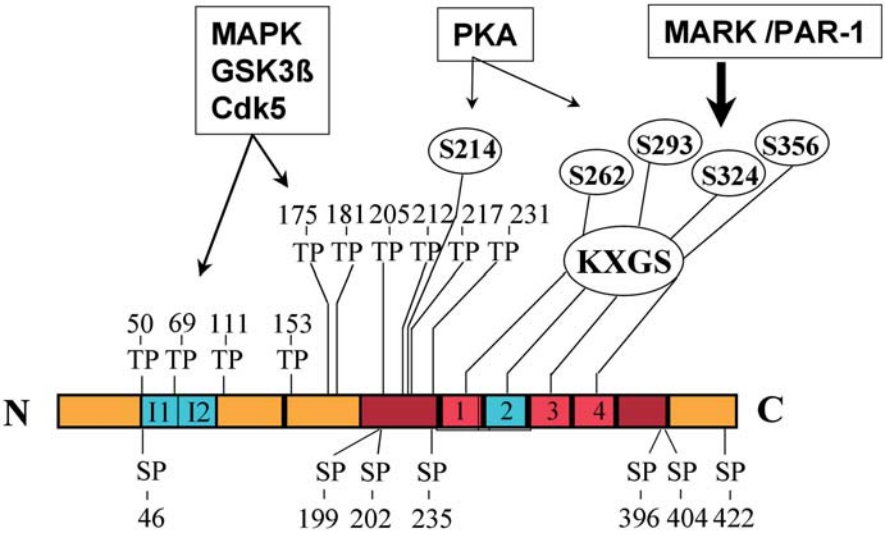

Fig. 1. Tau protein: domains, phosphorylation sites, and kinases. In CNS neurons, human tau occurs as six main isoforms derived from a single gene by alternative splicing (352–441 amino acid residues). The 3 or 4 repeats in the C-terminal half (~ 31 residues each) constitute the center of the microtubule-binding domain, as well as the core of Alzheimer paired helical filaments. Tau contains numerous Ser and Thr residues, many of which show abnormally high phosphorylation in Alzheimer's disease and are diagnostic of Alzheimer tau. Phosphorylation sites within the repeats (at KXGS motifs by the kinase MARK/Par-1) efficiently detach tau from microtubules

motifs in the repeat domain by the kinase MARK strongly disrupts tau-microtubule binding and leads to dynamic microtubules (Drewes et al. 1997). The interplay between tau and MARK becomes particularly noticeable in the case of neurite outgrowth, where activation of MARK has a similar effect as NGF signalling (Biernat et al. 2002).

A further unusual property of tau in AD is its aggregation, which is counterintuitive because of tau's excellent solubility. The aggregation is based on hotspots in the sequence that have an increased propensity for β-sheet interactions; they include the hexapeptide motifs ^{275}VQIINK^{280} and ^{306}VQIVYK^{311} (von Bergen et al. 2000). Thus tau aggregation is based on an "amyloid" principle, although the majority of the protein remains disordered, even when assembled into PHFs.

Transport inhibition by tau

Microtubules, motor proteins and tau

Intracellular traffic can be regulated at different levels, e.g., at the level of tracks (microtubules, tau), motors (kinesin, dynein), cargo adaptors (kinesin or dynein light chains or associated proteins), and by posttranslational modifications (phosphorylation; Mandelkow et al. 2004; Roy et al. 2005; Terwel et al. 2002). Among these proteins, tau is most clearly associated with AD, followed by kinases whose activities are again

noticeable in terms of tau phosphorylation. When studying the functions of tau in cells, one observes not only the expected stabilization of microtubules but also a general inhibition of intracellular traffic. For example, in cultured cells, mitochondria are distributed homogeneously (corresponding to the need for ATP), which is achieved by the network of microtubules that radiate out from the MTOC throughout the cell. If microtubules are destroyed (e.g., by nocodazol) or the motors are perturbed (e.g., dynein inhibition), the homogeneous distribution breaks down. If tau is elevated in such cells, the mitochondria congregate at the cell center, due to a preferential inhibition of kinesin-based transport towards the cell periphery. Similar observations can be made with other microtubule cargoes, such as peroxisomes, intermediate filaments, the endoplasmic reticulum (ER), exocytotic vesicles or recycling endosomes (Ebneth et al. 1998). A quantitative analysis of vesicle and organelle movements shows that tau does not change the instantaneous speed of a vesicle or organelle but reduces the attachment of vesicles to the microtubule tracks, the run lengths along microtubules, and the reversal frequencies (Trinczek et al. 1999). Thus tau imposes a bias for centripetal flow towards the cell center.

These results point to an unexpected property of tau: in AD, the abnormal changes of tau are usually thought to result from its phosphorylation and dissociation from microtubules, which therefore become unstable. However, the above experiments indicate that even "normal" tau bound to microtubules can perturb the cell's physiological functions by inhibiting the interactions of motor proteins and microtubules. This can become a serious problem for elongated cells, such as neurons, that are depend on an efficient transport system. As an example, Fig. 2 shows N2a cells differentiated by retinoic acid to develop neurites. They contain mitochondria for the generation of chemical energy, peroxisomes for detoxification of H_2O_2, neurofilaments and microtubules for structural stability and intracellular transport, and transport vesicles carrying supplies for the growth cone. In control cells, the mitochondria are distributed throughout the cell body and the neurites by microtubule-based transport (Fig. 2a), but when tau is elevated the mitochondria are nearly absent from the neurites and instead accumulate in the cell body (Fig. 2b). Fig. 2c and d shows an interpretation of the interference between motors and tau and the preferential inhibition of kinesin. In contrast to mitochondria, microtubules and tau are present throughout the neurites. Thus, even though the tracks for axonal transport are present in the neurite, the transport along them is impaired because tau and motors compete for overlapping binding sites on microtubules (Fig. 3; Seitz et al. 2002). This implies that neurites lack mitochondrial ATP synthesis and protection against oxidative stress (Stamer et al. 2002). Similar conclusions can be drawn from experiments with primary retinal ganglion cells transfected with tau. These neurons normally contain mitochondria throughout the cell body and the axons, but, after transfection with tau, the organelles disappear from the axon and congregate in the cell body. In all cases, there is a preferential inhibition of plus-end directed transport by kinesin so that minus-end directed transport by dynein dominates.

The disappearance of organelles from neuronal cell processes would be expected to cause deficiencies in local metabolism, leading to reduced ATP levels, Ca^{++} buffering capacity, or defense against oxidative stress. Indeed, when cells are exposed to H_2O_2, the degradation of neurites is much more rapid in tau-transfected neurons than in the non-transfected controls (Stamer et al. 2002). Rather than directly affecting biochemical

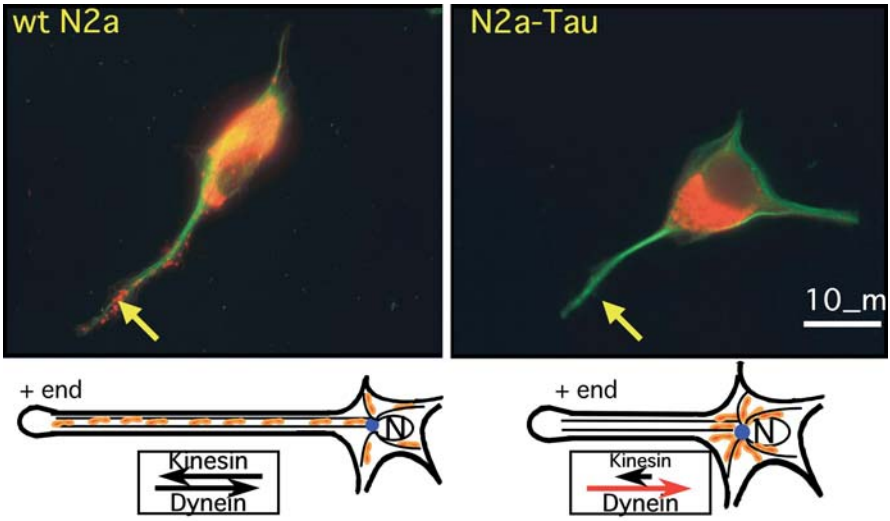

Fig. 2. Inhibition of microtubule-based transport by tau in neuroblastoma cells. (**a**,**b**) N2a cells, differentiated by retinoic acid and stained for microtubules (*green*) and mitochondria (*red*). (**a**) Control cell with mitochondria dispersed throughout the neurites. (**b**) Tau-transfected cell where the anterograde flow of mitochondria is inhibited, and mitochondria cluster in the cell body. (**c**,**d**) Diagrams of neurons (**N**) illustrating the particle flow along microtubules. (**c**) in a control cell, the anterograde and retrograde movements (by kinesin or dynein, resp.) are regulated by the cell to achieve a balanced particle distribution. (**d**) With excess tau on the microtubule surface, both types of movement are inhibited, but the effect is greater anterograde movements, resulting in a net retrograde flow of mitochondria

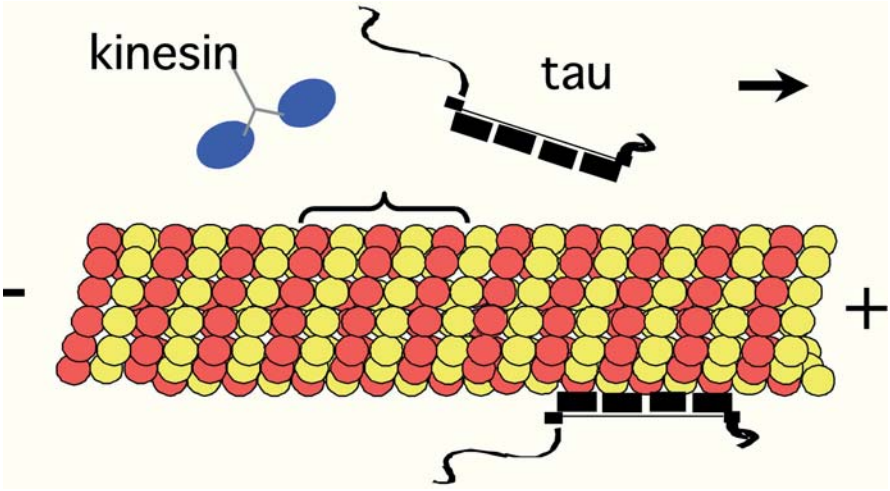

Fig. 3. Diagram illustrating the competition between motor proteins (kinesin) and tau protein for overlapping binding sites on a microtubule

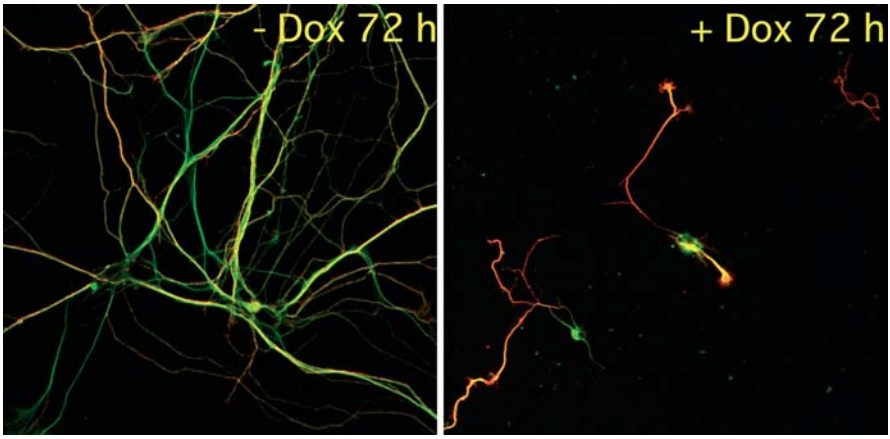

Fig. 4. Cortical neurons from a transgenic mouse with inducible expression of tau construct (tet-on system). **Left**: Neurons cultured for six days without induction of tau expression. Note the elaborate network of cell processes stained for microtubules (*green*). **Right**: Neurons cultured for three days, followed by induction of tau repeat domain (K18ΔK280) for three more days by doxycyclin (tet-on system). Note the enhanced expression of tau (*red*) and the pronounced decay of cell processes

pathways, tau reduces the viability of neurites by its effect on the transport of cell components. This process is illustrated in Fig. 4, where the expression of the tau repeat domain leads to the degeneration of cell processes and neurons.

Tau and APP trafficking

Amyloid precursor protein (APP) is transported into the axon by Golgi-derived vesicles along microtubules (Amaratunga et al. 1995). By analogy with the experiments described above, one would therefore expect that tau would be able to interfere with the anterograde transport of APP. This interference can be observed by expressing APP labeled with YFP in retinal ganglion axons, which have a defined polarity (Mandelkow et al. 2004). Without tau, APP vesicles move rapidly in both directions, but the anterograde direction predominates (Fig. 5). If the cells are co-transfected with tau, the APP vesicles become depleted from the axon and moving vesicles show predominantly a retrograde direction. A similar effect of tau on the transport of APP can be demonstrated in transfected hippocampal neurons. The results imply an intriguing link between tau and APP trafficking. APP is the precursor of the Aβ peptide, which aggregates into amyloid fibers in AD. It has been suggested that APP is a cargo adaptor for kinesin and that overexpression of APP leads to inhibition of transport and increased generation of Aβ, assuming that APP vesicles contain the β- and γ-secretases for Aβ cleavage (Kamal and Goldstein 2002). By this hypothesis, Aβ cleavage could occur in transit. On the other hand, the colocalization has not been verified by other investigators (Lazarov et al. 2005a). It is, therefore, an interesting question whether the retardation of APP vesicle trafficking by tau has an influence on the generation of Aβ. Our recent results show that the inhibition of APP transport and the

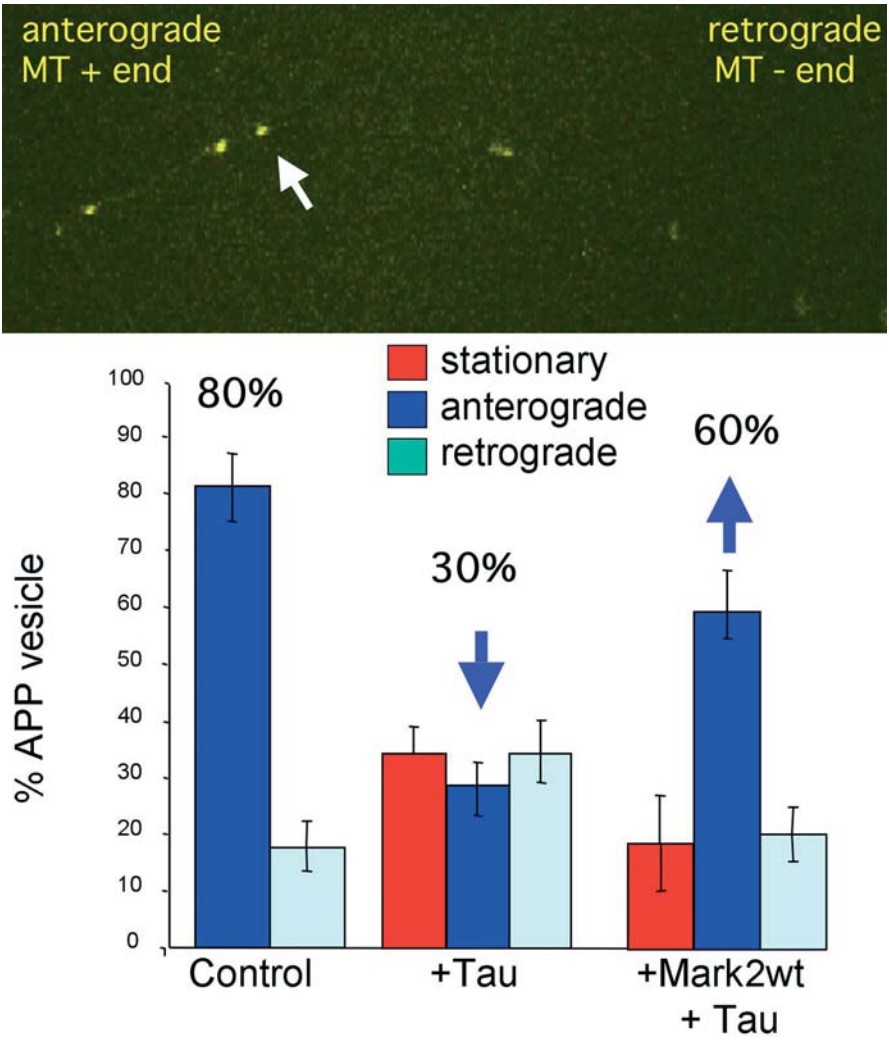

Fig. 5. Effect of tau on axonal transport of APP vesicles in retinal ganglion cells. **Upper panel**: Field of APP-vesicles (*arrow*) observed by confocal microscopy. Cells were transfected with APP-YFP by adenovirus. **Lower panel**: Quantification of movements of APP-YFP vesicles in retinal ganglion neurons. *Left*, control cell without transfected tau shows that most APP vesicles (~ 80%) move anterogradely. *Center*, in the presence of tau, many vesicles become immobile, anterograde movements drop to 30%, and net transport is now reversed. *Right*: When tau is phosphorylated by MARK, it becomes detached from microtubules, and anterograde flow is rescued again (60%)

longer dwell-time in the neuron do not lead to enhanced Aβ generation and that, in general, APP does not colocalize with BACE1 on the same vesicles (Goldsbury et al. 2006).

Rescue of transport inhibition by phosphorylation of tau

The effects of tau on traffic inhibition depend on its binding to microtubules; therefore, one might expect that a release of tau from microtubules would alleviate the traffic inhibition again. This can be tested in retinal ganglion cells, which in their normal state show rapid movement of cell organelles or vesicles in axons. The expression of tau causes the traffic inhibition described above; however, this can be rescued by transfection with MARK, the protein kinase that phosphorylates tau at the KXGS motifs of the repeat domain and thereby detaches it from microtubules (Fig. 5; Mandelkow et al. 2004). Taken together, these results suggest that tau must be able to fulfill seemingly opposite requirements, i.e., it should bind to the tracks to keep them intact but detach readily when a motor protein passes through. These relationships reveal puzzling contradictory aspects of the functions of tau. On one hand, the binding of tau enables traffic by generating stable microtubules. On the other hand, excess tau bound to microtubules can prevent motor proteins from attaching to microtubules. This spatial and functional paradox of tau arises because tau protein – unlike the ties of a railroad – lies on top of the tracks rather than below, therefore creating a stumbling block on the rails while at the same time tying them together. The solution to the structure-function paradox presumably lies in local regulation and equilibria: cargoes generally carry more than one motor, and even a single processive motor attached to a microtubule can propel the cargo. Therefore, traffic could remain functional if tau were removed locally, for example by phosphorylation.

Conclusions

The early appearance of tau abnormality in AD is linked to synapse loss and neurodegeneration. This is often interpreted in terms of two stages of tau toxicity, involving first the activation of kinases that "hyperphosphorylate" tau, causing its detachment from microtubules and hence microtubule breakdown. This stage is followed by the stage of tau aggregation into neurofibrillary tangles that obstruct the cell interior. Given the implications of transport inhibition discussed here, one can propose an extension to three stages of tau toxicity (Fig. 6):

- First, a local elevation of microtubule-bound tau could cause a reduction in the transport of vesicles, organelles, and other cargos, leading to starvation of cell processes, damage to synapses, and missorting of tau to the somatodendritic compartment. At this stage, the toxicity is based on normal functional tau, which could explain the toxicity of excess 4-repeat tau that occurs with some FTDP-17 mutations (Lee et al. 2001; Hutton et al. 2001). The traffic damage is most apparent in mice expressing tau in motor neurons (Terwel et al. 2002), or in Drosophila models by the degeneration of the neuromuscular junction (Chee et al. 2005).
- In the second stage, cells might mount a defense against excess and missorted tau in the form of kinase activation. A possible kinase that efficiently detaches tau from microtubules is MARK/Par-1, which would explain why the corresponding phosphorylation sites in the repeats of tau occur very early in AD (Augustinack et al. 2002). The detached tau can in turn be hyperphosphorylated by further kinases

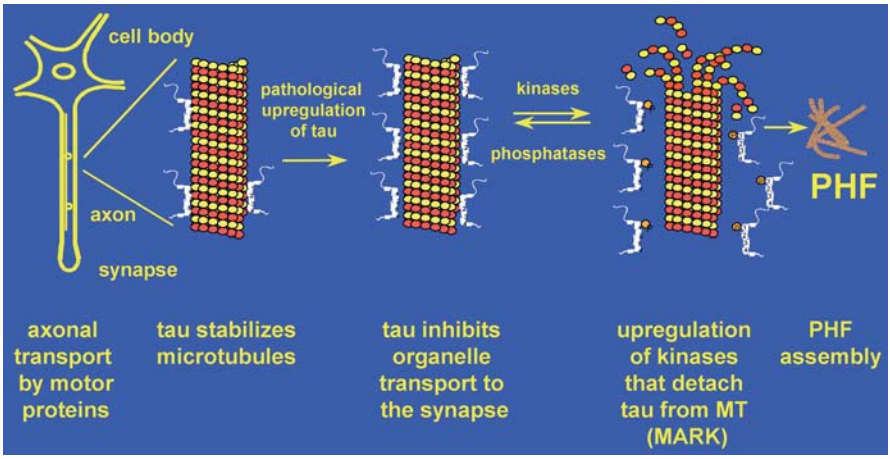

Fig. 6. Model of tau functions in neurodegeneration. *Left*: In a healthy axon, microtubules are stabilized by tau and serve as tracks for axonal transport. *Center*: Tau bound to microtubules is elevated early during neurodegeneration. Microtubules are stable, but the excess tau blocks traffic into axons because it interferes with motor proteins and thus makes axons and synapses vulnerable. *Right*: Phosphorylation of tau occurs because the cell attempts to remove tau from microtubules. Microtubules become unstable, and unbound tau aggregates into neurofibrillary tangles

(see Drosophila model of Nishimura et al. 2004). As a result of the overshoot kinase activity, microtubules become destabilized, and a pool of aggregation-competent tau is generated.

– The third stage of toxicity comprises the aggregation of tau into PHFs. The incipient aggregation of tau (oligomers, protofibrils) is still reversible, as demonstrated in our neuronal cell model (Khlistunova et al. 2006). Once the tangles become more developed (as in transgenic mice; Santacruz et al. 2005; Oddo et al. 2006), they gradually become irreversible due to the persistence of the aggregates, their chemical modifications, and the cell's inability to dispose of them by autophagy or proteasome activity.

In effect, this view shifts attention to functions of "normal" tau that are underappreciated at present but may explain very early stages of neurodegeneration. It is reminiscent of certain tau mutations in FTDP-17 that cause degeneration with little or no change in protein structure, simply by tipping the balance of tau isoforms to the tighter binding 4-repeat species (D'Souza and Schellenberg 2005). It remains to be seen if and where inhibitory concentrations of tau are generated, but, due to the extended nature of neurons that depend on an efficient traffic system, small and local effects would probably be sufficient. Likewise, it remains to be seen if and how a tau-induced retardation of APP transport alters APP processing and Aβ toxicity. At any rate, the early subtle changes in traffic efficiency should be reversible once the mechanism is known in more detail.

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E. Mandelkow

The Search for structure of tau and paired helical filaments

E. Mandelkow[1], *M. von Bergen*[1], *A. Marx*[1], *J. Biernat*[1], and *E.-M. Mandelkow*[1]

Summary

Tau is a microtubule-associated protein in neuronal cells. In Alzheimer's disease (AD), tau forms the subunits of the paired helical filaments. The protein adopts a "natively unfolded" structure. In paired helical filaments (PHFs), a small fraction of tau assumes a β-conformation that interacts with other tau molecules during aggregation. The core of a PHF has a cross-β structure similar to other amyloid fibers, whereas the major part of the protein retains its largely unfolded structure. Here we review the steps that have led to the current understanding of tau and PHF structure.

Tau and microtubules

The scientific history of tau protein is closely linked to the discovery of microtubule self-assembly in the early 1970s. Previously, tubulin was known as a major "colchicine-binding protein" in the brain (Borisy and Taylor 1967), but the conditions for assembling this protein into microtubules remained elusive until they were found by Weisenberg (1972) and others. This finding paved the way for identifying the central roles of microtubules for cell division, cell shape, and intracellular transport. An early key observation was that microtubule assembly was facilitated by microtubule-associated proteins, one of which was tau protein, isolated from brain by Kirschner's group (Weingarten et al. 1975).

The protein has unusual properties in that it is heat-stable and acid-stable, i.e., it is highly soluble so that it does not precipitate during boiling and treatment with acids. Its spectral properties are characteristic of a "random coil" protein (Cleveland et al. 1977). Imaging of tau by electron microscopy gave ambiguous results due to its low contrast (Zingsheim et al. 1979). Special preparation techniques such as quick-freeze deep-etching revealed a microtubule-bound "assembly domain" (so called because this domain promotes the assembly of microtubules) and a "projection domain" that protrudes away from the microtubule wall (Hirokawa et al. 1988). Image reconstructions from unstained microtubules decorated with tau molecules confirmed the largely disordered nature of tau on the surface (Santarella et al. 2004).

The cloning of tau from mouse and human (Lee et al.1987; Goedert et al. 1989b) revealed the sequence and domain composition (Fig. 1). Tau is unusually rich in polar

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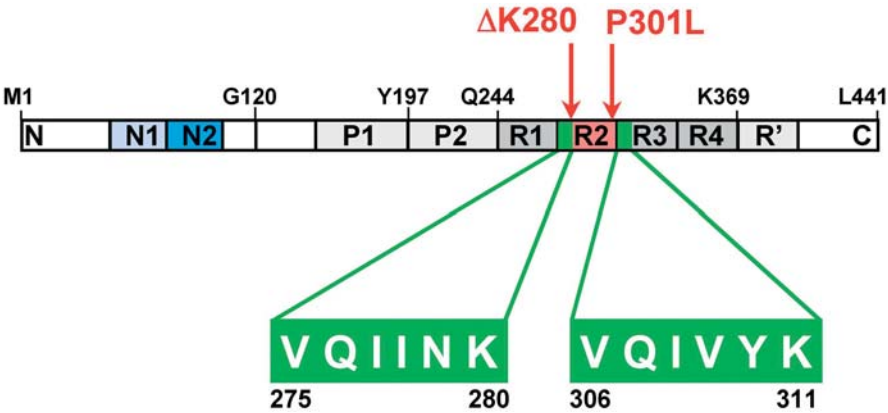

Fig. 1. Diagram of tau domains. The bar illustrates Tau441, the longest isoform in human CNS. Inserts N1, N2, and R2 can be alternatively spliced, giving rise to six isoforms. The N-terminal domain up to ~G120 has an acidic character; the other domains are basic. The left half (residues 1 to ~ 200) represents the "projection domain," the right half the "microtubule assembly domain". The 3 or 4 repeats R1-R4 comprise the core of the microtubule-binding domain as well as the core of the paired helical filaments (PHF). Two hexapeptide motifs at the beginning of R2 and R3 promote PHF aggregation by inducing β-structure. ΔK280 and P301L are two mutants from FTDP-17 cases that strongly enhance the rate of PHF aggregation by increasing the propensity for β-structure

and charged amino acids and has a basic character (except for the initial ~ 120 residues, where negative charges dominate). This explains the high solubility and the unfolded nature of the protein; however, it makes the aggregation of the protein in AD even more enigmatic. In particular, at first glance the sequence contains no elements that appear particularly amyloidogenic, such as exposed stretches of hydrophobic residues (as in the Aβ peptide of AD) or glutamines (as in huntingtin). Tau occurs in a number of isoforms, typically six in the human CNS, which arise from alternative mRNA splicing of exons 2, 3, and 10 and generate isoforms containing 352–441 amino acid residues. The N-terminal projection domain and C-terminal assembly domain can be separated by chymotryptic cleavage behind Y197 (Steiner et al. 1990); the C-terminal tail can be removed by caspase 3 behind D421 (Gamblin et al. 2003). The most conspicuous feature is the repeat domain within the C-terminal half (Q244-N368), containing three or four semi-conserved sequences of 31 or 32 residues: R1 = Q244-K274, R2 = V275-S305, R3 = V306-Q336, R4 = V337-N368. R2 is encoded by exon 10 and may be absent. The resulting isoforms can be designated as 0N3R, 1N3R, 2N3R, 0N4R, 1N4R, 2N4R, depending on the number of N-terminal inserts and repeats. In peripheral nerves, additional isoforms can occur by inclusion of more exons encoding ~ 300 further residues, generating "big tau" (Couchie et al. 1992). The general domain structure of tau is similar to other microtubule-associated proteins, such as the neuronal MAP2 or the ubiquitous MAP4, which, however, contain a much larger projection domain (Lewis et al. 1988). Its size determines the spacing between microtubules in cells (Chen et al. 1992). Fetal tau comprises only the shortest isoform (0N3R); the other isoforms are added during brain development (Drubin and Kirschner 1986). In the human CNS, the six isoforms are present in roughly equal amounts; in particular, there is an equal

balance of 3-repeat and 4-repeat isoforms (Goedert and Jakes 1990) that is perturbed in FTDP-17.

A notable feature of tau is its large number of potential phosphorylation sites, due to the frequency of S or T residues. Many of them (up to 17, depending on isoform) are part of SP or TP motifs and represent targets of proline-directed Ser/Thr protein kinases (e.g., MAP kinase, GSK-3β, cdk5, cdc2 etc.). Other sites are targetted by a variety of kinases, including PKA, PKC, CaMK, SGCK, AKT, MARK, SAD and others (for reviews, see Chen et al. 2004; Stoothoff and Johnson 2005). Notably, the KIGS or KCGS motifs in the repeat domain (e.g, S262) are phosphorylated by MARK, which strongly reduces the tau-microtubule interactions (Biernat et al. 1993; note that the same phosphorylation also inhibits PHF aggregation, illustrating the analogous role of the repeat domain in physiological and pathological functions of tau; Schneider et al. 1999). A further potent detaching site is S214, which can be phosphorylated by PKA and is upregulated during mitosis (Brandt et al. 1994; Illenberger et al. 1998). Tau contains five Y residues (residues 18, 29, 197, 310, 394), one of which (Y18) is phosphorylated by the Tyr-kinase Fyn (Lee et al. 2004a). Furthermore, tau contains one or two cysteines in the repeat domain (C291 in R2, C322 in R3) that can be engaged in intra- or intermolecular crosslinking, which affects conformation, dimerization, and aggregation (Schweers et al. 1995).

The main physiological function of tau, i.e., binding to microtubules, is achieved by the repeat domain and the adjacent proline-rich flanking domains. In general, 4-repeat tau binds microtubules more tightly (Butner and Kirschner 1991) whereas phosphorylation, especially in the repeat domain, tends to decrease the affinity (Biernat et al. 1993). Like soluble tau, microtubule-bound tau is mostly in a natively unfolded state (Fig. 2) and is, therefore, poorly visible by X-ray fiber diffraction or (cryo-) electron microscopy (Santarella et al. 2004; Al-Bassam et al. 2002). This is in strong contrast to other microtubule-interacting proteins, such as motor proteins, which show a periodic

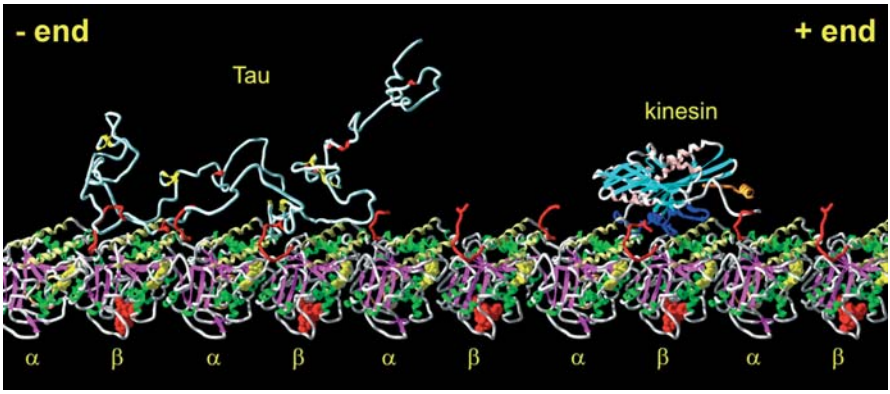

Fig. 2. Model of microtubule protofilament with bound kinesin and Tau. The protofilament consists of alternating subunits of α- and β-tubulin (∼ 450 residues each) arranged in a polar fashion ("plus" end pointing to the cell periphery). The head domain of kinesin, a microtubule-dependent motor protein (∼ 350 residues), has the compact folding typical of most cytoplasmic proteins. By contrast, Tau is natively unfolded; its structure is unknown in detail and modelled here as a random chain. Note that tau occupies a much larger volume than kinesin or tubulin

binding pattern to microtubules commensurate with the tubulin lattice (8-nm axial repeat; Santarella et al. 2004). However, it appears that tau binds in an extended fashion to the outer tips of microtubule protofilaments. When microtubules are disassembled by low temperature, tau stabilizes the ring-like disassembly products consisting of tubulin oligomers. Nevertheless, the binding of tau to microtubules must be highly dynamic since nuclear magnetic resonance (NMR) studies reveal a high mobility of most tau residues even in the bound state (Woody et al. 1983). Since tau and motor proteins both bind to the outer surface of microtubules, they compete for binding sites, explaining why tau can interfere with transport along microtubules, which leads to an inhibition of anterograde transport in axons (Stamer et al. 2002).

The conformation of tau in solution is unknown and presumably highly variable, as expected for a natively unfolded protein. An NMR analysis of secondary chemical shifts of the repeat domain reveals little secondary structure, except for some motifs of nascent β-structure near the beginnings of R2, R3, R4 (Mukrasch et al. 2005). These coincide with the regions involved in PHF assembly (see below). Nevertheless, FRET studies reveal a global (average) paperclip-like folding of tau in solution that results in a close juxtaposition of the repeat domain with the C-terminal and N-terminal ends of the molecule (Jeganathan et al. 2006; Fig. 3). This conformation is reminiscent of the discontinuous epitopes of certain antibodies (Alz50, MC1) that recognize early stages of AD and are generated by folding the N-terminus of tau over the repeat domain (Carmel et al. 1996; Jicha et al. 1997).

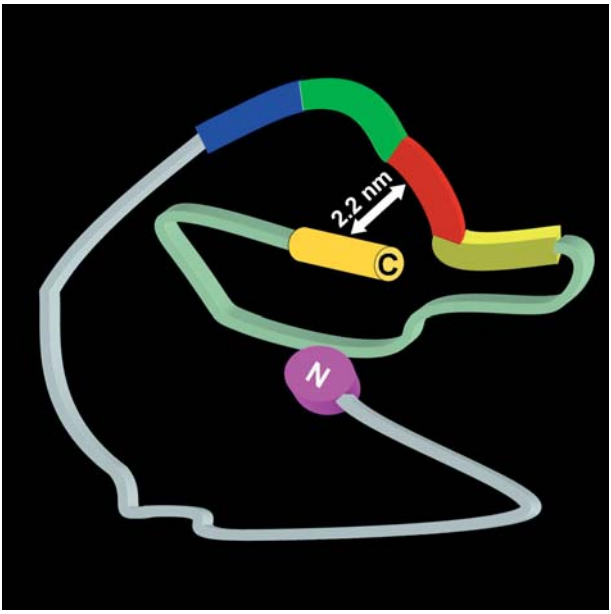

Fig. 3. Model of conformation of tau in solution deduced by FRET. The molecule shows a paperclip-like fold that brings the N- and C-terminal ends into the vicinity of the repeat domain. Similar folded conformations are recognized by several antibodies that recognize Alzheimer tau (e.g., Alz-50, MC1)

Tau and Alzheimer paired helical filaments

The molecular structure of PHFs is still unknown but determining it represents one of the major goals in the field, since this would greatly aid in the development of methods and drugs to prevent pathological aggregation. From a structural point of view, there was a long gap between Alzheimer's discovery of neurofibrillary tangles (Alzheimer 1907a) and the identification of PHFs as their basic elements, made possible by the advances in electron microscopy (Kidd 1963; Terry 1963). Another two decades passed with attempts to find suitable conditions for the isolation and characterization of PHFs (e.g., Ihara et al. 1983; Wisniewski et al. 1984). One important outcome was the reconstruction of PHFs from negatively stained electron micrographs, which showed each half of a PHF to be composed of three protein densities, with overall dimensions of $\sim$ 8 nm $\times$ 20 nm (Crowther and Wischik 1985); Subsequent work showed an analogous doubly tripartite structure for "straight filaments," a minor variant of Alzheimer PHF preparations (Crowther 1991). These variants appear to be caused by subtle changes in charge distribution around the β-structure forming motifs in the repeat domain at the beginning of R3 and R4 (DeTure et al. 2002).

With the development of specific antibodies against PHFs and microtubule proteins, the search for the protein composition of PHFs yielded tau protein as the major subunit (Brion et al. 1985d; Grundke-Iqbal et al. 1986b; Kosik et al. 1988). Shortly thereafter, molecular cloning resulted in the elucidation of tau sequences from mouse and humans and confirmed that the protein from Alzheimer PHFs was indeed tau (Lee et al. 1988; Wischik et al. 1988a; Goedert et al. 1988, 1989). This finding set the stage for the expression of recombinant tau protein and the structural and biochemical analysis of tau and PHFs.

A general difficulty in studying the formation of PHFs from soluble tau was the high solubility of the protein that counteracts assembly; the second problem was to derive criteria for the in vitro generation of bona fide PHFs. These problems were overcome by a search for appropriate tau constructs and assembly conditions, including the dimerization of the protein by disulfide crosslinking, which accelerates PHF assembly (Wille et al. 1992). The resulting fibers had the typical PHF morphology with an $\sim$ 80 nm crossover repeat (Fig. 4). A further step in accelerating PHF assembly and making it amenable for structural studies was the discovery that polyanionic molecules greatly facilitate PHF assembly. These include molecules such as sulfated glycosaminoclycans, heparin, RNA, acidic peptides, or fatty acid micelles (Goedert et al. 1996; Perez et al. 1996; Kampers et al. 1996; Wilson and Binder 1997).

An important issue concerning the substructure of PHFs was the question of whether they should be considered as "amyloid". The current definition of an amyloid, evolving from the analysis of several pathologically aggregating proteins, is that of an aggregating fibril whose backbone consists of β-sheets whose strands are oriented across (i.e., perpendicular to) the fiber axis ("cross-β-structure"). The distance between successive strands is $\sim$ 0.47 nm, so X-ray fiber diffraction patterns reveal a sharp meridional 0.47-nm reflection. Using this criterion, Kirschner et al. (1986) suggested a cross-β structure for both types of fibers isolated from Alzheimer brains, from amyloid plaques (containing the Aβ peptide) and neurofibrillary tangles (PHFs containing tau). In the case of PHFs, the diagnostic 0.47-nm reflection was weak, the purity of the preparation was somewhat uncertain, and later studies failed to confirm the reflection

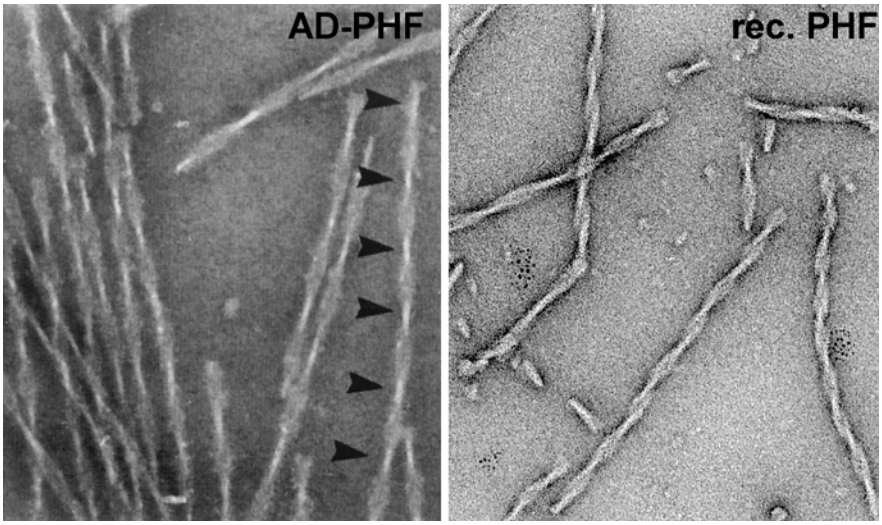

Fig. 4. Electron micrographs of paired helical filaments (PHF) isolated from Alzheimer brain (*left*) and reassembled in vitro from recombinant (rec) tau (repeat domain with pro-aggregation mutant ΔK280). Note the typical twisted appearance with crossover repeats of ~ 80 nm (*arrowheads*)

in Alzheimer PHFs (Schweers et al. 1994). Instead, other types of axial repeats were reported that suggested a non-amyloid packing of subunits (e.g., 3 nm; Crowther and Wischik 1986).

The resolution of this puzzle came with the realization that the aggregation of tau was based on very short motifs in the repeat domain (hexapeptide motifs ^{275}VQIINK^{280} and ^{306}VQIVYK^{311} at the beginning of R2 and R3, respectively; von Bergen et al. 2000, 2001; Fig. 1). These "aggregation motifs" tend to interact with a cross-β structure, forming the core of PHFs, whereas the bulk of the protein remains largely disordered. Thus the amyloid character of tau is poorly visible with full-length tau, but it becomes apparent with peptides derived from the aggregation motifs and/or improved procedures of specimen preparation (von Bergen et al. 2001; Gianetti et al. 2000; Berriman et al. 2003; Inouye et al. 2006; Fig. 5). The aggregation motifs coincide with sequences where nascent β-structure can already be detected in soluble tau by NMR spectroscopy, and indeed this region reveals a very low mobility, compared with the fuzzy coat (Mukrasch et al.2005; Sillen et al. 2005). The role of the hexapeptide motifs is further underscored by proline mutations that interrupt β-structure and thus inhibit aggregation ("anti-aggregation" mutants), or, conversely, by mutations that enhance the propensity for β-structure (e.g., P301L or ΔK280, both described for frontotemporal dementias) and thus promote aggregation ("pro-aggregation" mutants; Barghorn et al. 2000). On the basis of these data, it is possible to draw a rough outline of the steps involved in PHF aggregation (Fig. 6).

The next steps would be the determination of the packing of tau subunits within PHFs and their folding at high resolution. These goals have not yet been achieved, but they will likely occur in three stages:

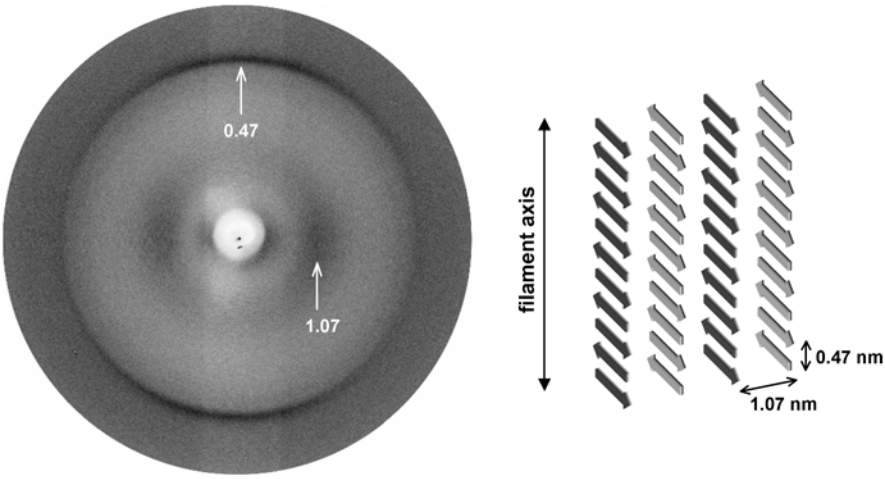

Fig. 5. Principle of PHF aggregation by forming cross-β structure. *Left*, X-ray fiber diagram of PHFs reassembled in vitro (from repeat domain, ΔK280 mutant). Note the meridional reflection indicating the 0.47-nm spacing typical of adjacent strands in a β-sheet, and the equatorial reflection at ∼ 1 nm typical of the separation between β-sheets. *Right*, illustration of a cross-β structure, with β-strands (*short gray arrows*) oriented perpendicular to the fiber axis (*vertical arrow*)

- First, since the backbone of PHFs consists of cross-β-structure, the analogy with other amyloid-forming proteins (e.g., Aβ peptide or peptides from yeast prion protein; for review, see Nelson and Eisenberg 2006) makes it likely that there will be protofibrils that are made up of pairs of juxtaposed β-sheets that interact axially by hydrogen bonding between their main chain strands and laterally through the sidechains across the sheets. This presumably includes hydrophobic interactions, as suggested by the nature of the pro-aggregation motifs. Since these residues are near one another, it is likely that their distances and interactions can be determined by spectroscopic methods, such as NMR or EPR. One example is the EPR study of Margittai and Langen (2004), who concluded that residues 301–320 in R3 must lie close to the corresponding residues in a neighboring molecule. This could be achieved by placing this stretch of residues in adjacent strands of a cross-β-sheet structure.
- The second level will be the arrangement of protofibrils within a PHF. Their number and interaction are currently unknown, but there are several constraints on possible arrangements: 1) The mass-per-length of the PHF core, determined by scanning transmission electron microscopy (STEM), is ∼ 60–70 kDa/nm, equivalent to roughly 3.5–4.5 repeat domain molecules per nm (Wischik et al. 1988a; von Bergen et al. 2006; for variations among PHFs, see Ksiezak-Reding and Wall 2005). Note, for comparison, that adjacent molecules in cross-β-structure are spaced 0.47 nm apart, equivalent to ∼ 2 molecules per nm, which would allow only ∼ 2 protofibrils. 2) The overall cross-sectional dimensions of the PHF core (comprising mainly the repeat domain) are ∼ 8 nm× ∼ 20 nm. This area is divided up into two halves, each containing three density peaks (and intervening valleys of lower density), so that the effective area is estimated at ∼ 80 nm^2 (Crowther 1991). These features, combined

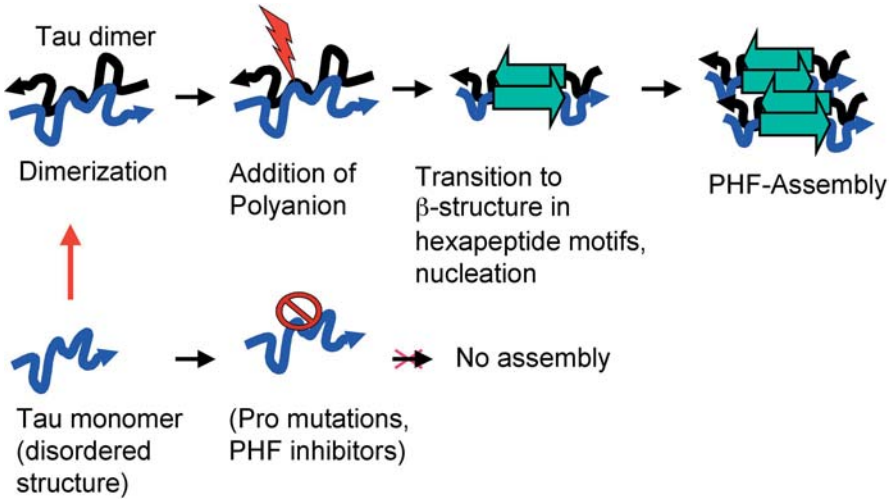

Fig. 6. Model of steps in PHF aggregation. The disordered tau monomer (*lower left*) initially dimerizes (*upper row*; this step can be enhanced by disulfide crosslinking), then partially converts to incipient β-structure around the hexapeptide motifs, followed by subunit addition to form a PHF nucleus and then a fiber with cross-β structure and several protofibrils. These steps can be accelerated by polyanions. If β-structure is prevented, e.g., by proline mutations in the hexapeptide motifs or by tau inhibitor compounds, the aggregation process is interrupted (*lower row*)

with the typical density of compact protein domains of $\sim$ 0.8 kDa/nm^3, represent boundary conditions that models of tau folding in PHFs will have to meet.

– The third and least well-defined aspect of PHF structure is the "fuzzy coat" surrounding the core (Wischik et al. 1988a). PHFs assembled from full-length tau and from the repeat domain have similar dimensions by electron microscopy, suggesting that the non-repeat parts of tau, comprising $\sim$ 70% of the protein (roughly residues 1–240, 370–441), make only a minor contribution to the apparent images, presumably because they retain their natively unfolded character (Barghorn et al. 2004). The extent of the fuzzy coat is best visualized by immunogold labelling, where antibody-binding sites can extend away from the center of the PHF. Nevertheless, a substantial fraction of tau molecules in PHFs must have a folded conformation, because PHFs can be immunopurified with antibody MC-1 whose epitope comprises tau residues near the N-terminus and within the repeat domain (Jicha et al. 1997).

Tau phosphorylation and aggregation

The two most noticeable changes of Alzheimer tau are its aggregation and extensive phosphorylation. Therefore, it is of great interest to test how these properties are related, and in particular, whether the phosphorylation of tau promotes the aggregation. The issue has been addressed by various authors using tau phosphorylated by different kinases and/or using pseudophosphorylated forms of tau, where certain residues were

exchanged for glutamate (for review, Stoothoff and Johnson 2005). In our opinion, these studies have yielded mixed results, i.e., the reported changes in aggregation kinetics caused by phosphorylation were, on the whole, quite variable. This variability is perhaps not surprising, considering the large number of potential phosphorylation sites on tau, the limited specificity of kinase preparations and the heterogeneity of phosphorylation states. In the case of some sites, e.g., the phosphorylation of the KXGS motifs in the repeat domain, phosporylation clearly inhibits aggregation rather than promoting it (Schneider et al. 1999). In the context of the present discussion, the important point is that aggregation of tau into bona fide PHFs can be achieved without any phosphorylation; therefore, it seems unlikely that phosphorylation has a major influence on PHF structure as such.

We also note that phosphorylation of tau in cells can change its properties on at least two different levels, tau-microtubule interactions and tau-tau interactions in PHFs. In the former case, phosphorylation generally tends to decrease the binding to microtubules (here again the phosphorylation at the KXGS motifs in the repeats and at S214 appear to have the most pronounced effects; Biernat et al. 1993; Brandt et al. 1994). The result is a decrease in microtubule stability but, perhaps more importantly, an increase in the cytosolic pool of tau, which can contribute to aggregation by mass action, independently of phosphorylation.

Implications for testing the role of PHFs in cell and animal models

Although the structure of PHFs is interesting from a biological point of view, the overriding goal is to use the structural information for preventing the neuronal degeneration in AD. The current insights can be used in two ways. First, one of the main obstacles to generating cell or animal models has been the exceptional solubility of tau (mentioned above), which means that simple overexpression of the protein (or modification by phosphorylation) is usually not sufficient to generate neurofibrillary tangles in a reasonable time frame. This problem can be overcome by using mutations that are known to enhance β-structure. Examples are the P301L and ΔK280 mutations, both known as FTDP-17 mutations, which modify the vicinity of the hexapeptide motifs to allow more extensive β-conformation (von Bergen et al. 2001). In both cases, transgenic mice readily develop neurofibrillary tangles (Lewis et al. 2000; and own observations, unpublished). With these models, it is possible to test hypotheses on tau-induced neurodegeneration such as the question of tau aggregation vs toxicity. For example, our tau-inducible cell models show that tau aggregation into PHFs is toxic, can be reversed by shutting down the expression of tau, and can be prevented by proline mutations in the hexapeptide motifs or aggregation inhibitor compounds (Khlistunova et al. 2006; Fig. 7).

Secondly, once the conditions for tau aggregation in vitro and in cell or animal models are known, it will be possible to search for compounds that are able to inhibit or reverse the aggregation process. Several reports on the screening of compound libraries and the identification of potential lead structures have appeared (Chirita et al. 2004; Pickhardt et al. 2005; Taniguchi et al. 2005). In some cases, low molecular weight compounds have even been efficacious in preventing tau aggregation in cell or animal models and in reducing tau-induced toxicity (Hall et al. 2002; Khlistunova et al. 2006).

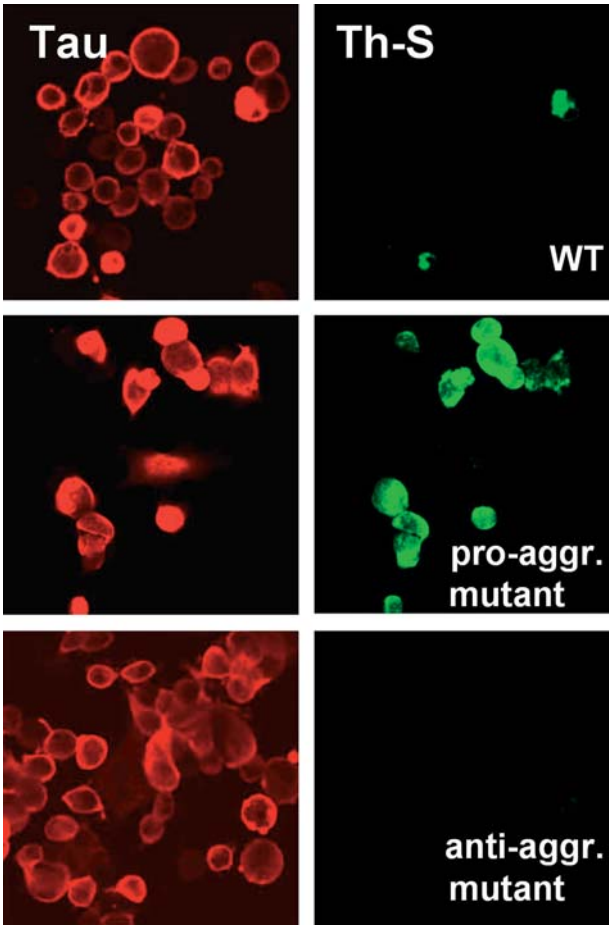

Fig. 7. Cell model of tau aggregation, based on the inducible expression of tau protein (repeat domain) in N2a cells. The red fluorescence (*left column*) shows the presence of tau repeat domain after switching on its gene expression. The green fluorescence (*right column*) illustrates aggregation (aggr.) with cross-β structure, visualized by the dye thioflavin-S. Note the pronounced aggregation with the "pro-aggregation" mutant ΔK280 (*middle row*), but much lower or no aggregation in the case of the wild-type (WT) sequence or the anti-aggregation mutant (ΔK280/I277P/I308P)

Thus there is hope that this search will one day lead to compounds that keep the buildup of tau aggregation under control in humans and thus contribute to the cure of the disease that A. Alzheimer identified 100 years ago.

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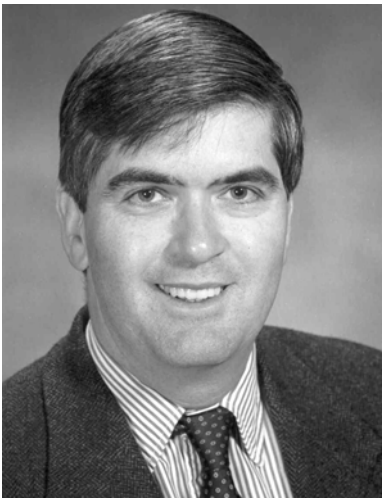
Oxidative Stress

Mark A. Smith



George Perry

The Changing landscape of Alzheimer's disease: From Insoluble to Soluble and from Pathogen to Protector

Mark A. Smith[1] and *George Perry*[1,2]

The new era of Alzheimer's disease (AD) research, catalyzed by the studies of Tomlinson, Blessed and Roth in the UK and Katzman in the US, can be broadly characterized into two major epochs. The first era involved studying the insoluble protein components of fibrils of the canonical lesions of AD, the senile plaques and neurofibrillary tangles (NFT). While classifying the pathology as causative in disease pathogenesis was noseological, the die was cast and, for the first epoch, research centered on why tau and amyloid-β aggregated to form fibrils and, in parallel, how fibrils elicited AD. The second epoch, only just begun, centers not on fibrils but on smaller microaggregates of amyloid-β and tau. Against this backdrop, our work on the cytoskeleton and mechanisms of insolubility led us from oxidative stress to metabolic and mitotic pathways. Our conclusions are that lesions of disease are pathognomonic, not pathogenic, and that both amyloid-β and phospho-tau are, if anything, to be revered, not rebuked.

Impure Thoughts on Cytoskeletal Composition

The fibers of NFT, paired helical filaments (PHF), are insoluble in denaturants (Grundke–Iqbal et al. 1981), prohibiting direct quantitative biochemical analysis. Nonetheless, evidence indicated that PHF were structurally similar and shared epitopes with two major cytoskeletal systems of neurons, microtubules (Iqbal et al. 1978; Grundke–Iqbal et al. 1979b) and neurofilaments (Gambetti et al. 1983a,b). While this early work was confounded by the low resolution of light microscopy, ultrastructural localization firmly established that neurofilaments and microtubule proteins were insoluble integral elements of PHF (Perry et al. 1985). In 1986, two articles claimed that neurofilament epitopes in PHF represented a cross-reaction of neurofilament antibodies to tau (Ksiezak–Reding et al. 1987; Nukina et al. 1987). Although subsequent work by at least three groups showed in fact that neurofilament epitopes were distinct from tau (Lee et al. 1988; Mulvihill and Perry, 1989; Zhang et al. 1989), neurofilament involvement in PHF was subsequently seldom studied. While great strides have been made with the "tau only" mechanism of PHF/NFT formation, it was the broader dissection of the processes involved in the pleotropic changes in cytoskeletal proteins that led to our work on oxidative abnormalities (Smith et al. 1994a). A new way to isolate PHF-tau fractions that were soluble became the standard preparation for biochemical studies on PHF (Greenberg et al. 1992). As with the tau-only hypothesis, this led to great strides

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in our understanding of PHF biochemistry, but at the cost of discontinuing analysis of the fibers that form NFT in vivo. NFT fibers vary from straight to PHF (Perry et al. 1991; Praprotnik et al, 1996), extend the length of the neurons, and are insoluble (Smith et al. 1996). In contrast, SDS-soluble PHF are homogeneous, and their isolation requires the addition of a detergent to a non-filament containing supernatant, suggesting that these filaments result from sarcosyl-induced assembly of tau present in brain extracts. Why the PHF in brain are insoluble and contain numerous components of the cytoskeleton beyond tau (e.g., Galloway et al. 1990) remains to be revealed.

Resolving Cytoskeletal Insolubility

The first suggested biochemical mechanism for PHF insolubility was crosslinking by glutaryl-lysine, bonds catalyzed by transglutaminase (Selkoe et al.1982a). In vitro, transglutaminase can crosslink tau or neurofilaments, but in vivo evidence such as detection of glutamyl-lysine has been scant. Insolubility resulting from crosslinking is a critical event, making polymers resistant to removal and thus inhibiting normal proteolytic pathways (Friguet et al. 1994). Ubiquitination of PHF in vivo (Mori et al. 1987; Perry et al. 1987b) and persistence in neurons for years suggest that NFT are resistant to proteolytic removal. Glycation of PHF (Smith et al. 1994b; Yan et al. 1994), discovered in the mid-1990s, provided a basis for insolubility and resistance to proteolytic removal, as did the subsequent findings of lipid peroxidation related modification (Sayre et al. 1997), all suggesting that reactive carbonyls play an important role in PHF biochemistry. Aldehyde modifications also play an important role in PHF-specific epitopes, since many antibodies raised to PHF recognize epitopes induced by the reaction of carbonyls with phosphorylated but not non-phosphorylated tau. Interestingly, some of the antibodies raised to PHF, e.g., Alz50, only recognize intermediates in the reaction of carbonyls with tau and not highly crosslinked tau (Takeda et al. 2000). Neurofilaments show similar properties, with some antibodies raised to NFT recognizing carbonyl-induced epitopes of phosphorylated neurofilaments but not tau (Perry et al. 1987a; Smith et al. 1995).

Oxidative Stress: From Secondary to Primary Event

Original descriptions of oxidative stress (Martins et al. 1986; Smith et al. 1991) were dismissed as epiphenomena (Mattson et al. 1995), based on the notion that plaques and NFT were long-lived and hence "expected" to accumulate oxidative damage similar to in other long-lived proteins such as collagen (Monnier and Cerami 1981). Amyloid-β in vitro was found to be toxic via an oxidative mechanism (Yankner et al. 1989), placing oxidative stress as a secondary event. This toxicity in vitro is related to its ability to bind to iron (Schubert and Chevion 1995; Rottkamp et al. 2001). Cell culture studies aside, what happens in vivo? Proponents of the longevity notion were somewhat correct; long-lived proteins do accumulate oxidative damage. However, the majority of the oxidative damage that is found in AD is short-lived. Indeed, oxidized RNA is markedly increased in neurons in AD (Nunomura et al. 1999) and Down syndrome

(Nunomura et al. 2000). Since RNA is rapidly degraded (i.e., turned over), looking at its oxidation state allowed the opportunity to see a "snapshot" of ongoing oxidative stress, as opposed to the cumulative history of such stress, and doing so revealed extremely novel insights regarding the pathogenesis of AD. First, oxidative stress is among the earliest, if not the earliest, change in disease pathogenesis (Nunomura et al. 2001). Second, RNA serves as a major iron-binding molecule in AD (Honda et al. 2005). Third, and perhaps most important, oxidative stress is inversely related to amyloid-β and tau pathology (Smith et al. 2002). The latter insight, that oxidative stress is lowest in neurons containing intracellular amyloid-β or phosphorylated tau, indicated that not only is pathology secondary to oxidative stress but that it serves to attenuate such stress. Indeed, in light of seminal findings by Jesus Avila showing that oxidative stress can lead to tau phosphorylation and aggregation of PHF (Gomez-Ramos et al. 2003), and by David Stern and Shi Du Yan showing that oxidative stress leads to increased levels of amyloid-β in culture (Yan et al. 1995), a picture emerges whereby oxidative stress both precedes amyloid-β and tau phosphorylation (Nunomura et al. 2001; Pratico et al. 2002) and serves to increase amyloid-β (Li et al. 2004) and tau phosphorylation (Perez et al. 2000) which are then, ultimately associated with decreased oxidative stress. Based on this we developed the concept that amyloid-β and tau are secondary, protective responses mounted by the brain in an effort to lessen oxidative damage (Joseph et al. 2001; Rottkamp et al., 2001; Smith et al. 2002; Lee et al. 2004b, 2005).

Pathogen or Protector?

Viewing amyloid-β and tau as antioxidants provides an explanation for the in vitro "oxidative" effects of amyloid-β (since all antioxidants are, by definition, also prooxidants dependent on environment) as well as the in vivo antioxidant properties of amyloid-β. Therefore, as we celebrate the 100th anniversary of Alois Alzheimer's original paper describing the pathological lesions, we find ourselves at a crossroads, namely, is the pathology a harbinger of disease or a protective response to the disease? The latter would represent a major paradigm shift but, after 100 years, is it not about time!?

Mark P Mattson

Molecular and cellular pathways towards and away from Alzheimer's disease

Mark P. Mattson[1]

Research in my laboratory is aimed at understanding the factors that determine whether neurons thrive or degenerate during aging (http://www.grc.nia.nih.gov/branches/lns/index.html). Our approach is to elucidate molecular and cellular mechanisms that regulate neuronal plasticity and survival, in the contexts of brain development and aging, and to determine if and how these mechanisms are altered in neurodegenerative disorders. We are particularly interested in signaling and metabolic pathways that are common to multiple neurodegenerative disorders (Fig. 1). Here I summarize some of

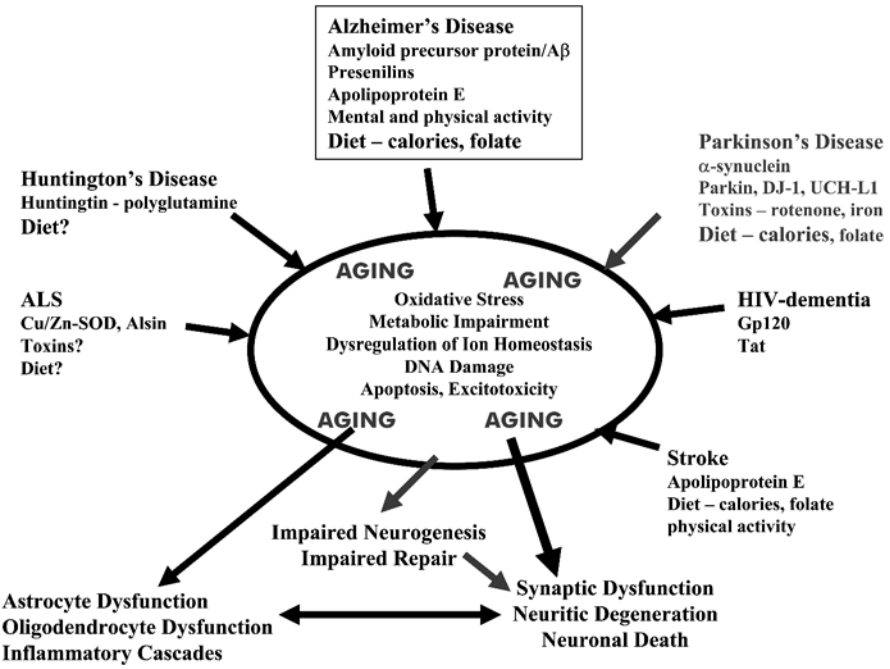

Fig. 1. Although genetic and environmental factors that lead to neuronal dysfunction and death may differ among neurodegenerative disorders, neurons suffer similar consequences (many of which occur during normal aging), including oxidative stress, energy deficits, perturbed ion homeostasis and accumulation of dysfunctional proteins

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the findings from my laboratory during the past three decades that have contributed to an understanding of the pathogenesis of Alzheimer's disease (AD), and to its prevention and treatment. The findings described below help to place key discoveries in other laboratories in the areas of genetics, amyloid and tau biology (Selkoe and Schenk 2003; Forman et al. 2004; Hardy 2004; Dermaut et al. 2005) within the broader context of mechanisms of aging, neuronal plasticity and cell death (Mattson 2004).

Two examples of concepts that have arisen from our research are 1) the same cellular signaling mechanisms that regulate the formation of neuronal circuits during development of the nervous system are intimately involved in the pathogenesis of neurodegenerative disorders, and 2) the calcium ion is a key regulator of neuronal plasticity and survival, and disruption of cellular calcium homeostasis plays a pivotal role in neuronal dysfunction and death in both acute and chronic neurodegenerative conditions. One of our early findings relevant to AD was that glutamate, the major excitatory neurotransmitter in the central nervous system, plays an essential role in sculpting the formation of neuronal circuits by precisely regulating dendrite outgrowth and synaptogenesis (Mattson et al. 1989). We established that glutamate regulates the architecture of neurons by activating receptors linked to calcium influx and that calcium, in turn, controls the state of polymerization of actin filaments and microtubules, thereby controlling growth cone behaviors. We also found that overactivation of glutamate receptors can cause neuritic degeneration and cytoskeletal alterations similar to those seen in neurons that degenerate in AD (Mattson 1990). In subsequent studies, we showed that neurotrophic factors can modify the effects of neurotransmitters on neurite outgrowth, synaptogenesis and cell survival, and that this is accomplished by regulation of the expression of genes that encode proteins that regulate cellular calcium homeostasis. The latter findings opened a new area of investigation in the neuroscience field, namely, the functions of neurotrophic factors in synaptic plasticity. Our work on neurotrophic factors (Cheng and Mattson 1991) has also led to clinical trials of neurotrophic factors in human patients, with a specific example being a trial of fibroblast growth factor in stroke patients.

Our research has increased understanding of the biochemical cascades responsible for neuronal dysfunction and death in AD. One example is our investigations into the links between oxidative stress and alterations in cellular ion homeostasis. It had been recognized that oxidative stress was involved in neurodegenerative disorders, but the causes of the oxidative stress and the specific ways in which oxidative stress results in synaptic dysfunction and selective neuronal degeneration were unknown. We established that amyloid beta-peptide (Aβ) induces oxyradical production, resulting in membrane lipid peroxidation in neurons. These effects of Aβ on the plasma membrane destabilize neuronal calcium homeostasis and render neurons vulnerable to excitotoxicity (Mattson et al. 1992). We showed that an aldehyde called 4-hydroxynonenal is liberated from peroxidized lipids and covalently modifies membrane ion- motive ATPases and glucose and glutamate transporters, impairing their function and thereby disrupting cellular calcium homeoastasis and causing ATP depletion (Mark et al. 1995, 1997). Moreover, we showed that this same lipid peroxidation cascade contributes to the degeneration of neurons in other neurodegenerative disorders, including stroke and amyotrophic lateral sclerosis. We also found that more subtle levels of membrane lipid peroxidation can impair coupling of membrane receptors to GTP-binding proteins, resulting in impaired synaptic transmission. This work established a previously unrec-

ognized link between oxidative stress and disruption of cellular calcium homeostasis in AD. We also identified several signaling pathways as being capable of protecting neurons from being damaged and killed by Aβ, with the transcription factor NF- κB being a key target of several such neuroprotective signaling pathways (Mattson and Meffert 2006).

More recently, we have revealed mechanistic links between oxidative stress, perturbed membrane lipid metabolism and neuronal death in AD. We found that alterations in sphingolipid and cholesterol metabolism during normal brain aging and in the brains of AD patients result in accumulation of long-chain ceramides and cholesterol (Cutler et al. 2004). Membrane-associated oxidative stress occurs in association with the lipid alterations, and exposure of hippocampal neurons to Aβ induces membrane oxidative stress and the accumulation of ceramide species and cholesterol. Treatment of neurons with alpha- tocopherol or an inhibitor of sphingomyelin synthesis prevents accumulation of ceramides and cholesterol and protects them against death induced by Aβ. Our findings suggest a sequence of events in the pathogenesis of AD in which Aβ induces membrane-associated oxidative stress, resulting in perturbed ceramide and cholesterol metabolism which, in turn, triggers a neurodegenerative cascade that leads to clinical disease.

Although the normal function of the amyloid precursor protein (APP) is not yet established, we have provided evidence that the α-secretase-derived form of APP (sAPPα) regulates neuronal excitability, synaptic plasticity and neuronal survival. Using whole cell perforated patch and single channel patch clamp analysis of hippocampal neurons, we showed that sAPPα suppresses action potentials and hyperpolarizes neurons by activating high conductance, potassium channels (Furukawa et al. 1996). Activation of potassium channels mediates the ability of the sAPPα to decrease intracellular calcium. In studies of hippocampal slices, we went on to show that sAPPα modulates synaptic plasticity in ways that strongly suggest a fundamental role for this APP-derived signaling protein in the regulation of learning and memory. We further showed that activation of the sAPPα signaling pathway can protect neurons against excitotoxic, oxidative and metabolic insults relevant to AD pathogenesis (Mattson et al. 1993). We also showed that the β-secretase-derived form of APP exhibits a marked decrease in physiological activity. The latter finding suggests that the shift in proteolytic processing towards increased β-secretase cleavage that may occur in AD may impair synaptic plasticity by decreasing levels of sAPPα.

Findings from our laboratory led to a new view of apoptotic biochemical cascades in the physiological regulation of synaptic plasticity and structural remodeling, and introduced the neuroscience field to the concept of "synaptic apoptosis." We showed that apoptotic cascades involving premitochondrial, mitochondrial and postmitochondrial components can be activated by physiological stimuli such as glutamate and trophic factor withdrawal in synaptic terminals and axons and dendrites. Moreover, the apoptotic cascades were shown to modify synaptic transmission and mediate structural remodeling of neuronal circuits, synapse loss and replacement. A specific example of our work in this area is the discovery that certain glutamate receptor subunits (AMPA receptor proteins GluR1 and GluR4) are direct substrates of caspase-3; cleavage of the subunits results in reduced AMPA currents and a resultant modification of synaptic function (Glazner et al. 2000). These findings reveal an entirely new function of apoptotic proteases as regulators of synaptic plasticity.

We have also contributed to the identification of the cellular and biochemical bases for the pathogenic actions of genetic mutations that cause early-onset inherited forms of AD. For example, we showed that presenilin-1 mutations cause synaptic dysfunction and increase the vulnerability of neurons to apoptosis and excitotoxicity by a mechanism involving an abnormality of calcium regulation in the endoplasmic reticulum (Guo et al. 1999). The calcium signaling defect was shown to involve overfilling of calcium pools. Presenilin-1 mutant knockin mice exhibited increased vulnerability to focal ischemic brain injury, suggesting a mechanism whereby presenilin mutations may promote neuronal degeneration under conditions of impaired energy metabolism (Mattson et al. 2000). Others had shown that presenilin-1 mutations and APP mutations result in increased production of Aβ and decreased production of sAPPα. Our work revealed how this altered processing of APP causes a disruption of neuronal calcium homeostasis that may contribute to synaptic dysfunction and cell death in AD. These findings have identified novel therapeutic targets for drug development, including enzymes that process APP, and proteins that regulate neuronal calcium homeostasis.

While most work on presenilins and γ-secretase have focused on APP as a substrate, we recently provided evidence that γ-secretase-mediated cleavage of Notch renders neurons vulnerable to metabolic injury (Arumugam et al. 2006). Notch antisense transgenic mice, and normal mice treated with inhibitors of γ-secretase, exhibit reduced brain cell damage and improved functional outcome in a focal ischemic stroke model. Notch endangers neurons by modulating pathways that increase their vulnerability to apoptosis and by activating microglial cells and stimulating the infiltration of proinflammatory leukocytes. These findings reveal Notch signaling as a novel therapeutic target for stroke and related neurodegenerative conditions. γ-Secretase inhibitors have been developed for the treatment of AD but side effects associated with the long-term treatments required for this disease render them unlikely to be used in patients. In the case of stroke, on the other hand, short-term treatment with γ-secretase inhibitors may prove effective in reducing brain damage without serious side effects. We also provided evidence that Notch signaling plays roles in synaptic plasticity in the adult brain (Wang et al. 2004). Mice with reduced Notch levels exhibit impaired LTP at hippocampal CA1 synapses. The Notch ligand Jagged enhances LTP in normal mice and corrects the defect in LTP in Notch antisense transgenic mice. Levels of basal and stimulation-induced NF-κB activity were significantly decreased in mice with reduced Notch levels. These findings suggest an important role for Notch signaling in a form of synaptic plasticity associated with learning and memory processes.

We have been working to identify dietary factors that may affect the risk of AD. We found that dietary restriction can increase the resistance of neurons in the brain to dysfunction and degeneration in animal models of relevance to the pathogenesis of Alzheimer's, Parkinson's and Huntington's diseases and stroke (Bruce–Keller et al. 1999; Duan et al. 2003; Maswood et al. 2004; Mattson 2005). The underlying mechanism was shown to involve increased production of neurotrophic factors, protein chaperones and mitochondrial uncoupling proteins, suggesting an hormesis response of brain cells to dietary restriction (Fig. 2). More recently, we have shown that dietary folic acid can protect neurons and improve behavioral outcome in an animal model of AD (Kruman et al. 2002). Folic acid deficiency results in elevated levels of homocysteine, resulting in an impaired ability of neurons to repair damaged DNA, which renders neurons vulnerable to being killed by Aβ. The implication of these findings is that dietary

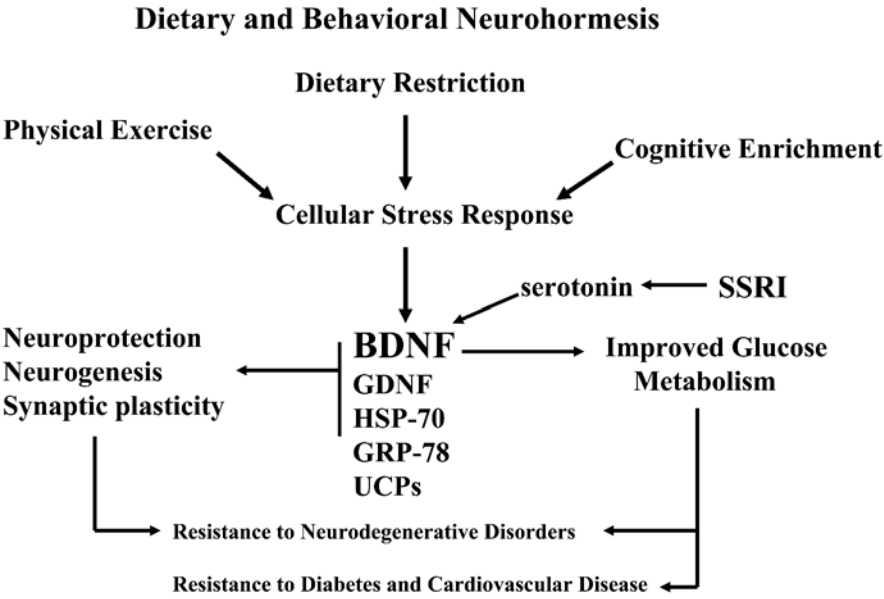

Fig. 2. Three different environmental factors that may reduce the risk of AD (exercise, cognitive stimulation and dietary restriction) may activate similar adaptive cellular stress response pathways in neurons. (Modified from Mattson et al. 2004)

restriction and folic acid supplementation will decrease the risk of neurodegenerative disorders in humans. These findings provide an example of how basic research into the biochemistry and biology of neuronal plasticity and death have resulted in information that individuals can apply to their daily lives to reduce their risk of AD.

Epidemiology

Jean François Dartigues

Paquid: an integrated, multidisciplinary, population-based approach to normal and pathological cerebral aging

Jean François Dartigues[1]

The study of cognitive decline in human beings during normal aging and during the morbid process leading to Alzheimer's disease, and of its consequences in terms of dependency, requires a certain number of methodological developments. Cognition is an abstract general concept characterized by several components: memory, language, attention, executive functions, sensory perception, visuo-spatial capacities, etc. Each of these components can be measured using more or less "pure" tests, i.e. tests that are more or less specific to the component. Unfortunately, the physiopathological process of decline that characterizes both normal and pathological aging cannot be directly measured, and researchers have to make do with surrogate markers that are assumed to reflect the process as closely as possible. It has been shown that some functions are more likely to deteriorate with age or in the preclinical phase of dementia (memory, attention, executive functions) (Masur et al. 1994). Therefore, the first step is to carefully select the functions tested and the test methods used, in order to minimize ceiling and floor effects, learning effects in repeated testing, and also the risk of refusal from subjects facing an excessive battery of tests (Morris M.C. et al. 1999). This first step requires neuropsychological expertise. The second step is to put together and monitor over time a sample of several thousand subjects, in order to obtain repeated measurements from several test runs and hence minimize variations due to chance, regression towards the mean and learning effects. This second step requires epidemiological and, more particularly, "geroepidemiological" expertise, due to the large number of epidemiological obstacles in elderly subjects. One of the most essential elements is to obtain and monitor a sample that reflects, as closely as possible, the phenomenon in the general population (Dartigues 2005). This implies minimizing participation refusals, which are always suspicious in studies of the aging process. The population-based approach is therefore unavoidable if we want to obtain a non-selected sample. This is true for both healthy and unhealthy subjects, given the many reasons for lack of medical care (denial or anosognosia of the disease, complaints to doctor not taken seriously, etc.).

The third step is to discern normal aging from dementia throughout the follow-up period. Dementia must be diagnosed by a specialist in clinical neurology. The fourth step involves analyzing data to 1) obtain a mathematical and longitudinal statistical representation that reflects the reality of the phenomenon as closely as possible and 2) estimate the impact of such and such a determinant. The fifth and final step entails analyzing the consequences of the results obtained, in terms of practical clinical reper-

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cussions and public health. In this way, we can hope to achieve a clear understanding of the physiopathology of the morbid processes leading to aging and dementia and, possibly, identify the factors on which we can act and determine when we should act on them to prevent or delay these processes. We should also be able to predict the cognitive future of the subjects according to these factors.

Obviously, to ensure that research is conducted efficiently, the protocols must be designed right at the very start and then implemented and analyzed jointly and integrally by neuropsychologists, epidemiologists, neurologists or geriatricians, biostatisticians and public health specialists. Therefore the five steps mentioned above are not successive but simultaneous and complementary. New theoretical developments are essential at every level, because this type of multidisciplinary approach has rarely been adopted anywhere in the world or because the approaches that have been adopted have not been satisfactory: biostatistical modelling of cognitive decline and dementia, including or not including death; development or improvement of clinical concepts such as "mild cognitive impairment" (MCI), a transition stage between normal cognitive function and dementia; development or improvement of neuropsychological theories explaining symptoms observed and their evolution; original analysis of the functional consequences of neuropsychological troubles at all stages of their development; development of an original epidemiological method for optimizing sample representativeness and the quality of data collection. Since 1988, such an integrated, multidisciplinary and population-based research approach has been developed through the establishment and follow-up of the Paquid cohort thanks to the simultaneous presence at the Bordeaux IFR (Public Health Research Institute) of INSERM E0338 biostatisticians, epidemiologists from INSERM units 593 and 657, clinicians from the Department of Geriatrics and the CMRR (Memory Resources and Research Centre) at Bordeaux University Hospital, and neuropsychologists and public health specialists from unit 593 and the ISPED (Institute for Public Health, Epidemiology and Development).

Paquid is still presently the only cohort of elderly subjects in France that satisfactorily represents the general population, to the extent that it can be used to estimate the prevalence, incidence, average duration, prognosis and consequences of dementia. All these data are essential to planning public health measures and organizing health care. The 10-year follow-up of the cohort (Ramaroson et al. 2003) provided estimations that greatly impressed political decision- makers and prompted the decision to set up two successive Government Plans on Alzheimer's disease and related syndromes. These included, in particular, the creation of CMRRs and "memory consultations". Paquid was the first cohort in France and one of the first cohorts in the world to study risk factors for dementia and Alzheimer's disease (Dartigues et al 1991), opening up new perspectives for preventing a disease that has for a long time been seen as an irreversible end-of-life phenomenon.

The most widely discussed part of the Paquid study (besides the EURODEM meta-analyses) concerns the relationship between moderate wine consumption and the risk of Alzheimer's disease (Orgogozo et al. 1997). For some epidemiologists, this work is detrimental to the reputation of the Paquid study and the researchers involved in it. Working on such a sensitive subject as alcohol in Bordeaux, and demonstrating the possible benefits of alcohol for the brain, could be seen as being slightly trivial, or even thoughtless. In fact, we continue to stand by these results, which have since been confirmed by many studies throughout the world, using other alcoholic beverages than

wine (Ruitenberg et al. 2002). Let's be perfectly clear: we are almost certain that there is a connection between moderate wine consumption and a decreased risk of dementia, but we believe that this decrease is due to the specific characteristics of the moderate drinker (and especially the non-drinker) rather than to the effects of the wine itself. Any adjustment is impossible, as what best characterizes the moderate drinker is precisely the fact that he drinks moderately. And a randomized trial is not possible, at least not in Bordeaux … The history of the effects of estrogen therapy on menopause (and that of statins) shows that we need to exercise great care when observing a relationship between a specific behaviour (use of medical care, consumption, leisure activities, etc.) and the risk of dementia (Shumaker et al. 2003).

Other risk factors have been investigated as part of the Paquid study. Two of them in particular continue to interest us: gender and the level of education. Most studies in the world have shown a higher incidence of dementia and Alzheimer's disease in women, (Fratiglioni et al. 2000) except for those conducted on the East coast of the United States, where women's activities and behaviors are closer to those of their male counterparts. An initial analysis, after eight years of cohort follow-up, showed that women in the Paquid study had a higher incidence of dementia and Alzheimer's disease after the age of 75 (Letenneur et al. 1999). However, in most cohort studies, death is a much more frequent event that competes with the risk of dementia, with life expectancy being much shorter in men. The use of multi-state models, which take death into account, confirms that women are more likely to suffer from dementia, but at a later stage in life (after 80) and less severely (Joly et al. 2002). A more recent analysis, based on age-related cognitive decline and not on the risk of dementia, seems to contradict this finding, however: gender does not impact either performance levels or the rate of age-related decline (Proust et al. 2006). It would therefore seem that the risk of dementia is higher in women, but that the age-related cognitive decline process combining both the effects of age and the morbid processes leading to dementia is no different from that observed in men. One explanation may be that dementia is more easily diagnosed or identified in elderly women, who are often widowed and live alone, taking full responsibility for everyday domestic activities.

The relationship between Alzheimer s disease and the level of education, which was revealed when the cohort was first put together (Dartigues et al. 1991) (contemporary with Zhang et al. (Zhang et al. 1990)) was confirmed subsequently. However, once again, this relationship has not been found everywhere in the world, in particular, not on the East coast of the United States. The most attractive theory is that brain reserve capacity is accumulated during childhood and adolescence (Stern 2002). In the Paquid study, the analysis of the incidence of dementia according to age shows that the risk of dementia is reduced by around half in subjects with a high level of education (Letenneur et al. 1999). In our study, the primary school diploma was used to separate subjects with a low education level from those with a high education level and hence to predict dementia. A sociological analysis of this diploma shows that, prior to 1945, it was an absolutely essential attainment for anyone wishing to pursue a non-manual profession. Unfortunately, in this respect, it is not really comparable with systems in other countries, and especially with the number of years of schooling offered in Anglo-Saxon countries. In fact, the attainment of a primary school diploma seems to delay the risk of dementia by five years, rather than really reduce it (Joly et al. 2002). The reserve capacity theory could therefore be validated by these data. We have also modelled the

effect of education level on the evolution of cognitive performance prior to the onset of dementia. It very clearly appears that the evolution of cognitive performance differs greatly depending on whether the subject has the primary school diploma or not. In subjects who develop dementia at the age of 75 and have a primary school diploma, the divergence from the rate of "normal" age-related cognitive decline occurs at 69, whereas in subjects without the primary school diploma it occurs much earlier at 57 (Jacqmin-Gadda et al. 2005). Hence, subjects with a low level of education experience "abnormal" cognitive decline at an earlier age, but the rate of "abnormal" decline is very slow. This finding probably explains why these subjects and their families are able to tolerate the cognitive troubles and do not seek medical assistance. On the other hand, subjects with a high level of education do not experience "abnormal" cognitive decline and dementia until a later stage. However, the rate of "abnormal" decline is abrupt, which probably explains why these subjects are not able to tolerate the related cognitive troubles and seek medical help (except in cases of denial …).

Many other results have been obtained through the Paquid study, especially with regard to the functional consequences of cognitive decline at all stages. We will continue to monitor the cohort, at least until the leading researchers retire.

Although population-based observation studies, which are often long and costly, are essential to obtain minimally biased estimations of the frequency of the disease as well as its duration, prognosis, secular trends and consequences in terms of dependency and public health, their utility in demonstrating the causality of a relationship between a risk factor and a disease is less obvious. In this context, the main advantage of these studies is that they identify factors that can be acted upon. However, the effect of these factors must then be confirmed by equally long and costly prevention studies, such as the one currently being conducted on EGb 761 (Extract of Gingko biloba 761), under the direction of B. Vellas in France and S. de Kosky in the United States.

Imaging

Mony J. de Leon

Hippocampal imaging in the early diagnosis of AD, 1988 to 2006

Mony J. de Leon[1]

Groucho Marx said, "Time flies like an arrow. Fruit flies like a banana;" such are my experiences in writing this summary. In 1977, as a tuition-poor Columbia University student, intrigued about the anatomical basis for dementia, I needed to develop a doctoral dissertation project. Computed Tomography (CT), introduced in 1972, was in its first generation in New York City. A fellow student told me, that at NYU, Steven Ferris was using CT scans to screen dementia patients for clinical trials. I was given the huge opportunity to examine the then short stack of NYU X-ray films and, after a chance meeting in 1978 in the hallway with a young NYU resident neurologist, Irwin Blau, and with neuroradiologist Ajax George, although I did not realize it then, my career had begun. We examined all possible scans from patients with senile dementia, patients with cognitive dysfunctions not severe enough to be called dementia which was then referred to as questionable dementia, other poorly understood dementias, such as normal pressure hydrocephalus, vascular, and Pick's, and as many of the normal spouses and patient care-givers that we could convince to stick their heads into the CT scanner. NYU neuropathologist John Pearson gave us specimens to scan and cut, and lessons at the microscope. The dementia field before 1980 did not consider imaging a "legitimate" research domain, and for several years we had no funding and no students. Virtually all clinical imaging in dementia was done to rule out stroke and other mass lesions. Of the few groups engaged in neuroimaging research, in the US and UK, the focus was on CT ventricular enlargement and cortical sulcal prominence (Roberts et al. 1976) and, in Scandinavia, xenon cortical perfusion imaging (Ingvar et al. 1975). We made hundreds of measurements on CT scans, using rulers, and temperature-controlled planimeters, even cutting out paper-traced ventricles with scissors and weighing them to learn: 1) if we could distinguish senile dementia of the Alzheimer type (SDAT) from normal, and 2) which regional measurements were best associated with cognitive impairment. In the process, we developed methods to evaluate cortical atrophy (de Leon et al. 1979) and periventricular white matter pathology (George et al. 1986a,b). With our rulers, scissors, and weighing scales, we learned that measurements of the temporal horn and third ventricle were better than those of the frontal horn in discriminating SDAT. This finding put us on the trail of temporal lobe pathology.

In 1980, the NIH funded five FDG-PET centers, one of which included Brookhaven National Laboratories (BNL) in partnership with NYU. Also in 1980, I began an NYU post-doctoral neuroimaging fellowship in Psychiatry and Radiology and got a salary. Now Al Wolf (BNL), Ajax George, David Christman (BNL), Steven Ferris, and Joanna

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Fowler (BNL) and I were working together. Thus began a period of great excitement and activity, in part made possible by my finding a rent-stabilized walk-up apartment in Manhattan and a shared desk at NYU. In the absence of computers to assist with image analysis, using draftsman tools, David Christman and I developed CT and PET image registration protocols to map regional tissue glucose metabolism in 2-D. Later, Henry Rusinek and Wai Tsui of NYU computer automated this coregistration process and compiled a library of 3-D image analysis tools, called MIDAS, that continues to grow. However, in 1980, samples were outlined on hard-copy CT films and mechanically transferred to paper FDG PET scan printouts to yield the tissue sample coordinate values that were phoned in to BNL. Days later, we received back the regional metabolic rates. We published the first FDG PET paper in 1980 in which we demonstrated widespread metabolic reductions in SDAT relative to normal control (Ferris et al. 1980). In London in 1981, Frackowiak demonstrated that oxygen metabolism is reduced in SDAT using PET (Frackowiak et al. 1981), and within five years, there were reports from Berkeley University (Friedland et al. 1983), NIH (Cutler et al. 1985; Duara et al. 1983), University of Pennsylvania (Alavi and de Leon 1985) and UCLA (Benson et al. 1983; Kuhl et al. 1982) that cortical glucose metabolism reductions in AD were observed using PET. Frackowiak et al. (1981), Metter et al. (1985), Foster et al. (1984), and Friedland et al. (1983) refined this view to highlight the now well-known temporoparietal deficit characteristic of AD. Yet, in spite of Ball having pronounced in 1985 that AD was a "hippocampal dementia" (Ball et al. 1985), there was still no mention in the structural or metabolic imaging literatures of a hippocampal examination.

In 1984, Ajax and I began experimenting with scan angles in the acquisition protocols. We developed the so-called "negative angulation" to visualize the temporal horns on fewer tomographic slices. This protocol was also applied to the PET to enable more accurate sampling of the lateral temporal cortex glucose metabolism (the 1.5-cm resolution of the early PET cameras prohibited accurate isotope recovery from the hippocampus). This extra acquisition protocol also added to the cost of the CT scans, and with the help of Zaven Khachaturian at the NIH-NIA, we received funds to develop our structural imaging research. It was somewhat of a surprise for us to find that the negative angle CT scan acquisition revealed changes in the region of the hippocampus. We first termed these changes, which appeared somewhat like a dark blur on CT, peri-hippocampal lucency, reflecting our uncertainty as to whether the attenuation change was due to (CSF atrophy) or to tissue damage (see Fig. 1). After several years of studying the location and histology of the hippocampal lucencies, made possible in large measure with the post-mortem materials provided by neuropathologists Henryk Wisniewski at the Institute for Basic Research and Gleb Budzilovich and Douglas Miller from NYU, and the timely availability of a few good MRI scans, in 1988 we wrote a paper (de Leon et al. 1988) reporting that moderate to severe hippocampal atrophy was more common in SDAT [now called Alzheimer's disease (AD)] than in normal controls, and the magnitude of the atrophy was associated with hypercortisolemia following i.v. glucose infusion (see Fig. 2 adapted from de Leon et al. 1997). Also in 1988, Seab et al. wrote the first hippocampal volume paper demonstrating that AD patients have smaller hippocampi than controls. We later published anatomical validations reporting that hippocampal lucencies were caused by enlargement of the hippocampal, choroidal, and transverse fissures of Bichat secondary to the loss of hippocampal volume (de Leon et al. 1993; George et al. 1990; Narkiewicz and de Leon 1992; Narkiewicz et al. 1993)

(see Fig. 3 and (Petrella et al. 2003 for review). But, the proof for an early diagnosis still required longitudinal clinical prediction.

In 1989, in a combined, cross-sectional and prediction CT study of 175 patients, including 76 with AD, 27 with mild cognitive impairment (MCI) and 72 normal elderly, we reported that hippocampal atrophy was found in 87%, 70%, and 22% of the groups, respectively. But, perhaps our most relevant clue to the early diagnostic potential of hippocampal atrophy was that, unlike normal controls, who showed an increasing prevalence of hippocampal atrophy with age, hippocampal atrophy was independent of age in AD (de Leon et al. 1989). In other words the greater majority of AD patients consistently had hippocampal atrophy, whereas in normal subjects, hippocampal atrophy only became prevalent at great age. These findings encouraged us to examine whether hippocampal atrophy was a predictor of the decline to AD in "questionable dementia" patients (named mild cognitive impairment [MCI] by Barry Reisberg). In the prediction part of the study, we observed that 11/20 cases of MCI deteriorated to AD after three years, whereas 28/28 controls did not decline. The presence of hip-

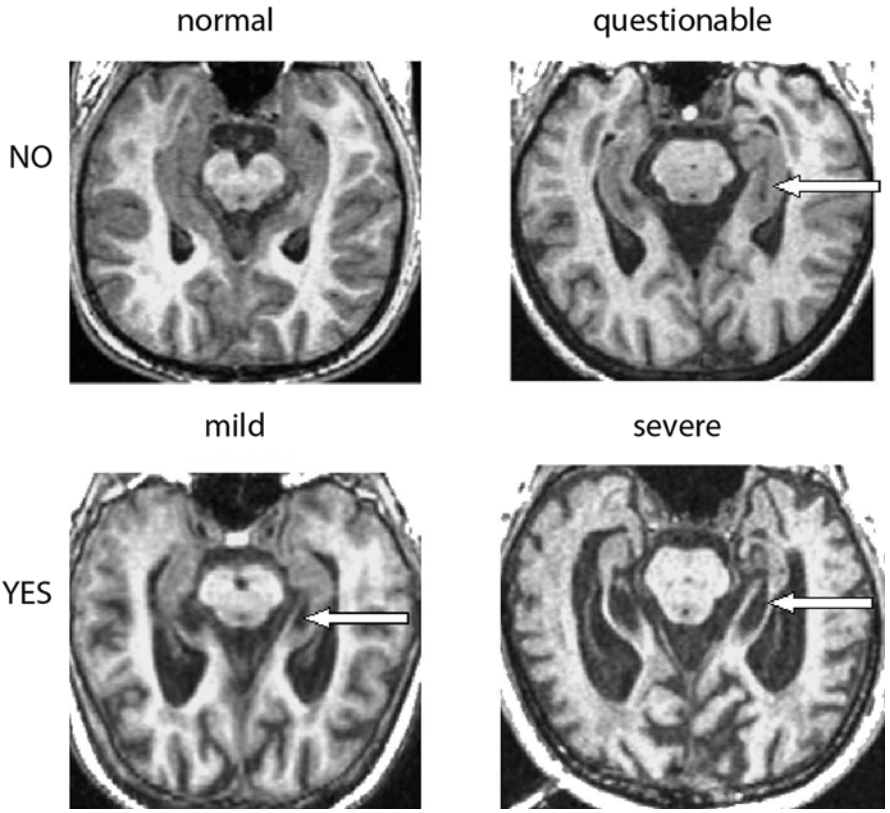


Fig. 1. Hippocampal atrophy rating scale as shown on MRI (de Leon et al. 1993, as derived from de Leon et al. 1989)

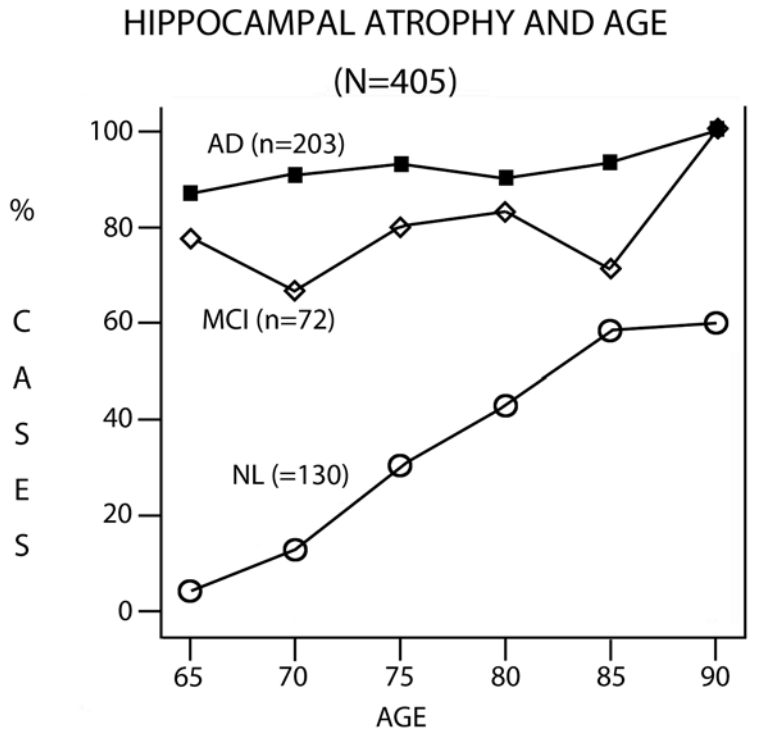


Fig. 2. Hippocampal atrophy as a function of diagnostic group and age (de Leon et al. 1997)

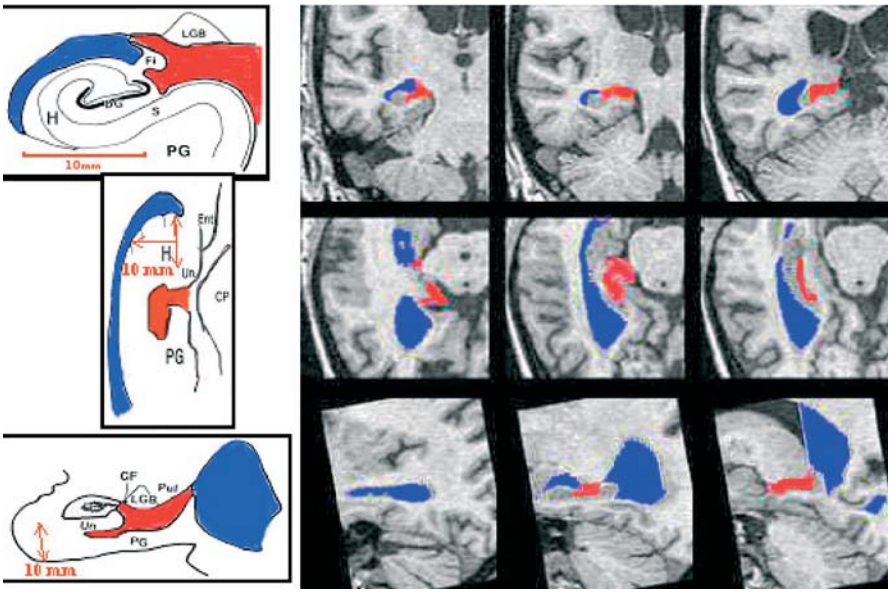


Fig. 3. Hippocampal fissures in *red* and ventricle in *blue* (de Leon et al. 1993)

pocampal atrophy correctly identified 91% of the decliners and 81% in each of the two non-declining groups (de Leon et al. 1989).

In 1993, we replicated our CT MCI to AD prediction finding using MRI (de Leon et al. 1993) and, in 1994, Jobst et al. in the UK demonstrated with CT marked hippocampal atrophy progression in AD. Our group, (Convit et al. 1993) showed that the MRI hippocampal volume loss was anatomically specific in MCI, and after 1997, others demonstrated the MCI to AD predictions using the MRI hippocampal volume (Jack et al. 1999; Kaye et al. 1997; Visser et al. 1999). In 2000, Bobinski et al. working with both Henryk Wisniewski's lab and our group reported that the hippocampal volume loss in AD was highly correlated with the number of hippocampal neurons. In parallel to the brain imaging work, in 1991 Flicker et al. at NYU demonstrated that declarative memory impairments predicted the transition from MCI to AD. Soon after we showed that memory function in normal aging (Golomb et al. 1993 and MCI (de Leon et al. 1993) was related to the hippocampal size. Thus, the earliest links between declarative memory changes, hippocampal size, and early AD pathology were made almost 15 years ago. Of immense value to this and the more recent imaging work were the pioneering observations of Heiko and Eva Braak (1991), who, in staging neurofibrillary pathology, demonstrated that the hippocampus and entorhinal cortices are among the early sites of damage in AD. Structural imaging has continued to contribute to the recognition of early clinical disease. MRI measured reductions in entorhinal cortex volumes were found to predict the MCI decline to AD (de Toledo-Morrell et al. 2000; Dickerson et al. 2001; Killiany et al. 2000) and perhaps to be even more useful than hippocampal volume measurements (Stoub et al. 2005). Most exciting, it was first reported by Rusinek et al. 2003 that accurate predictions of the decline from normal to MCI, or to AD, were possible with hippocampal measurements. This was later confirmed in studies by Rusinek et al. 2003, den Heijer et al. 2006 and Fox et al. 1996.

With improved spatial resolution of PET cameras (4–6 mm) and with the availability of the image registration algorithms developed by Pelizzari et al. (1989), Woods et al. (1993), and Rusinek et al. (1993), in 1997 we applied hippocampal imaging to PET (de Leon et al. 1997). While even today, the overwhelming majority of FDG-PET studies of MCI and AD rely on automated techniques that do not specifically examine the hippocampus, MRI-based FDG-PET sampling protocols consistently identify hippocampal metabolic abnormalities (see Nestor et al. 2003 and Mosconi et al. 2004 for review). Interestingly, De Santi showed that the PET hippocampal measurements were diagnostically superior to those obtained from MRI in classifying NL, MCI, and AD patients(De Santi et al. 2001). In 1998 Johnson et al. demonstrated with SPECT that hippocampal perfusion deficits predicted the transition between MCI and AD, and, in 2001, we showed with PET that entorhinal cortex and hippocampus glucose metabolism reductions predicted the transition between normal and MCI (de Leon et al. 2001).

Most recently, computerized image analysis approaches to examine the hippocampus have been developed. MRI approaches by Rusinek et al. (2003), Csernansky (Wang et al. 2003) and Thompson et al. (2004) and PET-based solutions by Mosconi et al. (2004) point to a new era. This new generation of automated tools will provide the opportunity for large-scale investigations to use standardized sampling of this difficult to measure, yet highly informative brain region.

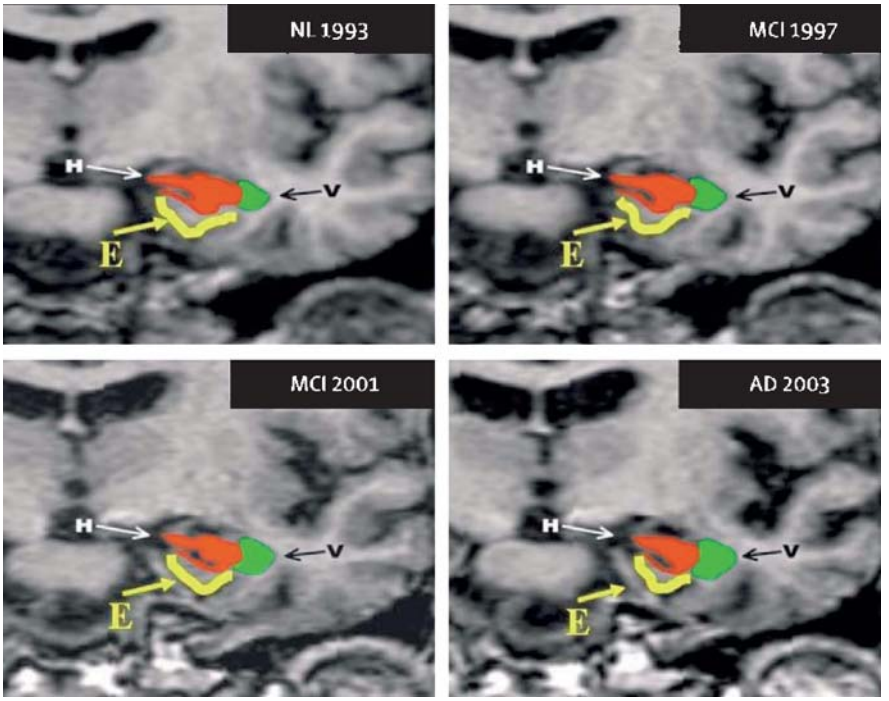
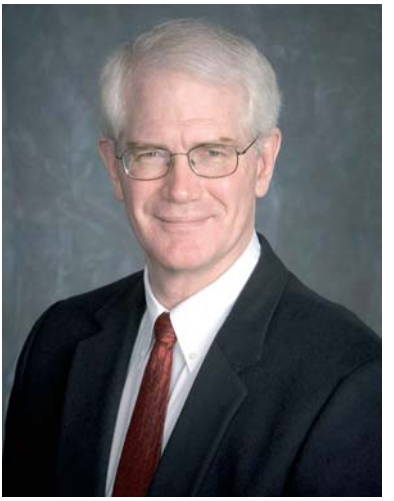


Fig. 4. Ten-year time series demonstrating on MRI, hippocampal (*red*), entorhinal cortex (*yellow*), and ventricular (*green*) changes in association with clinical decline from normal in 1993 to MCI to AD in 2003

In summary, over the past 18 years, structural and glucose metabolism imaging studies of the hippocampus and entorhinal cortex (see Fig. 4) have contributed to the early diagnosis of AD. The next horizon will be the use of imaging to select presymptomatic patients and to monitor a therapeutic course in primary prevention studies.

Acknowledgements. I am in debt to my brilliant colleagues: Ajax George, Henry Rusinek, Wai Tsui, Barry Reisberg, Steven Ferris, Susan De Santi, Antonio Convit, Maciek Bobinski, L.A. Saint Louis, and Lisa Mosconi. My appreciation goes to the many friends and patients who stuck their heads in scanners at my request. With gratitude for the support of Zaven Khachaturian and Neil Buckholtz from the NIH. In memory of my gifted mentors and pioneers: Alfred Wolf, David Christman, Henryk Wisniewski, and Jacob Cohen.



John C. Morris

Detecting Early-Stage Alzheimer's Disease in MCI and PreMCI: The value of informants

John C. Morris[1] and *Martha Storandt*[2]

In the century since Alzheimer's seminal presentation of the original patient with Alzheimer's disease (AD; Alzheimer et al. 1987), emphasis now is being given to the recognition of early-stage AD in comparison to nondemented aging. As a consequence, there is intense interest in mild cognitive impairment (MCI). The concept of MCI was introduced to characterize older individuals with cognitive deficits that, although abnormal for age, fell short of overt dementia (Flicker et al. 1991). [Related terms such as "age-associated memory impairment" (Crook and Bartus 1986) and "age-associated cognitive decline" (Levy 1994) were proposed as variants of normal cognitive aging, and "cognitive impairment, no dementia" (Graham et al. 1997) overlaps MCI but may characterize a broader array of cognitive dysfunction.] The MCI construct was further developed by Petersen and colleagues to feature memory deficits (amnestic MCI); this group proposed MCI as a transitional state between the cognitive changes of normal aging and AD (Petersen et al. 1999; 2001a). The influential work of Petersen and coworkers resulted in the recognition of MCI as a pathological condition imparting increased risk for AD (Bennett et al. 2002), and it now is a target of treatment trials that aim to prevent the conversion of MCI to clinically diagnosed AD (Petersen et al. 2005).

The MCI construct has engendered controversy, however, because not all individuals with MCI are at increased risk for AD; some individuals meeting criteria for MCI do not progress to AD and some even revert to normal over time (Ritchie et al. 2001; Larrieu et al. 2002; Fisk et al. 2003; Ganguli et al. 2004). Thus MCI is a heterogeneous condition with both neurodegenerative and other etiologies (Ancelin et al. 2006). This heterogeneity contributes to the variability in published outcomes for MCI, particularly for the rates of progression to AD. Other factors contributing to this variability include differences in case ascertainment (e.g., population-based studies versus samples from specialty clinics), nonuniform diagnostic criteria for MCI, and different implementation strategies for the criteria. The criteria for MCI recently have been refined to address its heterogeneous etiologies and identify subtypes without memory deficits or with deficits in multiple cognitive domains (Winblad et al. 2004; Petersen 2004). The major interest in MCI, however, remains focused on its relevance as a prodromal state for AD.

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Both the original and revised criteria for MCI have inherent conceptual difficulties and problems in operationalization that notably limit the value of the MCI construct. The representation of MCI as a "transitional state between the cognitive changes of normal aging and the earliest clinical features of Alzheimer's disease" (Petersen et al. 2005) disregards the basic pathobiology of AD, wherein synaptic and neuronal degeneration occur along a continuum from minimal to extensive. The initial clinical correlates of this progressive neuronal deterioration include mild impairment of memory and other cognitive abilities that may only subtly affect everyday function. That is, the initial clinical manifestation of AD is MCI. The very mild deficits associated with the early symptoms of AD may be insufficient to reach the current clinical detection threshold for many physicians, but nonetheless these deficits represent the earliest clinical features of AD. Stated another way, conceptually MCI is not a risk factor for AD, it already *is* AD in its earliest symptomatic stage.

In addition to the flawed premise that MCI predates the early symptoms of AD, its characterization remains problematic. The MCI criteria (original and revised) and their application are insufficiently sensitive to distinguish nondemented aging from prodromal AD. Hence, MCI samples include a mixture of both normal and impaired individuals. Observed outcomes (e.g., rates of progression to clinically diagnosed AD) vary as a function of the proportion of nondemented individuals in the sample.

A key factor in the failure to discriminate normal aging from MCI is that MCI criteria characterize "impairment" by comparing an individual's cognitive test performance with normative values (Petersen et al. 2001b; Winblad et al. 2004). Using neuropsychological criteria to define MCI poses methodological problems because there is no consensus on the number and types of cognitive measures needed to characterize each cognitive domain, the quality of the reference normative values may vary, and factors such as education, race, ethnicity, and culture affect neuropsychological test performance (Manly et al. 2005; Teng and Manly 2005). Another problem is the age-related variability in cognitive test performances for nondemented elderly (Morse 1993). This variability contributes to an extensive degree of overlap of performances of normal and MCI groups such that absolute levels of performance on psychometric tests do not reliably distinguish these conditions (Storandt and Hill 1989; Sliwinski et al. 1996).

The criteria for MCI require a comparison of an individual's cognitive performance at a point in time to the performance of (presumably) demographically similar individuals. This *interindividual* comparison does not indicate whether cognitive function has declined for that individual; it simply places the individual's performance in relation to that of others. Performance below arbitrarily defined cut-points (e.g., 1.5 standard deviations below normative values) determines "impairment." This approach is inconsistent with the definition of dementia, which requires cognitive decline in relation to *the individual's previously attained levels* (American Psychiatric Association 1994). The clinically relevant information needed to determine that an individual is demented is not how the individual performs in relation to other people but rather that the individual has experienced cognitive loss relative to his/her prior abilities.

Detecting *intraindividual* change can be accomplished in two ways. One is serial neuropsychological testing. Confounding factors associated with cognitive tests, including statistical issues related to the reliability of the measures (signal-to-noise ratio) and nonlinear patterns of progression, require an observation period of greater

than 1 year to infer a change in cognitive status (van Belle et al. 1990; Morris et al. 1993). This duration, of course, is impractical for clinical practice and also for many research programs in which diagnostic classifications are made at time of enrollment.

The other method of detecting cognitive loss involves interviews with someone who knows the individual well (typically the spouse, adult child, other relative, or close friend). The observations of an informant or collateral source permit the individual's current cognitive and functional abilities to be judged in relation to the individual's previous status (Table 1). This longitudinal perspective uses the individual as his/her own control in assessing whether cognitive decline has occurred. Moreover, informant-based observations are "face valid" as they relate to the everyday performance of the individual and are sensitive to even very mild impairment (i.e., avoid the "ceiling" effects of cognitive tests). Informant observations also are unaffected by scale restriction ("floor" effects) and minimize demographic and cultural factors. Most important, informant reports of cognitive change are accurate (Cacchione et al. 2003; Harvey et al. 2005) and have been shown to be highly sensitive to even very mild cognitive decline (McGlone et al. 1990; Morris et al. 1991; Koss et al. 1993; Jorm 1997; Jorm et al. 2000; Ready et al. 2004; Galvin et al. 2005).

The value of informant observations has long been recognized; Alzheimer's case history of the original patient with AD begins with an interview of her husband (Maurer et al. 2000). Standard dementia assessment protocols such as the Dementia Scale (Blessed et al. 1968), the Global Deterioration Scale (Reisberg et al. 1982), the Clinical Dementia Rating (CDR; Hughes et al. 1982; Morris 1993), and the Cambridge Examination for Mental Disorders of the Elderly (Roth et al. 1986) include semistructured informant interviews. Surprisingly, neither the original (Petersen et al. 1999) nor the revised (Winblad et al. 2004) criteria for MCI mandate informant observations; self-reported memory complaints alone are sufficient for a MCI designation. The subjective complaints of memory impairment in normal elderly, however, neither correspond with actual cognitive function nor predict future dementia (Flicker et al. 1993; Jorm et al. 1997; Carr et al. 2000), and the subjective reports of cognitively impaired individuals of course are unreliable (Ganguli et al. 2006). The reliance on interindividual comparison of neuropsychological test performance as a criterion for MCI and the

Table 1. Advantages and disadvantages of informants in dementia diagnosis

Advantages
Assess change (longitudinal perspective)
Observations about cognitive abilities are relevant to everyday function (face validity)
Culturally fair
Absence of ceiling and floor effects
No practice effects
Accurate and sensitive for dementia in its earliest stages
Disadvantages
Observant and reliable informant not always available
Time for informant interview
Some cultures discourage reporting "negative" information (e.g., dementia symptoms) in elders

failure to require informant observations regarding the cognitive and functional status of the individual result in the inclusion of "false positive" and "false negative" cases in MCI samples. Such cases include nonimpaired individuals who selfreport memory complaints or perform poorly on neuropsychological tests and individuals who are experiencing meaningful cognitive decline but still perform above the cutoff values on neuropsychological tests. The current conceptual and methodological approach to MCI thus ensures its heterogeneity and limits its clinical and research utility.

The principle of intraindividual change permits the recognition of the earliest symptomatic stages of AD in MCI individuals (Morris et al. 2001; Storandt et al. 2002), even in individuals who are not yet sufficiently impaired in their neuropsychological test performance to meet criteria for MCI ("preMCI"). We recently reported (Storandt et al. 2006) findings from 388 individuals clinically diagnosed with AD at the CDR 0.5 stage (identical to the CDR stage used for the characterization of MCI individuals for a multicenter trial of donepezil and vitamin E; Grundman et al. 2004). Thirty-two of the 388 individuals met neuropsychological criteria for amnestic MCI, 90 met revised criteria for MCI, and 276 were too minimally impaired cognitively to meet either set of MCI criteria (preMCI). The diagnosis of AD was validated by progression to a CDR 1 or greater stage of dementia with a median survival of about four years for the amnestic and revised MCI groups, comparable to reported rates of progression of 12%–15% per year for MCI to clinically probable AD (Petersen et al. 1999). The preMCI group also progressed to CDR 1 or greater stage of dementia but, because these individuals were less cognitively impaired at baseline, the median survival was about eight years. Additional validation was provided by the neuropathological diagnosis of AD in nine of nine amnestic MCI, 18 of 20 revised MCI, and 43 of 47 preMCI individuals who came to autopsy; overall, AD was confirmed in 92% of the autopsied sample. This neuropathological perspective provides additional evidence that the initial cognitive symptoms that are characterized as MCI in reality represent AD (Markesbery et al. 2006).

Our sample is not unique (Table 2). It is comparable to other reported MCI samples on demographic features, apoE genotype, level of impairment as measured by the MMSE and CDR-SumBoxes, and rate of progression to greater dementia severity. What is different is our clinical detection method that utilizes an informant interview in addition to an examination of the individual. Although informant-based methods may not be appropriate in all settings, an informant interview that can be completed in less than three minutes (Table 3) provides a positive predictive value of 87% in discriminating nondemented aging from dementia at the CDR 0.5 or greater stage (Galvin et al. 2005).

Not all individuals meeting criteria for MCI have AD (or another dementing disorder). The subset of MCI that eventually is recognized as clinically probable AD, however, can be distinguished from non-AD causes of MCI using usual clinical methods, because the phenotype of the subset of MCI caused by AD is identical to that of more overt AD, only milder. The initial deviations from an individual's own baseline that represent cognitive decline caused by AD may not place that individual below some value on a scale determined by group norms. AD can be detected in MCI and even preMCI if the diagnosis is based on intraindividual, not interindividual, comparisons. The sensitivity and accuracy of clinical detection methods suggest that it is time to move beyond the MCI concept in favor of etiologically based classifications, most notably early-stage AD.

Table 2. Demographic and clinical features of MCI and PreMCI samples

	Washington University (Storandt et al. 2006)			Mayo Clinic (Petersen et al. 1999)	Multicenter Trial (Grundman et al. 2004)
	Amnestic MCI N = 32	Revised MCI N = 90	preMCI n = 276	Amnestic MCI N = 76	Amnestic MCI N = 769
Age (years)	69.9 (6.2)	73.3 (8.9)	75.7 (8.9)	80.9 (1.0)	72.9 (7.3)
Education (years)	13.2 (3.0)	12.5 (3.4)	14.2 (3.0)	13.7 (0.4)	14.7 (3.1)
M/F	12/20	32/60	143/133	30/46	417/352
MMSE	27.5 (2.2)	25.4 (1.8)	27.9 (1.6)	26.0 (0.3)	27.3 (1.9)
CDR-SB	2.0 (0.9)	2.2 (1.1)	1.6 (1.0)	1.5 (0.2)	1.8 (0.8)
% apoE ε4 carriers		51%		56%	55%

Legend: Mean values are given +/− standard deviations. M = male, F = female. MMSE = Mini-Mental State Examination (Folstein et al. 1975), where the range of possible scores is from 30 (best) to 0 (worst). CDR-SB = Clinical Dementia Rating, SumBoxes, where the range of possible scores is from 0 (best) to 18 (worst). Carriers of the apolipoprotein E (apoE) ε4 allele for the three samples (Washington University, Mayo Clinic, Multicenter Trial) are given in percentages.

Table 3. Eight-item informant interview to differentiate aging and dementia

Report only a **change** caused by memory and thinking difficulties:

1. Is there repetition of questions, stories, or statements?
2. Are appointments forgotten?
3. Is there poor judgment (e.g., buys inappropriate items, poor driving decisions)?
4. Is there difficulty with financial affairs (e.g., paying bills, balancing checkbook)?
5. Is there difficulty in learning or operating appliances (e.g., television remote control, microwave oven)?
6. Is the correct month or year forgotten?
7. Is there decreased interest in hobbies and usual activities?
8. Is there overall a problem with thinking and/or memory?

(Adapted from Galvin et al. 2005).

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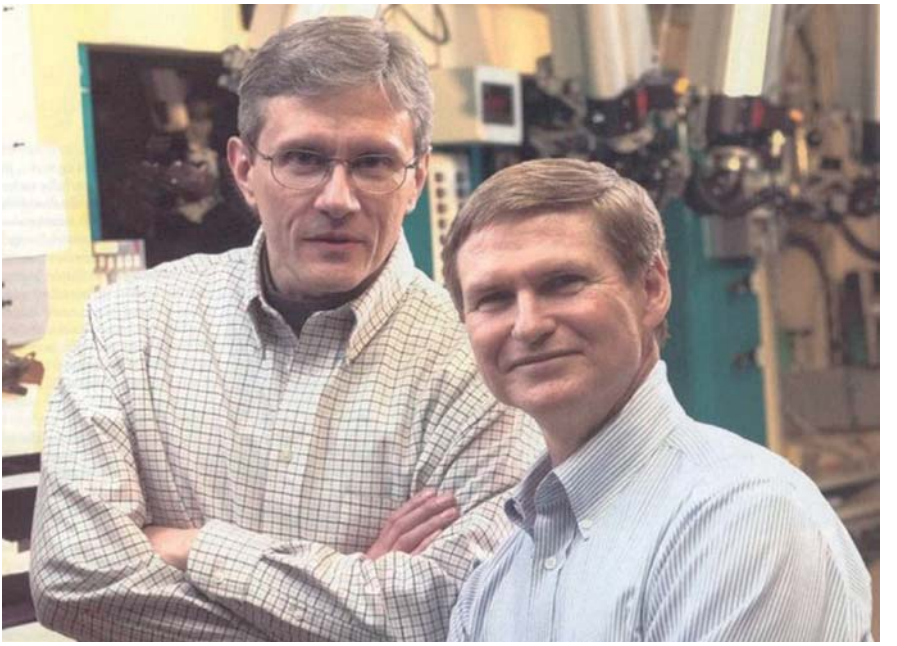

William E. Klunk (*left*) and Chester A. Mathis (*right*)

Imaging the pathology of Alzheimer's disease: Building on a century-Old blueprint

William E. Klunk[1] and *Chester A. Mathis*[2]

Alois Alzheimer carefully described the clinical and histological findings of an unusual case of pre-senile dementia in his benchmark presentation in Tübingen, Germany, in 1906. He meticulously documented the presence of "tangled bundle[s] of fibrils" and "miliary foci … of a peculiar substance." With this description of neurofibrillary tangles and amyloid-beta (Aβ) plaques, the basis for the characteristic neuropathology that still defines Alzheimer's disease (AD) 100 years later was born. In recent efforts to image this pathology in living patients, we had several advantages over Alzheimer; most importantly, he had told us exactly what pathology to target. Although it took almost 100 years to develop an effective in vivo tool to image this pathology, Alzheimer had effectively laid out the "blueprint" in his classic paper published in 1907 (Alzheimer 1907a).

The first documented suggestion to image Aβ plaques and tangles made general reference to the use of Congo red as a contrast agent for MR imaging (Khachaturian 1985). Efforts are underway in this area, but large technological hurdles remain (Poduslo et al. 2004; Jack et al. 2005; Wadghiri et al. 2005). In contrast, most of the fundamental tools necessary to image the pathology of AD became available with the advent of successful positron emission tomography (PET) in the 1970s (Wagner 1998). While PET cannot resolve structures as small as individual plaques and tangles, the technique is capable of imaging the regional abundance of micro-structures such as neuroreceptors (Wagner et al. 1983). As with neuroreceptors, the key to imaging the pathology of AD was the development of specific radiotracers. An important step to successfully developing these AD-specific tracers was viewing the labeling and quantitation of plaques and tangles as analogous to those of neuroreceptors (Eckelman 1986).

It is important to have a basic understanding of the nature of the amyloid fibril in order to appreciate the development of the tools for imaging the pathology of AD. Among AD researchers, "amyloid" is often equated with Aβ, but amyloid is a more general term. Amyloid (from the Greek amylon, meaning starch) refers to the "starch-like" staining properties of this substance. This notion derived from Virchow's term, "Cellulose-Frage," used when describing the substance he stained with iodine in peripheral tissues (Virchow 1854). Amyloid deposits were soon understood to be composed mainly of protein (Friedreich and Kekulé 1859) and were later shown to exist in a cross beta-sheet fibril conformation (for a review, see Uversky et al. 1999).

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All beta-sheet fibrils consist of a regular, repeating, linear array of peptide backbones spaced 4.76 Å apart (Pauling and Corey 1951). Histologic dyes bind to most amyloid deposits as a result of this beta-sheet fibrillar nature, without specificity for any particular amyloid protein (Glenner et al. 1972). Several models have been proposed to explain this binding in molecular terms (Klunk et al. 1989; Carter and Chou 1998; Krebs et al. 2005). Beta-sheet specificity appears to be a property of all known in vivo imaging probes for amyloid. The specificity for AD comes from the fact that extensive accumulation of amyloid is largely due to the massive build-up of Aβ. However, AD is often a triple amyloidosis comprised not only of Aβ amyloid but also amyloid in the form of hyperphophorylated tau in neurofibrillary tangles and alpha-synuclein in the form of Lewy bodies (Trojanowski and Mattson 2003). In most cases of AD, the Aβ amyloid component far outweighs the other amyloid components (on a total mass or molar basis), and it may be that the particular tracers used bind inherently better to Aβ or that Aβ presents more available binding sites. The specific amyloid imaging agent we discuss below, Pittsburgh Compound-B (PiB), appears to reflect mainly Aβ amyloid deposits under the conditions relevant for PET studies (Klunk et al. 2003).

Considering all of this, the concept of imaging the pathology of AD is really a very simple one: start with a histological dye known to bind specifically to amyloid and chemically modify it so that it 1) rapidly crosses the blood-brain barrier in large amounts; 2) has increased specificity and affinity for Aβ (to enhance specific binding signal); and 3) clears rapidly and completely from all non-amyloid components of brain (to decrease background signal). There are other more detailed criteria as well, and these have been reviewed elsewhere (Mathis et al. 2003, 2004). For the Congo red series of compounds, it proved very difficult to reach the desired degree of brain entry, so brain clearance never became a focus. With the initiation of work on the thioflavin-T derivatives (Klunk et al. 2001), the brain entry goals were quickly attained and more focus was placed on affinity and clearance (Mathis et al. 2003). Other investigators also have taken compounds unrelated (Shoghi-Jadid et al. 2002) or distantly related to PiB (Verhoeff et al. 2004) into human trials. These compounds showed less striking differences between AD patients and healthy controls (i.e., lower effect size). This difficulty may be primarily due to slower clearance from amyloid-free brain, but lower affinity for amyloid also may play a role.

A major hurdle in making the translation from pre-clinical work to human imaging studies was the lack of a good animal model for PET imaging studies, despite the availability of good transgenic mouse models of Aβ plaque deposition (Duff et al. 1996; Hsiao et al. 1996; McGowan et al. 1999) and microPET scanners capable of imaging mice (Cherry and Gambhir 2001). Recent work has shown that the transgenic mouse models have, per mole of Aβ present, less than 1% of the PiB binding capacity (i.e., Bmax) found in AD brain (Klunk et al. 2005). This finding, coupled with the volume-averaging inherent in imaging such a small brain, has prevented the transgenic, Aβ-depositing mouse models from being useful for microPET studies of Aβ-imaging PET tracers (Toyama et al. 2005). In constrast, transgenic mouse models have proven very useful for high resolution multiphoton microscopic imaging studies using macroscopic amounts of PiB and other fluorescent Aβ-binding compounds and have helped establish the in vivo specificity of these agents for Aβ plaques (Bacskai et al. 2003). However, the lack of a good PET model for Aβ imaging meant that we could not directly compare the in vivo performance of these compounds in preclinical studies. Extrapolations

had to be made from the in vivo brain entry and clearance studies in normal rodents (Mathis et al. 2003), along with the specific binding of the radiotracers to synthetic Aβ or to homogenates of post-mortem AD brains known to have high Aβ content (Klunk et al. 2003). This multi-factorial approach was used as a surrogate for direct in vivo comparison, and PiB was chosen as the radiotracer to take into human studies (Mathis et al. 2003, 2004).

The results of several early human imaging studies using PiB have been reported (Klunk et al. 2004; Lopresti et al. 2005; Price et al. 2005; Fagan et al. 2006; Mintun et al. 2006). These and other studies presently performed in 16 PET centers around the world have consistently shown that, compared to cognitively normal control brain, AD patients show approximately 1.5 to 3.0 times greater retention of PiB in brain areas known, from post-mortem studies, to have heavy Aβ deposition (Arnold et al. 1991; Thal et al. 2002). The areas of highest PiB retention are typically the frontal cortex/anterior cingulate and the precuneus/posterior cinculate (Fig. 1). Parietal, lateral temporal and anterior/ventral striatum also show high retention. Areas of the occipital lobe are typically involved as well. The mesial temporal and sensorimotor cortices have little (or no) increase in PiB retention in AD. White matter regions and cerebellum show no increase in AD over normal controls.

The pharmacokinetic behavior of this tracer has proven itself to be very amenable to the standard methods of quantification of reversibly bound tracers (Price et al. 2005). Furthermore, simplified methods of analysis that will facilitate clinical applications have proven very reliable (Lopresti et al. 2005). The goal of these early studies was more to show consistency with known post-mortem data than to provide new insights about Aβ pathology, but several intriguing findings have begun to emerge. First is the concept that Aβ deposition can be identified in cognitively normal controls, setting the stage for longitudinal follow-up studies of the natural history of Aβ deposition and the relationship of the earliest stages of Aβ deposition to clinical symptoms (Klunk et al. 2004a; Lopresti et al. 2005; Price et al. 2005; Mintun et al. 2006). Second is the finding that approximately 30% of subjects classified with mild cognitive impairment (MCI) show no evidence of significant Aβ deposition in their brains. This finding is of interest given the fact that it is of great importance to distinguish those MCI patients that will never progress to the diagnosis of AD from those that will (Gauthier et al. 2006). Third, comparison of PiB imaging and CSF measurements of Aβ have shown that the CSF measurement is a very good predictor of brain Aβ deposition, even when it does not predict clinical diagnosis (Fagan et al. 2006).

An immediate application of Aβ imaging would be facilitation of the development of new, effective anti-Aβ therapies for AD. The same would apply to tau imaging and anti-tau therapies if this modality can be developed and validated. Knowing whether a drug does or does not effectively lower brain Aβ levels will be essential in interpreting the clinical results of these studies. However, not only removal of the pathology but also the timing of the removal of pathology is likely to be an absolutely critical issue in the success of anti-Aβ therapies, and in vivo imaging may be helpful in this aspect as well. For example, examination of the reports on the three available post-mortem brains from the AN-1792 trial (immunization with Aβ plus QS-21 adjuvant) seems to illustrate this concept (Nicoll et al. 2003; Ferrer et al. 2004; Masliah et al. 2005a). All three reports of autopsies from the AN-1792 trial pointed out that, despite the apparent focal clearance of Aβ pathology, the neuritic and neurofibrillary pathology remained. This

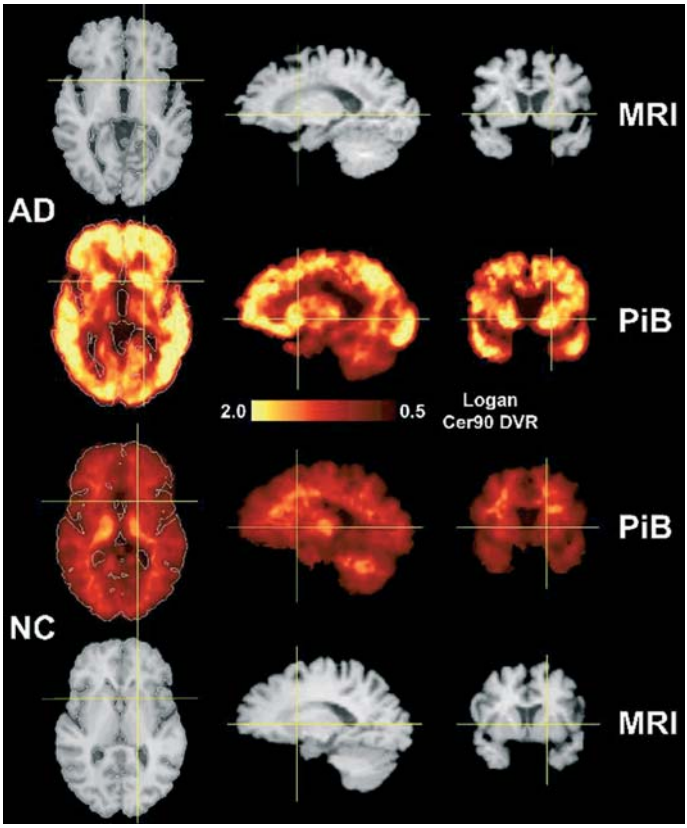

Fig. 1. MRI (*top* and *bottom rows*) and PiB images (*second* and *third rows*) from a mild AD patient (AD; *top two rows*; MMSE = 27) and a cognitively normal control (NC; *bottom two rows*). Transaxial (*left*), sagittal (*center*) and coronal views (*right*) are shown. The crosshairs are for reference within each subject and the striatum is outlined in the transaxial images for reference. The parametric PiB images represent a Logan analysis of the 90-min dynamic PiB PET scan with the cerebellum as reference (CER90), as described in detail by Lopresti et al. (2005), and are displayed as the distribution volume ratio (DVR)

neuritic pathology is undoubtedly related to the cytoskeletal changes espoused by many to be an important, proximate cause of the cognitive changes that clinically characterize AD (Terry 1996). This may explain why, despite the strong post-mortem evidence of effective Aβ plaque removal, the clinical effects of AN-1792 were modest (Gilman et al. 2005). That is, too much cortical damage may have already occurred prior to therapy and the therapy did little to reverse the neuritic pathology – at least in the short term. Taken together, these findings suggest that if, anti-Aβ therapies are to be optimally effective, they may have to be initiated at a very early, perhaps even pre-clinical phase of AD. This notion is daunting to those who design drug trials because of the implications for 1) subject numbers required to design such a "preventive" trial; 2) ethical issues attendant with initiating potentially harmful therapies in asymptomatic subjects; and

3) the difficulty in defining an endpoint if the groups have few clinical symptoms to begin with.

The answer to this very difficult issue may bring us back to Dr. Alzheimer's very first patient, Auguste D., who had an early-onset form of dementia (Maurer et al. 1997). It may be that this early-onset AD subtype could hold the answer to solving this problem – in particular, the early-onset, autosomal dominant familial AD (eoFAD) form of AD (St George-Hyslop 1995; Tanzi et al. 1996). Recent preliminary work in asymptomatic carriers of the most common form of eoFAD, the presenilin-1 mutations on chromosome 14, shows the presence of focal, clinically silent Aβ deposition in the striatum (Johnson et al. 2006). These individuals, by virtue of the certainty of their prognosis, juxtaposed with the absence of clinical symptoms and presumably little cortical pathology, may present the ideal cohort in which to determine the true effectiveness of anti-Aβ therapy. The numbers involved in trials could be small and there is an imaging marker to follow as a short-term primary endpoint. Ethical considerations are still an issue, but the certainty of developing AD at an early age makes this a very different ethical question than the one raised by the treatment of asymptomatic individuals who may never develop the disease for which the experimental therapy is targeted.

In recent years studies of eoFAD families have led us to a deeper understanding of the genetics and molecular biology of AD (Hardy et al. 1998). It would be fitting for this group of individuals to lead us to the recognition of effective treatments for AD in much the same way as Auguste D. led Alois Alzheimer to the pathology of this terrible disease 100 years ago. It also would be fitting if elimination of the very pathology that Alzheimer first saw using silver staining techniques new to the 20th century (Bielschowwsky 1902) – and now imaged in living patients using technology new to the 21st century (Engler et al. 2002) – led the way to elimination of this disease as the major public health threat it stands to be later in this century (Lobo et al. 2000; Hebert et al. 2003).

The neuropathological blueprint of "tangled bundle[s] of fibrils" and "miliary foci … of a peculiar substance" that Alzheimer observed under the microscope and reported in his landmark presentation in Tübingen must have been striking to those who attended that meeting in 1906. This blueprint formed the basis of much of what was to follow in the next 100 years. This is nowhere more true than in the case of the in vivo imaging of AD pathology.

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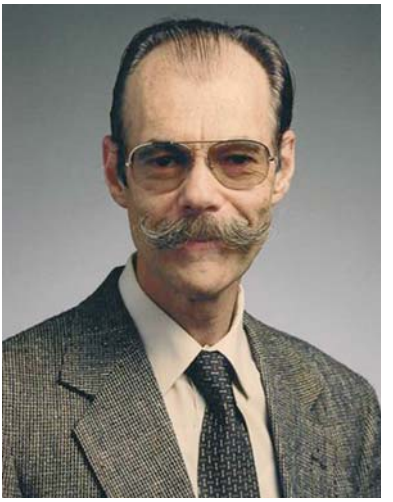
Therapeutical Perspectives



Leon J. Thal

How do we test drugs?

Leon J. Thal[1]

Current Drug Development

At the present time, the drug development process is broadly broken down into two phases: preclinical and clinical. Preclinical drug development generally begins with the designation of a target. The target may be an enzyme, a receptor, or a biological process. Compounds are first developed and optimized for the target of interest. They are subsequently tested in a series of models to examine the biological activity that might be expected from the agent. Testing may begin in cell culture systems and then advance to animal models. If animal models of the disease exist, the drug will be tested in suitable and appropriate animal models. In many cases, only partial animal models exist, or in some cases no animal models exist at all and decisions to move forward regarding drug development depend on target identification and activity at that target. Once evidence of biological activity in in vivo systems exists, preclinical toxicology is performed. For neurodegenerative diseases, this procedure generally requires the testing of the agent at a variety of doses in two animal species for a duration of at least six months of time in order to support chronic exposure in humans.

Once preclinical toxicological studies have been completed and sufficient drug is available and has been produced according to good manufacturing practices, human clinical trials can begin. Phase 1 trials are generally performed to test for toxicity and to determine pharmacokinetics in small numbers of subjects, generally numbering under 100. This process usually consists of single and multiple dose testing of the agent. Testing often begins in young normals, proceeds to elderly normals, then proceeds to the population of interest. A maximum tolerated dose is sought so that doses lower than the maximum tolerated dose can be tested in later efficacy studies. Many drugs will fail in phase 1 trials because of either toxicity or poor pharmacokinetic profiles.

In phase 2, a variety of doses are generally tested in dozens to several hundred subjects to determine safety and tolerability over a longer period of time and to gain an initial indication of efficacy. Several doses are generally tested in phase 2 trials to narrow the dose range that needs to be tested in phase 3 studies. Once an initial indication of efficacy is obtained, power calculations for phase 3 trials can be performed based on the initial indication of efficacy.

Phase 3 trials are the final, pivotal trials that are performed to demonstrate efficacy of an agent. In general, two phase 3 trials are usually required to demonstrate replication of a successful experiment. AD phase 3 trials generally include 400 to

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1,000 subjects, depending on the number of arms and the effect size sought. Most contemporary phase 3 trials are enrolling 200 to 300 subjects per arm in trials lasting from 6 to 18 months, depending on the presumed mechanism of action of the agent.

Symptom Treatment vs. Disease Modification

Contemporary drug testing for AD drugs began in 1986 with the testing of tetrahydroaminoacdridine (Cognex), the first cholinesterase inhibitor approved for the treatment of AD. Cholinesterase inhibitors were developed to produce symptom improvement. Dual outcome measures were utilized, requiring a positive study to demonstrate drug-placebo difference on both a cognitive outcome measure that captures the core phenomena of AD and a global measure to ensure that the effect size is large enough so that drugs with trivial effect sizes would not be approved. The concept of dual outcome measures was eventually codified in a set of draft guidelines for the clinical evaluation of anti-dementia drugs (Leber 1990). These guidelines have worked well, and five drugs with an anti-dementia claim have been approved and marketed between 1993 and the present time. All of the drugs developed are marketed for the symptomatic treatment of AD.

At the present time, the testing of drugs designed to improve symptoms in patients with AD continues. However, there is increasing interest and focus on the development of drugs designed to alter the underlying pathology of the disease to produce "disease modification." Our ability to prove disease modification has been problematic and a variety of approaches are being explored at present.

Current Status of Testing Drugs for Disease Modification

In testing drugs that may modify the disease process, phase 1 trials are run in an essentially identical manner to the trials used for drugs that treat symptoms. This approach is logical since the main goal of phase 1 testing is to determine the maximum tolerated dose and pharmacokinetics. In addition, however, some phase 1 clinical trials of disease-modifying drugs are also seeking to determine whether or not a biological signal can be detected in plasma cerebrospinal fluid (CSF) or other available tissues.

Phase 2 testing of drugs designed to modify disease in AD remains quite enigmatic. Classical phase 2 designs expose modest numbers of subjects to a variety of doses, and the subjects are followed for relatively brief periods of time (generally up to six months) to determine drug-placebo effects to estimate the efficacy of the drug and its safety. However, AD progresses at a relatively slow rate. Disease-modifying drugs will produce slope differences rather than acute improvement. The ability to detect a slope difference depends on the rate of decline in the untreated population and its standard deviation. Since AD patients decline slowly and quite variably, the length of time necessary to determine a true slope difference using current methodology is approximately 12 to 18 months. Classic phase 2 trials testing of many different doses would require extremely large, long, and costly trials to determine slope differences. Thus, alternate trial designs are currently being investigated.

In some cases, biomarker trials are being conducted as phase 2 studies. For example, if a drug lowers the production of amyloid, lower levels of α-beta should be found in

the CSF in a dose-dependent fashion. Similarly, if a drug blocks the production of tau, lower levels of CSF tau should be found in a dose-dependent manner. The need to find a biological signal rather than a clinical signal in phase 2 studies to make a go, no-go decision to phase 3 has fueled the development of a series of biomarker studies (Mueller et al. 2005b). Unfortunately, to date, no biomarker has been validated in AD (Katz 2004).

Phase 3 testing for drugs designed to alter rate of change in AD also differs from the designs used to test drugs with symptomatic effects. First, the trials are much longer, since a separation of slope between drug-treated and placebo subjects is needed. These trials are generally 12 to 18 months in length or longer. Secondly, clinical trial maneuvers are often utilized to demonstrate a disease-modifying effect (Mani 2004). Three clinical maneuvers most commonly utilized involve the use of the randomized withdrawal design, the randomized start design, or the presence of a persistent difference in slope over time. It is hypothesized that if a drug has a disease-modifying effect and it is withdrawn, the individuals treated with the drug throughout the trial will have gained an advantage over those on placebo that will be sustained and will not disappear upon drug withdrawal. Thus, a persistent drug placebo difference should persist even after withdrawal of the agent. Similarly, in a randomized start design, individuals randomized to placebo will not gain the benefit of the drug during the early treatment phase and therefore will never catch up to an individual who has been on drug throughout the entire duration of the experiment. Finally, a persistent difference in slope argues that the drug is having an increasing and prolonged effect that must be interpreted as disease modification. In addition, a series of biochemical markers are being used to infer disease modification. These include measurements of β peptide, tau protein, and isoprostanes. Volumetric magnetic resonance imaging (MRI) is also being used in an attempt to demonstrate a decrease in the rate of atrophy in individuals treated with drugs as a surrogate marker for neuroprotection (Mueller et al. 2005b). Functional imaging using positron image tomography, single photon emission computerized tomography, and functional MRI are also being pursued as possible biomarkers.

To date, no drug for AD has been approved with a label of disease modification. It is clear that the hurdle to achieve this goal will be substantial.

Drug Testing in the Future

How will this current model of drug testing be altered in the future? A few examples and speculations will be provided.

Preclinical drug testing currently involves target validation and preclinical toxicology. One potential improvement in this area might involve the use of human cell lines with appropriate genes as targets of interest inserted so that the cells could be used for both screening of compounds and preclinical toxicology. One example would be the development of human embryonic stem cell lines containing genes from familial AD subjects resulting in over-expression of amyloid in a human cell line. These cell lines could then be used to screen for both the efficacy and toxicity of drugs designed to reduce the production or accumulation of amyloid.

Phase 1 testing will likely remain quite similar to the way that it is currently being done. However, the use of additional biomarker measurements designed to determine whether or not the compound of interest either inhibits enzymes, attaches to a receptor, or otherwise alters biochemistry is likely to become more widespread. Molecular imaging is also likely to become more widespread to determine receptor occupancy.

In phase 2 testing, if biomarkers can be developed, drugs designed to alter rate of change will be examined primarily for their effect on biomarkers, which will provide an indication of how the drugs might perform over longer periods of time in populations of interest. These biomarkers are likely to include not only CSF tau, α-beta, and imaging but also changes in protein expression, gene regulation, and metabolomics.

Phase 3 clinical trials are likely to remain large and long. Established biomarkers will be utilized to support a claim of disease modification. In addition, rather than including all subjects with AD, subsets of patients with specific disease profiles are likely to be enrolled both in early phase 2 studies and in initial phase 3 studies. Once efficacy is demonstrated in more homogeneous populations, trial populations will be broadened to include less homogeneous populations and subjects with significant concomitant comorbidites. Examples of patient selection may be use of subjects with the Apolipoprotein E_4 genotype, inclusion of individuals known to be rapid decliners, and inclusion of individuals with a higher genetic load based on discovery of the residual late-onset AD risk factors.

At the present time, only a very limited number of primary prevention trials have been carried out in AD. Few have been sponsored by the pharmaceutical industry, largely because of the high cost, long testing time, and large sample size needed for these trials. There are numerous methodological advances that need to be accomplished before more agents can be tested in a primary prevention paradigm. Some improvements might include the following: 1) development of simplified home-based instruments that can be completed without necessitating a visit to the clinic, 2) use of local community physicians to follow subjects during the trial, and 3) enriching the sample studied by including subjects likely to develop AD during the trial. Our ability to predict AD is likely to be based on a combination of risk factors including family history, presence of the gene for Apolipoprotein E_4, plasma α-beta levels, and presence or absence of late-onset risk factors, as well as future genomic, proteomic or metabolomic approaches. Once risk factor profiles are identified, individuals at risk who develop AD can be enrolled in primary prevention trials for proof of efficacy.

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Inflammation and the lesions of Alzheimer's disease

Edith G. McGeer[1] and *Patrick L. McGeer*[1]

Introduction

There is abundant evidence that neuroinflammation is involved in the progression of Alzheimer's disease (AD). This finding has been the subject of many recent and extensive reviews (e,g., Blasko et al. 2004; Hoozemans and O'Banion 2005; McGeer and McGeer 2004a, 2005, 2006; Neuroinflammation Working Group 2000; Rozemuller et al. 2005; Streit et al. 2005; Walker and Lue 2005). Here we briefly summarize the evidence and then advance some hypotheses as to how the inflammation may be connected to the primary lesions that Alzheimer first described. We also review evidence indicating the importance of inflammation in the pathogenesis of other diseases of aging such as Parkinson's disease (PD), macular degeneration, atherosclerosis and heart disease.

Evidence for Inflammation in Alzheimer's Disease

The first indication of chronic inflammation in AD came with reports of a profusion of activated microglia associated with AD lesions (McGeer et al. 1987; Rogers et al. 1988). In the 19 years since the first report, evidence has accumulated that many other inflammatory markers are greatly elevated. These include all of the classical complement proteins and many inflammatory cytokines, as well as miscellaneous materials such as C-reactive protein (CRP), substance P, intercellular adhesion molecule-1 (ICAM-1) and its receptors, lymphocyte function-associated antigen 1 (LFA-1) and Mac-1 (for references, see the reviews cited above). Measurement of mRNA levels for many of the inflammatory markers indicates the inflammation is more severe than in badly diseased rheumatic joints (Fig. 1). The inflammation is silent because the brain has no pain fibers.

Polymorphisms in the promoter and untranslated regions of such cytokines as TNF-α and IL-1β, which favor increased expression of these inflammatory mediators, increase the risk of AD. Thus the presence in AD brain of a prominent inflammatory reaction is well established. But does it affect the progression of the disease?

Activated microglia are professional phagocytes that are equipped with the NADPH oxidase system. Consequently, they are capable of oxidative burst activity that generates

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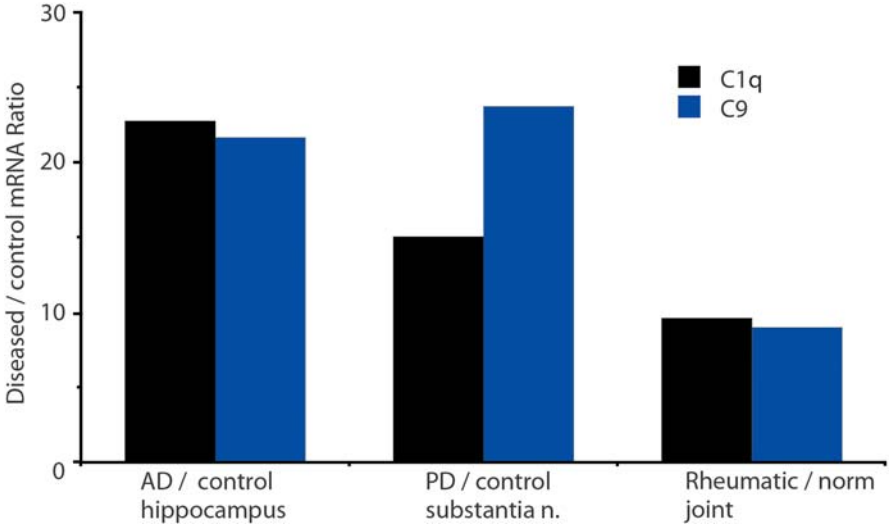

Fig. 1. Ratio of mRNAs in inflamed and normal tissue for C1q and C9 in AD compared to normal hippocampus, PD compared to normal substantia nigra, and rheumatic joint tissue compared to normal (norm) joint tissue. The rheumatic joint tissue was removed surgically because of intractable pain; other tissues were postmortem specimens (McGeer and McGeer 2004a)

large numbers of free radicals. This may well account for the oxidative stress believed to be responsible for much of the neuronal death in AD. Activated microglia produce other neurotoxic materials. Their secretions have repeatedly been demonstrated to kill neurons in tissue culture (McGeer and McGeer 2004a).

The complement system also makes a substantial contribution to neuronal death in AD brain. When the complement system is fully activated, the terminal components (C5b, C6, C7, C8, C9) are assembled into the lytic macromolecule C5b-9, known as the membrane attack complex (the MAC). The MAC is intended to insert itself into foreign bacteria and viruses but, if host cells are inadequately protected, it may damage them as well in a process called bystander lysis. Dystrophic neurites in AD brain are immunostained for the MAC, indicating autolytic attack. Such staining is not seen in control brains. The MAC has a very short half-life, so the abundant staining for the MAC in AD brain suggests such an attack contributes substantially to neurite loss in AD (McGeer and McGeer 2004a)

The evidence that chronic inflammation may be responsible for much of the neuronal death in AD led to the suggestion that anti-inflammatories might slow progression of the disease (McGeer and Rogers 1992). This possibility has gained support from more than 20 epidemiological studies that indicated that chronic use of classic non-steroidal anti-inflammatory drugs (NSAIDs) greatly reduced the risk of AD (McGeer and McGeer 2006). Administration of NSAIDs such as ibuprofen has also been reported to reduce the amyloid burden and to improve open field activity in APPsw transgenic mice (Lim et al. 2001b; Heneka et al. 2005; Jantzen et al. 2002; Yan et al. 2003). Three small pilot trials with such drugs, which are mixed COX-1/COX-2 inhibitors, obtained

promising results (Rogers et al. 1993; Scharf et al. 1999; Arai et al. 2000), but larger scale trials with selective COX-2 inhibitors, steroids or subclinical doses of a classical NSAID failed (McGeer and McGeer 2006). The failure of selective COX-2 inhibitors is not unexpected since COX-2 is constitutively expressed in neurons rather than microglia in brain (Hoozemans et al. 2003). It is of some interest that treatment of APPsw mice with the selective COX-2 inhibitor, celecoxib, led to an increase in the amyloid burden (Kukar et al. 2005).

Larger scale trials of clinically effective doses of a classic NSAID such as ibuprofen may still be warranted. But there is the problem of gastric toxicity. Moreover, the prostaglandins are probably minor actors on the inflammatory stage. A search for drugs that inhibit the activation of microglia or the complement system might yield better candidates.

Inflammatory Markers and the Classical Lesions Described by Alzheimer

The aggregated amyloid of senile plaques also has active roles in the inflammatory process: it can both activate microglia and stimulate the complement system (McGeer and McGeer 2004a). Thus one can hypothesize a vicious circle: an initial event causes some amyloid to be deposited; this stimulates the inflammatory reaction; the oxidative stress from the activated microglia and the MAC of complement kill more neurons in the vicinity; and this causes the release and deposition of more amyloid.

Antibodies have been classically considered to be the chief activators of complement. It has now become clear, however, that there can be vigorous activation of complement in the absence of antibodies. A key finding regarding the mechanism of complement activation in AD was made by Rogers et al. (1992), who demonstrated that amyloid protein, when aggregated, was a strong complement activator. Thus, the senile plaques of AD have a unique activator of complement. In addition, the complement cascade can be activated by the pentraxins, amyloid P and C-reactive protein, which are both upregulated in affected regions of AD brain (McGeer et al. 2001).

Possible Similarities to Other Diseases of Aging

Chronic inflammation appears to be an important factor in many of the important diseases of aging. Perhaps the strongest evidence, apart from AD, is seen in PD. The substantia nigra stains heavily for activated microglia, reactive astrocytes, all the complement proteins, including the MAC, and various other inflammatory mediators such as ICAM-1 (Miklossy et al. 2006; McGeer and McGeer 2004b). The mRNAs for the complement proteins and such inflammatory indicators as C-reactive protein are greatly elevated in the basal ganglia (Fig. 1; McGeer and McGeer 2004b). Studies in both humans (Langston et al. 1999) and monkeys (McGeer et al. 2003) dying years after exposure to MPTP have shown that inflammation can persist for years after the precipitating insult. A form of IL-1β that increases production of this inflammatory cytokine may increase the risk of PD (McGeer and McGeer 2004b), just as it does AD, and one

epidemiological study suggests that use of NSAIDs reduces the risk of PD (Chen et al. 2003).

Another similarity between AD and PD is that each involves a misfolded protein that is capable of stimulating the inflammation. α-Synuclein, a major protein in Lewy bodies, may play the same role in PD as beta-amyloid seems to play in AD. α-Synuclein is released from damaged dopamine neurons and is capable of activating microglia and stimulating astrocytes to produce IL-6 and ICAM-1 (Klegeris A, private communication). This combination is capable of attracting further microglia to the site and activating them. Whether or not α-synuclein also stimulates the complement cascade is not yet established. Thus, as in AD, one can hypothesize a vicious circle in PD with an initial insult causing extracellular deposition of some α-synuclein, which stimulates the inflammation that, in turn, leads to attack on further dopaminergic neurons, with further deposition of α-synuclein.

Immunohistochemical evidence for chronic inflammation in affected tissue is seen not only in AD and PD but also in other central disorders, such as ALS and multiple sclerosis, and in more peripheral conditions, such as atherosclerosis, heart disease and macular degeneration (Bok 2005; Kuehn 2005). Finch (2005), for example, has recently reviewed the "remarkable convergence of inflammatory mechanisms in the etiology of cardiovascular disease and Alzheimer disease." One epidemiological study has been published indicating that NSAID use reduces the risk of macular degeneration (McGeer and Sibley 2005). Moreover, as in AD and PD, forms of inflammatory mediators that favor their production have been reported to increase the risk of macular degeneration (Scholl et al. 2005).

Aggregated proteins are also seen in many diseases other than AD and PD. Skovronsky et al. (2006) list 10 diseases where aggregation of misfolded proteins may be part of the pathogenetic mechanism. The diseases (and proteins) they list other than AD and PD are ALS (superoxide dismutase), progressive supranuclear palsy (tau), Lewy body dementia (α-synuclein), Huntington's disease (Huntington), multiple system atrophy (α-synuclein), Pick's disease (tau), prion diseases (protease-resistant prion protein), and spinocerebellar degeneration (ataxin).

It is tempting to hope that if the intensive research into AD or PD leads to a useful therapeutic approach, parallel approaches may also be found for therapies for these other diseases that seem to have many similarities in pathogenetic mechanism.

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Dale Schenk

Aβ immunotherapy prevents and Reverses Alzheimer's disease neuropathology

Dale Schenk[1], *Dora Games*[1], and *Peter Seubert*

By the late 1990s, research into the role of beta amyloid peptide (Aβ) in Alzheimer's disease uncovered a number of important findings. It was a key pathological feature of the disease and nearly all known familial forms of the disorder appeared to either directly or indirectly increase levels of the more amyloidogenic Aβ 42 in the brain tissue of affected individuals. The precise details of the Aβ generating proteases, beta and gamma secretase, were not yet understood, but their existence was fairly certain. Most importantly, a few transgenic mouse models finally existed that demonstrated fairly robust progressive amyloidosis.

In the mid 1990s, we were fortunate enough to be working with one of these models, the PDAPP mouse (Games et al. 1995), and we asked the question of what experiments might be reasonable to do with the model to better understand beta amyloid plaque formation and neuropathology or, more ambitiously, if it was possible to intervene therapeutically in these pathogenic processes. Many ideas were put forth for possible experiments in this mouse model: for example, trying to understand whether the cholinergic system or glutamatergic systems were dysfunctional as well as testing a number of compounds, such as NSAIDS, for which there already existed compelling epidemiological efficacy data for AD. We certainly did not have enough animals to test all of the ideas put forth, since breeding mice has always been a challenge for large-scale experiments; thus we had to rank various experiments for their potential merit and likelihood of success. It was against this backdrop that the idea of immunizing the mice with the Aβ peptide itself was suggested.

The rationale behind this idea as a potential therapeutic approach was that binding of antibodies to antigens could effectively reduce the free levels of unbound peptide. This idea, coupled with the fact that virtually all plasma proteins enter the brain at approximately 0.3% of the plasma levels, led to calculations that a circulating antibody titer of 1:50,000 would achieve a titer of 1:1,500 in the brain, possibly enough to exert a biological effect. Also, if antibody did reach the brain in sufficient quantities, it was conceivable that it might disrupt the formation of fibrils, as had been shown previously in vitro (Solomon et al. 1997). Despite supportive data at the time, one did have to make the leap to assume that the reduction in free levels of Aβ would be sufficient to reverse or reduce plaque burden. This was indeed a reasonable assumption to prevent plaque formation, but perhaps far less likely to reverse any existing plaque. The hurdle of the blood-brain barrier, together with the insolubility of amyloid plaques and the frank probability of failure of such an experiment, resulted in the bestowing of the lowest

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priority ranking by our internal review process to this effort, out of all of the numerous suggestions put forth to be tried in mice.

Fortunately, through tenacity, a few extra animals were secured and the experiments were performed (Schenk et al. 1999). Specifically, PDAPP mice were immunized with Aβ 42 plus adjuvant beginning at a very young age (6 weeks), prior to any plaque deposition, and were boosted monthly with immunogen until the mice were sacrificed at the age of 13 months. As a control, we immunized a group of mice with a fragment of serum amyloid protein (SAP). At the conclusion of the experiment, the brains of these mice were examined for plaque burden, astocytosis and microgliosis. Surprisingly, the mice that had been immunized with Aβ peptide were essentially devoid of amyloid plaques. The result was so striking that our first reaction was to reconfirm the transgenic status of the Aβ-immunized animals. We also examined alternative plaque detection methods, such as Congo red and thioflavin stains, which were also negative on the Aβ-immunized brains. Remarkably, the simplest conclusion for this first experiment was that the immunization with Aβ peptide had resulted in antibodies that had somehow blocked the formation of amyloid plaques. Immediately upon seeing these results, we set out to do an even more critical and difficult experiment. Rather than immunizing very young mice that had no pathology at the beginning of the experiment, we initiated immunization with Aβ peptide at the age of 11 months (i.e., plaque-bearing animals) and continued treatment until the age of either 15 or 18 months. The expectation was that this would be a much more stringent test of the therapeutic potential of Aβ immunotherapy, since the low concentration of antibodies expected to enter the brain would now have to block elongation of existing fibrils of Aβ rather than stop the initiation of new ones. Analysis of the brain tissue of the PDAPP mice that had been immunized again demonstrated that not only did immunization with Aβ peptide block the increase in further plaque formation but it also appeared to have actually eliminated existing plaques. This impression, based on histological images from single time points, would be elegantly confirmed in vivo (Backsai et al. 2001).

From the perspective of the role of amyloid burden in the PDAPP mouse and its relevance to AD, we also observed in these studies that, when amyloid burden was reduced, both dystrophic neurites burden and astrocytosis were simultaneously reduced. Reassuringly, this remarkable early preclinical observation would also be found years later to hold true for patients suffering from the disease who were treated with Aβ immunotherapy (Ferrer et al. 2004; Masliah et al. 2005a; Nicoll et al. 2003).

Perhaps the most unexpected finding from these studies was that, in the Aβ-immunized mice, microglia appeared to have taken up Aβ peptide in far greater amounts than previously seen in either the transgenic mice or in AD brain tissue itself. Another result suggested in this initial report, and demonstrated to be true in subsequent papers, was that Fc receptors on microglial cells engaged via antibodies that were bound to Aβ plaques represent a powerful plaque-clearing mechanism.

It is fair to say that the two experiments described in the original publication inspired many further studies in laboratories around the globe that were interested in Aβ peptide and AD (Arendash et al. 2001; Janus et al. 2000; Morgan et al. 2000; Schenk et al. 2004; Wilcock et al. 2004b). Perhaps most importantly, it was now possible to specifically reduce the burden of beta amyloid plaques in the APP transgenic mice and to assess a variety of outcomes. These studies also allowed numerous laboratories to better understand factors involved in the process of amyloidosis and its reversal.

Finally, they opened a potentially rich and complex therapeutic avenue for treatment or even prevention of AD that was unanticipated. Each of these areas will be briefly discussed here, though they are sufficiently complex that space will not allow a truly appropriate review.

Perhaps the most immediate question raised by the experiments described in the first paper was whether the reduction in plaque burden was attributable to anti-Aβ antibodies or somehow caused by a T-cell response to Aβ. This question was unambiguously resolved by showing that passive treatment of PDAPP mice with monoclonal antibodies to Aβ could demonstrate reduction in plaque burden and related neuropathologies (Bard et al. 2003). This same paper also demonstrated that anti-Aβ antibodies do enter the brain and bind to plaques and then engage Fc receptors that mediate plaque removal. In fact, the antibody-mediated process of Aβ removal and elimination could be demonstrated in vitro using brain sections and microglial cells.

The question of what plaque burden might have to do with cognitive capabilities – a key burning question, and one that is dealt with elsewhere in these reviews – was quickly and accurately assessed by two groups simultaneously, with similar results, in 2001 (Janus et al. 2000; Morgan et al. 2000). Both groups nicely demonstrated that Aβ immunization could reduce the loss of cognitive performance typically seen in a number of different APP/presenilin mouse models. These important findings provided further impetus for testing the immunotherapeutic approach in AD patients. Many immunotherapy-based papers have followed. Most of these have attempted to address which forms of Aβ are exerting various cognitive impairments and to determine the mechanism by which various anti-Aβ antibodies exert their beneficial effects. Use of different mouse models and various anti-Aβ antibodies has proven valuable in unraveling these questions.

The question of precisely why plaques form initially has not yet been resolved, but the underlying mechanisms involved in the reduction of Aβ plaque burden by immunotherapy have been partially resolved. Current findings suggest that, in vivo, at least three mechanisms are responsible for the ability of anti-Aβ antibodies to reduce plaques. The first is simple destabilization of existing plaques by physically binding to them, as most directly shown in vivo by Backsai et al., who demonstrated that injection of Fab fragments of anti-Aβ monoclonal antibodies removed existing plaques, as shown in living animals by dual photon confocal microscopy (Backsai et al. 2001). The precise biophysical principles of this phenomena are not fully understood, but it is conceivable that antibodies binding to the free C- or N-terminal regions of Aβ, which are accessible even when the peptide forms a fibril, change the conformation of the peptide such that the fibril is destabilized and disassociates. The second mechanism, already discussed and cited, is Fc-mediated phagocytosis by microglia cells. This is likely to be catalytic in the sense that a few antibodies bound to a plaque are likely sufficient for the microglial cells to engage and engulf a significant fraction of the entire plaque, making the process fairly efficient. A third component is simple reduction in the free concentration of Aβ peptide, as was initially anticipated in the rationale for the first immunization experiment. This mechanism has been coined the "sink" hypothesis, with the concept that since antibodies remain predominantly in the blood, they essentially serve to draw out the Aβ from the brain through mass action (De Mattos et al. 2001). Though the concept is attractive, it is difficult to test in vivo since all antibodies tested enter the

brain at some low level and the other mechanisms described above will likely also enter into the observed effects in vivo.

Perhaps the most important question underlying Aβ immunotherapy is determining its potential clinical utility. The first clinical test of the potential of immunization of Aβ as a possible treatment for AD used a synthetic version of Aβ 42 termed AN 1792. This agent was used in a number of phase 1 clinical trials where its tolerability and safety were investigated (Bayer et al. 2005). Following these early studies, it moved into a relatively large, phase 2 multicenter, placebo-controlled, double-blind study to investigate additional safety and pilot efficacy of the approach, although it was not powered to test for efficacy on the standard clinical endpoints in Alzheimer's disease such as ADAS-COG. Early in the phase 2 study, after a large majority of patients had received two doses of AN 1792, two cases of meningo-encephalitis occurred followed rapidly by two more. Dosing was immediately halted, although the study remained blinded and was converted essentially into a monitoring safety study that would still investigate exploratory endpoints at the 12-month time point (Gilman et al. 2005). The results of the study have been extensively discussed elsewhere, but several biological signals occurred in this study that represent very original observations that have not been seen before in therapeutic trials in AD patients. For example, in patients that generated reasonable antibody titers to Aβ (greater than 1:2,200), CSF levels of tau where reduced towards normal values, volumetric MRI values were reduced (Fox et al. 2005), a composite neuropyschometric battery of tests showed improvement and autopsy analysis showed plaque burden to be reduced and evidence of active amyloid clearance (Ferrer et al. 2004; Masliah et al. 2005a). Collectively, these results are consistent in many respects with what has been seen in APP transgenic mouse models of AD, with the notable exception that the meningo-encephalitis had not been predicted. The effects on cognition in AD patients were far too preliminary, given that drug dosing in the study was interrupted, to infer whether AN 1792 did or did not have a convincing effect overall on the patients' performance. Nonetheless, this initial clinical testing, in addition to all the progress since the initial preclinical report (Schenk et al. 1999), has resulted in a large number of ongoing clinical trials worldwide. The most advanced of these is Bapineuzumab, a humanized anti-Aβ antibody, currently in phase 2 studies in the US and imaging studies in Europe, to investigate safety, tolerability and initial exploratory efficacy in mild to moderate AD.

The initial preclinical study (Schenk et al. 1999) has indeed initiated a great many studies in both the discovery and applied clinical fields of AD. Relative to the lon-g-term investigation of the role of Aβ in AD, which is now almost 20 years of age, we should have an unambiguous answer regarding the clinical utility of Aβ immunotherapy within the next five years.

The general concept of immunizing with an amyloidogenic protein or peptide for treatment of a disease has also been recently expanded to a number of different disease classes, such as prion protein biology (Heppner et al. 2001) and Parkinson's disease (Masliah et al. 2005b). It is earnestly hoped that this general approach will generate a number of additional new therapeutic strategies for a class of diseases that have remained refractory to a large number of potential treatments thus far.

Christian Haass (*left*) and Roger M. Nitsch (*right*)

Immunotherapy of Alzheimer's disease

Roger M. Nitsch[1] and *Christoph Hock*[1]

Early in April 1906, Auguste Deter's brain was obtained by autopsy after four and a half years of progressive dementia that ultimately led to her death. Her doctor, Alois Alzheimer, who had cared for her, examined and photographed her since the initial admission in November 1901, stained microscopic sections of her brain by using his colleague's Franz Nissl newly established protocols. Besides profound neuron loss and fibrillar tangles, he found multiple deposits of a "peculiar substance" β-amyloid. Several months later, in November 1906, he reported this finding at a meeting in Tübingen (Alzheimer 1907a), and in the 1910 edition of his psychiatry textbook, Alzheimer's mentor, Emil Kraepelin, coined the term, "Alzheimer's disease," for the discovery (Kraepelin 1910). Today, 100 years after the initial report, β-amyloid has become the most promising target for curing the disease, and it appears as if immunotherapy can remove this "peculiar substance" from the brain (Weiner and Frenkel 2006). The major proteinaceous component of β-amyloid, the Aβ peptide, was characterized biochemically and was used to formulate vaccines designed to generate antibodies against β-amyloid but the provocative challenges about this concept lie in the fine line between tolerance and immunity, physiology and pathology, drawn by the transition of the soluble Aβ peptide into toxic, oligomeric aggregation products that finally assemble into β-amyloid fibrils (Walsh and Selkoe 2004a; Schenk et al. 2004; Nitsch 2004). Autoimmunity against self antigens, combined with the risk of fragile amyloid-laden brain blood vessels prone to bleedings, constitutes the next major hurdle to be overcome in the development of a safe immunotherapy. In fact, 18 of 298 patients who had received the first experimental vaccine, consisting of synthetic β-amyloid fibrils, developed autoimmune disease with clinical signs of aseptic meningoencephalitis (Orgogozo et al. 2003), and 3 of 22 patients who had received passive immunotherapy with humanized antibodies against Aβ developed MRI signs possibly consistent with microhemorrhages (Black et al. 2006). Despite these inherent risks, a large number of pharmaceutical and biotech companies initiated development programs for Aβ immunotherapy because of a compelling body of evidence from preclinical validations and clinical observations that provided proof of the concept with remarkable consistency.

β-amyloid plaques and some of their aggregated, oligomeric precursors form structural and possibly pathological neoepitopes that differ from unstructured, alphahelical, ransom coil or beta-sheet conformations in soluble Aβ monomers or the related sequences in the amyloid precursor protein (APP). Neoepitopes related to Aβ fibrillization develop late in life and are partially invisible to the peripheral immune system because they are confined to the brain. Therefore, they do not generally induce strong immune

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responses beyond low abundance of autoantibodies against Aβ aggregates in elderly subjects and in patients with dementia. However, the immune response against β-amyloid can be induced and strongly boosted in humans by active immunization with β-amyloid fibrils combined with an adjuvant resulting in high titers of antibodies with the ability to bind β-amyloid plaques with high affinity (Hock et al. 2002). Because the formation of oligomeric Aβ fibrillization intermediates is most likely a primary event in the pathophysiology of the disease, followed by a cascade of secondary events including synaptic dysfunction, neurofibrillar and cytoskeletal abnormalities, inflammation, oxidative stress, disrupted neurotransmission, homeostasis of ions and metabolism, their removal by immunotherapy can be expected to halt the detrimental and partially self-fuelling cascade of pathogenic events. The vast majority of data obtained in experimental animals support this theory: both active Aβ immunization and passive transfer of antibodies against Aβ consistently restored neuron morphology, synaptic plasticity, the phosphorylation status of tau, and neurotransmission and behavior (Lombardo et al. 2003; Oddo et al. 2004).

To analyze both the quality and the titers of antibodies against β-amyloid-related neoepitopes in patients who received active Aβ immunization, we developed a tissue amyloid plaque immunoreactivity (TAPIR) assay that allows for the quantitative identification of antibodies directed against β-amyloid plaques in brain tissues (Fig. 1). In contrast to conventional ELISA assays with immobilized synthetic peptides, commonly used to determine humoral reposes in vaccination trials, TAPIR assays use bona fide tissue β-amyloid plaques that developed over long time periods in living brains, within the complex tissue situation of the neuropil and in close contact with neurite membranes, reactive astrocytes and activated glial cells.

We found significant increases in serum titers of TAPIR-positive antibodies against β-amyloid plaques in 20 of 30 patients in the Zurich cohort of patients who participated in the vaccination trial (Hock et al. 2002), demonstrating that humans can mount an active polyclonal humoral immune response against β-amyloid-related epitopes without inducing unwanted antibodies that cross-reacting with APP in other, unaffected, tissues. Remarkably, some of these antibodies were able to cross the blood-brain barrier, as indicated by their presence in CSF without signs of intrathecal generation of antibodies. The mechanisms for blood-brain barrier passage of plasma-derived IgG may include increased blood-brain barrier permeability, passive diffusion and active transcytosis.

To determine whether antibodies against β-amyloid were associated with slowed progression of dementia in AD, we followed the patients of the Zurich cohort over a three-year time interval after the initial vaccination. Both cognitive functions and capacities of daily living declined less in patients with increased serum titers of antibodies against β-amyloid as compared to patients without such antibodies (Hock et al. 2003). Clinical outcomes were correlated with the immune response within the first three months following immunization. Our findings in the Zurich subcohort of AD patients were confirmed by the demonstration of better composite scores of memory performance in patients with high antibody titers of the world-wide AN-1792 multicenter cohort (Gilman et al. 2005). Together these observations provided clinical proof of concept for immunotherapy of AD. The published clinical data were supported by neuropathological analyses in four brains obtained at autopsy. Consistently, these studies showed reduced β-amyloid pathology in extended brain areas, accompanied by

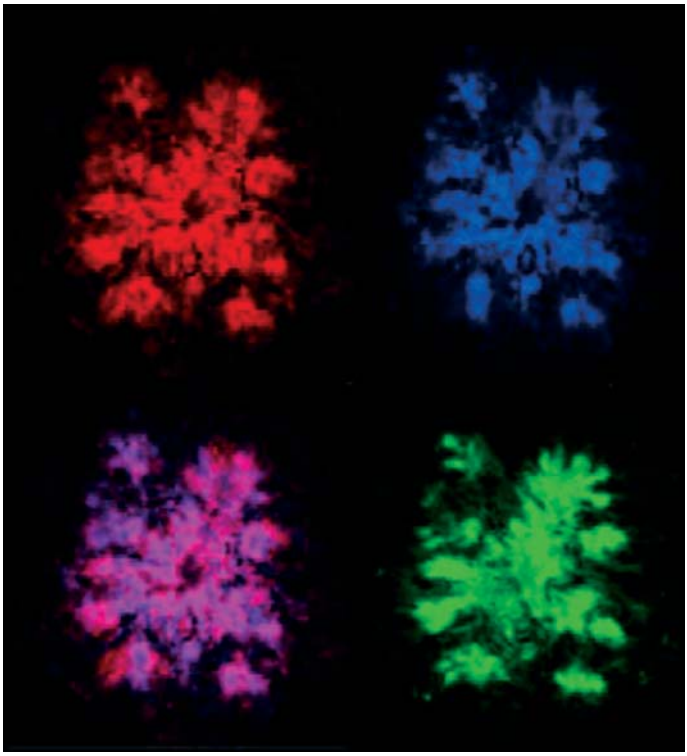

Fig. 1. Confocal immunofluorescence image of β-amyloid plaques stained with diluted cerebrospinal fluid (CSF) obtained from a patient vaccinated with aggregated Aβ. *Left upper panel*: immune CSF, *red*. *Right upper panel*: monoclonal antibody 4G8, *blue*. *Left lower panel*: double staining with human immune CSF and 4G8, *purple*. *Right lower panel*: Thioflavin S, *green*. Reprinted from, Hock et al. (2003), with permission from Elsevier

reduced astrogliosis and maintained tangle pathology (Nicoll et al. 2003; Ferrer et al. 2004; Masliah et al. 2005a). There are three mechanisms currently proposed to explain the therapeutic response to immunotherapy, and all involve removal, or reduction, of brain β-amyloid: microglia-mediated removal of amyloid, removal by peripheral amyloid sink, and antibody-mediated disaggregation of amyloid. These mechanisms may play in concert, but microglia-mediated uptake is almost certainly involved, as indicated by the presence of microglia filled with β-amyloid in brain areas that have been cleared of β-amyloid following immunotherapy (Nicoll et al., 2003).

Because beneficial cognitive effects were strongest in hippocampus functions, we measured hippocampus size over three years following Aβ immunization. We observed larger hippocampal volumes in patients who had generated antibodies against β-amyloid at the end of the three years follow-up period. Hippocampal volumes were correlated with better cognitive outcome. Stabilized hippocampus volumes were preceded by greater volume losses during the first year in patients with antibodies against β-amyloid, consistent with a previous report (Fox et al. 2005). These data may re-

flect a biphasic course of volume changes with initial losses followed by subsequent recoveries. In future studies, combined PET β-amyloid and MRI volumetric imaging will answer the question whether reduction in β-amyloid is paralleled by initially greater volume losses followed by subsequent increases without recurring β-amyloid formation.

If clinical safety can be improved, the available evidence strongly suggests that immunotherapy directed against brain β-amyloid plaques remains one of the most promising therapeutic approaches our field has seen over the last 100 years since Alois Alzheimer first observed the initial case of the disease that carries his name.

Acknowledgements. Supported by SNF grants 3200B0-112616 and 51NF40-111381 (NCCR Neuro), EU LSHM-CT-2003-503330 (APOPIS), DFG SFB 6027. We thank D. de Quervain, E. Gruber, A. Hänggi, K. Henke Westerholt, U. Konietzko, C. Mondadori, A. Papassotiropoulos, D. Schenk, A. Signorell, J. Streffer, and A. Wollmer.

Todd E. Golde

"Magic" bullets, shotguns or cocktails to treat or prevent Alzheimer's disease?

Todd E. Golde[1]

For the most part, the single target approach to drug discovery has failed to identify "magic bullets" that significantly impact the clinical manifestations of CNS diseases (Roth et al. 2004). Even when a "magic bullet" that is purportedly selective for a single target proves efficacious in a certain disease setting, in time, a more complete understanding of its pharmacology often shows that efficacy can be attributable to 1) interaction with several molecular targets or 2) a more complex physiologic effect than was originally intended. For example, it is now clear that "selective" drugs targeting G-protein coupled receptors (GPCRs) are, for the most part, not nearly as selective as previously thought; they bind with high affinity to a number of GPCRs (Roth et al. 2004). In addition, there is growing evidence that the beneficial effect of HMGCoA reductase inhibitors (statins) is not completely attributed to its cholesterol lowering effects and may be enhanced by effects on isoprenoid levels and protein prenylation, which can be both anti-proliferative and anti-inflammatory (Liao 2002). Indeed, as the appreciation of the complexity of targets that mediate the efficacy of many current CNS drugs grows, the idea of intentionally developing "magic shotguns" (single compounds that interact with multiple targets) or "magic cocktails" (optimized cocktails composed of multiple "magic bullets") for CNS disorders becomes more attractive (Roth et al. 2004).

The molecular dissection of the pathogenic cascades that result in Alzheimer's disease (AD) has led to the identification of multiple potential AD therapeutic targets that appear "druggable" (Golde 2003, 2005; Hardy and Selkoe 2002). Based on results from proof of concept preclinical studies in amyloid precursor protein (APP) and tau transgenic mouse models, a degree of cautious optimism is warranted with respect to a number of "magic bullet" therapies for prevention of AD. Less optimism is warranted with respect to such "magic bullets" showing marked efficacy in patients with AD. Pharmacologic or immunologic treatment of AD mouse models initiated when the mice show limited pathology can significantly attenuate the development of additional AD-like pathologies (Schenk et al. 1999); however, the same treatments initiated when the mice have more robust pathology typically show limited efficacy (Das et al. 2001). Though it is imperative that we test single target therapies for AD in the clinic, we need to realistically assess what kind of clinical benefit might be expected. In the meantime we need to consider the development of "magic shotguns" or "magic cocktails."

The basic tenet of the Aβ hypothesis of AD is that the process of Aβ accumulation as amyloid triggers a complex pathological reaction that leads to tau and in

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rarer cases synuclein aggregation, inflammation, oxidative stress, neuronal death and dysfunction, and ultimately clinical dementia (Hardy and Selkoe 2002). The evidence supporting this hypothesis is extensive. Pathological, genetic, biochemical, and modeling studies all point to a critical role of Aβ aggregation in AD and also show that the downstream pathological reaction appears to be complex. Because of the complexity and uncertainty regarding cause and effect in the downstream pathology, most "magic bullets" that are being considered as ideal AD therapies target Aβ by influencing either its production or subsequent aggregation. As Aβ aggregation is both concentration-dependent and regulated by relative levels of Aβ42, strategies targeting production of total Aβ or Aβ42 are rational approaches for therapeutic development. It is also possible to directly alter Aβ aggregation or possibly enhance clearance of Aβ or Aβ aggregates. Single target mono-therapy strategies include β- and γ-secretase inhibitors, selective Aβ42 lowering agents, Aβ aggregation inhibitors, and, arguably, various forms of Aβ immunotherapies.

Though many in the field have suspected that Aβ accumulation as amyloid precedes clinical symptoms by years or decades (Golde 2003), it has not been until recently that limited data directly supporting this assertion have begun to emerge. Indeed, amyloid imaging and biomarker studies do suggest that amyloid deposition precedes even early signs of cognitive dysfunction (Fagan et al. 2006). It also appears that, at least in mice, amyloid deposition is rather irreversible, even in the absence of ongoing Aβ production (Jankowsky et al. 2005b). If clinical AD and the accompanying neuronal loss are the result of many years or decades of Aβ accumulation in the brain, it is only reasonable that we consider the question, "Will 'magic bullets' targeting Aβ have marked efficacy in patients with AD?" In any case, no amount of theoretical information will substitute for well-designed prospective studies in humans that must ultimately be undertaken to prove the efficacy and safety of an anti-Aβ therapy. However, if such trials do not show marked efficacy or even show no efficacy, assuming the therapy did produce the desired effect on the target, we must consider how we develop and test preventative anti-Aβ therapies. Indeed, most AD researchers would endorse the notion that a safe anti-Aβ therapy given early enough would significantly delay the onset of AD. Unfortunately, economics, ethics, regulatory concerns, uncertainty regarding timing (e.g., how soon is soon enough), sample size, and perhaps some element of dogma deter efforts to launch primary prevention trials.

To enhance the chance that the "magic bullet" approach to AD will be successful, we must optimize preventative trial design so that the time and costs of such studies are reduced. We can only hope that genetic, biomarker, and imaging studies will enable the selection of an at-risk population so that these trials become more feasible. It is also clear that we need to lobby the regulatory bodies to at least consider non-cognitive and non-functional clinical end points with respect to initial "magic bullet" studies. If a therapy safely targets Aβ, it should be evaluated in a prevention trial, even if it shows limited or no efficacy in the therapeutic trial. Indeed, statins were initially approved for human use based on their ability to lower cholesterol. Only in phase IV was it shown that they had significant effects on morbidity and mortality.

Early clinical data from phase II trials do suggest that treatments targeting Aβ, such as Flurizan (R-Fluribiprofen), a compound that selectively lowers Aβ42 (Eriksen et al. 2003), and Alzhemed (Aisen 2005), a small molecule reported to inhibit Aβ aggregation, may be beneficial as AD therapies, especially, at least for Flurizan, when administered

in the earlier stages of the disease. Similarly, there is some very anecdotal data from human Aβ immunotherapy trials suggesting that Aβ immunotherapy may have some limited benefit (Gilman et al. 2005; Hock et al. 2003). However, what can be inferred from these ongoing therapy trials (and applies even more so to currently approved therapies) is that none of these therapies, at least in the context of AD therapy, is a true panacea. Though it is possible, it is unlikely that anti-Aβ therapy will cure those already affected with AD. Anti-Aβ therapies may slow the course of the disease, but it may be overly optimistic to expect a significant reversal of cognitive symptoms.

Given the complexity of pathological changes present in the brain even in very early stages of AD, it may be that, in order to dramatically alter the course, we need to explore alternatives to the "magic bullet" approach. Indeed, some of the more successful preclinical studies provide a strong rationale for a "magic shotgun" or "magic cocktail approach." Indeed, a number of safe compounds that almost certainly have a multitude of complex pharmacologic targets have shown efficacy in AD mouse models. Compounds such as curcumin, garlic extract, various fatty acids, lithium, various kinase inhibitors, some natural products, and perhaps statins and NSAIDs might be considered potential "magic shotguns" (Chauhan 2005; Lim et al. 2001a; Nakashima et al. 2005; Phiel et al. 2003; Refolo et al. 2001; Wolozin 2001). Because of the predominance of the "magic bullet" mentality for drug discovery and the uncertainty regarding mechanisms of action of these "magic shotguns," there is reluctance to clinically develop these types of therapies.

An impressive number of drug-like compounds of diverse structural classes can modulate Aβ production and aggregation. If we can begin to identify and validate other targets for AD therapy, either by employing a proof of concept "magic bullet" approach or dissecting out the mechanisms of action of a "magic shotgun," we may be able to develop more potent "magic bullets" that non-selectively alter multiple targets. Such an approach, at least in the near future, will be an iterative process involving screening compounds for desired mechanism of action and will inherently involve some element of chance. We might nevertheless consider that it may be possible to identify compounds that interfere with Aβ production or aggregation, also target tau, and perhaps have anti-oxidant or anti-inflammatory properties or other multi-target properties. Ultimately, it is likely that, to most effectively treat AD, we will identify some "magic cocktail" that simultaneously targets both the initiating factors in the disease and downstream pathways. Such a cocktail might also consist of anti-Aβ and anti-tau therapies, cognitive enhancers, and anti-inflammatory or anti-oxidant compounds and some form of regenerative therapy, such as a growth factor.

Given the time it takes to develop even a single new drug for AD, unless we identify known drugs that might safely work together to create a "magic cocktail," it may be that before we develop therapeutic "magic cocktails" we will have developed and successfully tested "magic bullets" or "magic shotguns" as preventative therapies. Indeed, I for one believe that we will prevent AD long before we develop effective stem cell therapies. In any case, 100 years of AD research have identified targets that I and many in the field believe will lead to effective preventative therapy for this disease. It is my hope that, within the next 10 years, we can translate target discovery into therapeutics that profoundly alter the clinical course of AD.

Dennis J. Selkoe

The seventh age of man: Solving senility

Dennis J. Selkoe[1]

Less than three years prior to his death at age 52, William Shakespeare remained hard at work, co-authoring *The Two Noble Kinsmen* with John Fletcher. Although he lived relatively long for his time, the Bard escaped the ravages of late-life dementia that he described briefly but poignantly in Jacques' soliloquy in *As You Like It*. The loss of memory and intellect that often accompanies great age must have been experienced throughout human history. Thus, the specific disorder that Alzheimer described was surely known to the ancients. But the remarkable increase in life expectancy since Shakespeare's time has made senile dementia commonplace.

Given the seeming inevitability that many long-lived individuals will develop profound dementia, it is enormously exciting to think that our generation may be about to witness a substantial reduction in this scourge. Following upon the success of biomedical research in reducing or eliminating certain infectious diseases, it appears that similar inroads into chronic, non-infectious diseases such as atherosclerotic cardiovascular disorders and Alzheimer's disease are within reach. There will no doubt be further fits and starts along the way, but it is my opinion that the concepts and tools necessary to slow, and ultimately prevent, Alzheimer's disease are almost in hand.

This volume chronicles the salient contributions made by physicians and scientists during the last half century that have brought us into an era of therapeutics for Alzheimer's disease. Having experienced the excitement and satisfaction of participating in this worldwide endeavor, I am grateful for the opportunity to provide a personal perspective on how the remaining battle to defeat Alzheimer's disease might unfold in the next few decades.

Not surprisingly, one's level of optimism about solving Alzheimer's depends very much on one's opinion about the probable causes of this complex and multifaceted syndrome. Since my earliest conversations with George Glenner and other leaders in the field of human amyloidotic diseases, I have believed that the gradual accumulation of an amyloidogenic protein was likely to have dire consequences for the structure and function of surrounding cells. Among nervous system diseases, Alzheimer's disease has emerged as perhaps the most compelling example of a disorder that may be precipitated by the accumulation, misfolding and progressive aggregation of an otherwise soluble protein. The last decade has seen the extension of this concept to a number of other previously enigmatic neurodegenerative disorders, including Parkinson's, Huntington's and Lou Gehrig's diseases. While much further research will be needed to support the hypothesis that these disorders are fundamentally due to accumulation of

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misfolded proteins, the evidence that this is indeed the case in Alzheimer's disease is more advanced and convincing. Many steps in the pathogenic cascade remain murky and require careful elucidation, but findings from human neuropathology, the study of genotype-to-phenotype conversions and, most importantly, the initiation of therapeutic trials have combined to support the hypothesis that lowering or neutralizing amyloid β-protein (Aβ) should slow or prevent the clinical progression of Alzheimer's disease.

My predictions about the road ahead are predicated on the assumption that further careful clinical testing will reveal that anti-Aβ therapies influence the course of cognitive decline in Alzheimer's disease and in its harbinger, mild cognitive impairment, amnestic type. If this assumption proves true, one can also predict that a sizable fraction of more subtle, age-linked impairment of declarative memory – particularly episodic memory – will also be amenable to anti-Aβ therapies. Certainly, many humans who experience subtle cognitive impairment in late life have explanations other than Alzheimer-type Aβ accumulation, and such patients will presumably receive no benefit from anti-Aβ therapeutics, unless their symptoms are due to a combination of Aβ- and non-Aβ-driven alterations in hippocampal circuitry. But advances in functional magnetic resonance imaging and the in vivo detection of subtle forms of Aβ deposition may allow us to distinguish patients who lose memory function via Aβ-related events from those who have other mechanisms.

If current and planned therapeutic trials of anti-Aβ agents that unfold during the next decade show the clinical promise that very early studies suggest, we are likely to experience a paradigm shift in how humans confront and deal with the prospect of Alzheimer's disease and its antecedents. Because Aβ accumulation in limbic and association cortices is linked to age, even in the absence of clinically detectable impairment, it is reasonable to offer people the opportunity to estimate – first crudely and later with increasing precision – the likelihood that they will experience a moderate or severe rise in cerebral Aβ levels in late life. As it becomes refined, this risk screening could be applied to individuals in middle or late-middle age, well before they would be expected to develop Alzheimer-type cognitive impairment.

The risk assessment I envision will be multifactorial (Fig. 1). Around age 50 or thereafter, primary care providers will obtain a brief neurological history, attempting to elicit a personal and/or family history of subtle or profound cognitive impairment. Special attention will be paid to the family history in view of the likelihood that a large fraction (in my opinion, more than half) of Alzheimer's disease will be shown to have complex genetic determinants. The physician will then perform a brief cognitive screen with an emphasis on questions that assess declarative memory, particularly episodic memory. It seems likely that current, widely used office instruments for screening subjects for the presence of dementia, such as the Blessed Dementia Rating Scale or the Mini Mental State Exam, will be supplanted by tests that focus more specifically on verbal declarative memory, face-name recognition and other memory-related functions that are known to be sensitive to the earliest phase of Alzheimer-type dementia, even preceding MCI-amnestic type. The idea here is to focus on rapid but sensitive office tests that will reveal AD-type dementing symptoms. Broader and deeper neuropsychological assessment can be performed if a suspicion of an Alzheimer-like dementia emerges. The physician will also perform a standard neurological exam.

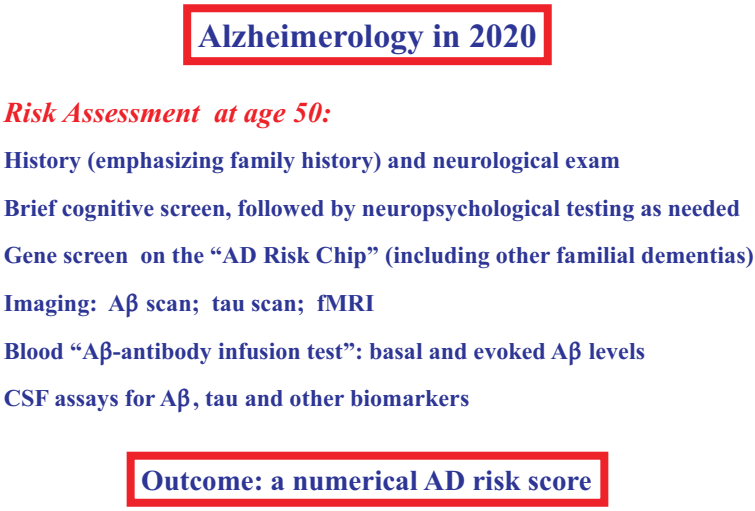

Fig. 1. Alzheimerology in 2020

Next, subjects with suspicious histories and exams will undergo an imaging procedure that, by the year 2020 or earlier, will be both sensitive and specific for the detection of Alzheimer-type neuropathology, particularly early Aβ deposition. One can safely speculate that positron emission tomography and even structural MRI will be performed using agents that will sensitively detect diffuse Aβ deposits. An additional imaging strategy that may be employed will be functional MRI during the administration of simple memory and cognitive screening tasks. Clearly, whichever imaging procedure has been shown between now and then to be sensitive to early Aβ deposition and its cellular consequences will be employed. Therefore, the scenario I outline here contemplates only one imaging procedure to be used during initial screening of healthy 50-plus year olds. Depending on the ease and cost of performing this procedure, one will be able to determine whether imaging will be done routinely in patients after the age of 50 who show positive risk on the aforementioned historical and mental status screening procedures.

An additional risk assessment screen that will likely be widely used will be an "AD risk chip" or something similar. A chip- or solution-based hybridization screen to detect all of the known genetic mutations causing dominantly inherited disease as well as polymorphisms serving as risk factors for AD will be conducted. Obviously, this screen can also include genes implicated in other inherited dementias, including the tau mutations. In view of the likelihood that this type of analysis will be feasible before the year 2020, governments will need to have established formal safeguards against discrimination of patients with positive genetic risk profiles, particularly as regards employment and health insurance.

An additional feature of the risk assessment I envision will be direct measurement of Aβ in plasma by mass spectrometry to reveal all of the heterogeneous Aβ peptides present in biological fluids. It is unlikely that, at that point in time, we will rely solely on Aβ42/Aβ40 ratios; rather, we will be experienced in analyzing a more refined spectrum

of Aβ species that can reveal a propensity to AD-like disease. Thus, we will be able to detect absolute decreases in Aβ40 peptides and shorter species as well as increases in Aβ42 or longer species to more accurately assign risk. While it may turn out that only a minority of middle-aged humans prone to AD-type dementia will have an abnormal Aβ profile in the plasma, one could then – as now – select certain patients for CSF analyses, including Aβ, tau and other biomarkers that have been validated as specific and sensitive for AD. In this regard, it is of great interest that European clinicians have for some time routinely obtained CSF for measuring Aβ, tau and other analytes in patients with MCI or AD, something that American clinicians have been far more conservative about, in my view unnecessarily.

There will no doubt be other imaging methods and fluid biomarkers that will have been proven to help discriminate the earliest stages of AD-type cognitive function from other kinds of impairments. One of these, I think, will be the measurement of an acute rise in plasma Aβ levels induced by the administration of a single dose of an anti-Aβ monoclonal antibody, as originally described by David Holtzman and colleagues. Those subjects who show an acute rise in plasma Aβ above the levels seen in middle-aged normal individuals without known risk of AD will have this quantitative value incorporated into their formal risk assessment.

Through the process described in the preceding paragraphs, a healthy individual after age 50 will be assigned a numerical "AD Risk Score" based on a weighting of the different quantitative and semi-quantitative values obtained in this screening process. The outcome will be a quantitative estimate of the likelihood of developing AD or another Aβ-mediated dementing illness. Obviously, this estimate will be quite crude at first, but after extensive experience applying such quantitative assessments to various populations around the globe, one will be able to obtain an increasingly refined estimate of AD risk. A series of categories of relative risk will be established and modified over time, and we will determine into which category a subject falls.

Based on the outcome of this screening process, non-symptomatic individuals with low risk will be told this and will be assessed again after a certain interval, perhaps in five years. This paradigm would be not unlike today's use of screening colonoscopy on a five-year basis in otherwise healthy individuals after age 50. Those pre-symptomatic subjects who have various positive results on the risk assessment profile, e.g., altered plasma Aβ peptide ratios or a positive Aβ antibody challenge test, but who have negative or borderline scans for diffuse Aβ deposition will be offered prophylactic treatment (Fig. 2). This treatment might constitute a β-secretase inhibitor or possibly a γ-secretase modulator. I assume that, by some years from now, small molecules that can selectively modulate γ-secretase to lower Aβ42 – or else Aβ42 and Aβ40 plus other minor species – but do not interfere significantly with the processing of Notch and most other γ-secretase substrates will be available. The pharmacological complexities of inhibiting β-secretase with a blood-brain barrier-permeable small molecule will presumably also have been resolved. Yet another alternative may well be an Aβ oligomerization inhibitor that can stabilize the Aβ monomer and decrease the likelihood of monomer-to-dimer conversion, the initial and critical step in Aβ aggregation in the brain. Or perhaps such an agent will stabilize and mask Aβ dimers. The choice of preventative agents will obviously depend on the overall efficacy versus safety profile of these various approaches; the approach that has the least risk for side effects will be the most appropriate for use in presumptive pre-symptomatic subjects.

Alzheimerology in 2020
A New Way to Manage Alzheimer's Disease

Determine the risk category into which a person falls:

Pre-symptomatic subjects with negative Aβ Scans:
β- or γ-secretase inhibitor
or
Aβ oligomer inhibitor/stabilizer

Pre-symptomatic subjects with positive Aβ Scans:
β- or γ-secretase inhibitor
or
Aβ oligomer inhibitor/stabilizer
plus
Aβ immunotherapy (passive or active)

Symptomatic subjects (with positive Aβ scan):
β- or γ-secretase inhibitor
plus
Aβ immunotherapy (passive or active)
plus
Symptomatic agents
(e.g., acetylcholinesterase inhibitor; NMDA antagonist;
other neurotransmitter modulators; psychotropic drugs)

Fig. 2. New Way to manage Alzheimer's disease

Those pre-symptomatic subjects who have altered test results as above but also have a clearly positive Aβ brain scan will be categorized as harboring active AD-type disease that requires even more vigorous treatment. This treatment may include a β- or γ-secretase modulator plus a form of anti-Aβ immunotherapy. At this writing, one can still speculate that an active vaccine employing a small N-terminal Aβ peptide might turn out to be both safe and efficacious in lowering cerebral Aβ levels. But one persistent theoretical concern is that any active Aβ vaccine, even one that eliminates the major T-cell epitope in the peptide, will still lead to some T-cell activation during the process of B-cell stimulation and antibody production, raising the specter of a T-cell meningitis in those individuals who already have some level of peripheral T-cell reactivity to Aβ. The latter possibility can, of course, be tested in the subject's peripheral blood; we already know that a fraction of the elderly population harbors a small number of T-cell clones that are endogenously reactive to Aβ. If active vaccination, which is desirable in terms of ease of administration to a very large population and relatively lower cost, is shown not to be feasible as a result of safety concerns, then passive Aβ immunotherapy for pre-symptomatic subjects with positive Aβ brain scans may be reasonable. The latter could turn out to be a monoclonal antibody, an Fab fragment thereof, or perhaps

even a non-Aβ immune modulator (i.e., another protein) that can be shown to lower Aβ levels in the brain without initiating a T- or B-cell response to Aβ per se.

If the AD Risk Score indicates that the patient is not only at risk for the disease but already has telltale symptoms of mild, intermittent memory impairment, then full therapy would be instituted. This therapy would likely include a β- or γ-secretase inhibitor/modulator, an oligomer neutralizer, and/or a form of anti-Aβ immunotherapy. In addition, the patient would be given certain symptomatic treatments as needed, including those currently used, such as acetylcholinesterase inhibitors and NMDA antagonists, but presumably other neurotransmitter modulators. Psychotropic agents that could ameliorate early behavioral symptoms would be offered on a case-by-case basis.

It is impossible to predict accurately which of the aforementioned Aβ-lowering strategies will turn out to be the safest, most efficacious and least costly to administer. Indeed, it is in this area that our field will encounter its greatest challenge during the next two decades: devising therapeutic strategies that can be administered to large numbers of still-healthy subjects to *prevent* AD, rather than to wait until it requires treatment. We are all well aware of the complexity and cost of performing primary prevention trials for chronic, late-life disorders such as Alzheimer's disease. Only after there is considerable experience with the benefits and risks of Aβ-lowering agents in already symptomatic subjects will one feel comfortable in exploring these in presymptomatic individuals. Although the challenges of developing preventative agents that can be widely applied may seem formidable, this is a task that has been successfully accomplished for a number of other chronic, widespread diseases, and I believe that many of the goals outlined herein can be reached by around 2020.

It is important to emphasize that the kind of risk assessment/preventative treatment paradigm that is envisioned above cannot be applied to Alzheimer-type dementia in isolation. One will need to take advantage of the progress in deciphering the molecular mechanisms of other neurodegenerative disorders that impair cognition with age in order to broaden our risk assessment to non-AD dementias and offer specific therapies for these disorders as appropriate. But I believe that progress towards these goals will occur more quickly and more substantively for the management of Alzheimer's disease per se, with other less frequent dementias building on the experience with Alzheimer's disease to move in parallel directions.

One can be certain that there will be many permutations and alterations in the kind of diagnostic/therapeutic schema proposed above, but the central question is not whether we will reach such a goal but how long it will take us. The development of disease-modifying agents based on the best current understanding of mechanism always takes much longer than one initially surmises. Many unforeseen setbacks can and will occur. But given the wonderful successes in the scientific assault on Alzheimer's disease during the last three decades that we are celebrating at this 100th anniversary, it can be safely predicted that we will achieve a substantial level of prevention of Alzheimer's disease in the next few decades.

Forum and Network

Jacqueline Mervaillie

Fondation Ipsen and Alzheimer's disease: a 20-year relationship

Jacqueline Mervaillie[1]

Shortly after its founding in 1983, Fondation Ipsen's destiny became intertwined with that of alzheimerology and although its activities extend to fields as diverse as endocrinology, oncology, neuroscience and cardiovascular pathologies, the foundation is most often associated with its focus on Alzheimer's disease.

Chance, rather than strategy, was the main engineer at work. As the foundation published the first issue of *Alzheimer Actualités* [Alzheimer News] in June 1986, and later organized its first *Colloque Médecine et Recherche* on Alzheimer's disease on September 14, 1987, the importance that the theme was to take on was only occasionally visible. Little known to the medical community, particularly in France, and often considered a pathology more or less on a par with cognitive aging, the disease's neuropsychological signs had elicited only a limited number of studies while on the research side, the molecular lesions were not yet identified. Conference speakers broaching the topic were in the habit of starting their lectures by admitting to a certain degree of ignorance. Alzheimer's disease was a pathology with no known cause, no genetic origin, no predictability, in that it did not have any biological markers, and not even any serious therapeutic avenues. None of these statements is true today. Twenty years have gone by and considerable efforts have been made by the best teams across the world to address these challenges.

1987: The Turning Point

When Fondation Ipsen began focusing its attention on Alzheimer's disease, the predominant topic in the field was cholinergic deficit. Yet by the time the first *Colloque Médecine et Recherche*, entitled, "Immunology and Alzheimer's Disease." was organized, the focus was on β-amyloid (Aβ) and its precursor, APP. Konrad Beyreuther and Benno Müller-Hill had just published their article on the APP gene in the February 19,1987 edition of Nature (Kang et al. 1987); the papers showing that tau protein was the main component in paired helical filaments had come out shortly before that. A new era was dawning. As of yet, it had not given rise to much discussion. The joint efforts of immunologist Annick Pouplard-Barthelaix – one of the first to highlight the immune and inflammatory aspects of Alzheimer's disease – and neurologist Jean Emile, both from Angers and organizers of the first meeting, were to change that. Participating as speakers were: Henry Wisniewski, George Glenner, Colin Masters, Konrad Beyreuther, Dennis Selkoe, Tsuyoshi Ishii, Piet Eikelenboom, H Hugh Fudenberg, Taihei Miyama

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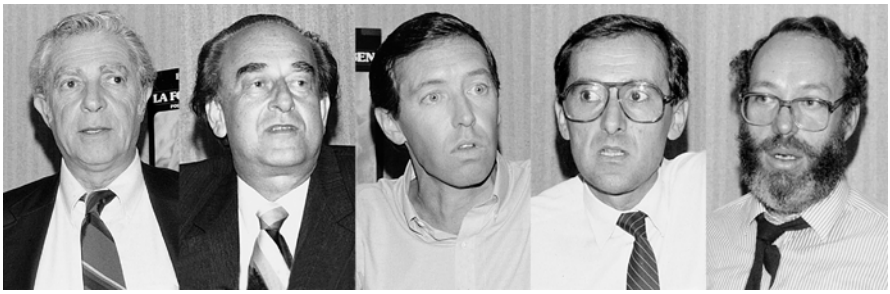


Fig. 1. From *left* to *right*: George Glenner, Henry Wisniewski, Dennis Selkoe, Konrad Beyreuther and Colin Masters at the first Fondation Ipsen Alzheimer meeting in Angers (September 14, 1987)

and André Delacourte. Glenner and Wisniewski were then seen as leaders in the field, whereas Colin Masters, Dennis Selkoe and Konrad Beyreuther did not yet have the authority that was soon to become theirs (Fig 1).

20 *Colloques Médecine et Recherche* to help communicate knowledge

Since that day and in a pioneering spirit, Fondation Ipsen has strived to bring to light new developments in science's understanding of Alzheimer's disease, detecting the promising topics and devoting to them, in many cases, some of the leading international conferences available to researchers. On each occasion, it has chosen to bring together Alzheimer specialists and researchers from various fields, with the aim of sparking new ideas and enhancing debate. Twenty *Colloques Médecine et Recherche* (Table 1) have since been held, each another opportunity to focus on the best teams and the most promising research avenues. Following every conference, a publication was issued and the series *Research and Perspectives in Alzheimer's Disease* (Springer) is now a valued part of any university library's collection, each volume a reference point in the advancement of Alzheimer research (Fig. 2).

The speakers include the vast majority of those who have made or are still making Alzheimer history. With only a few exceptions, all of the Potamkin Award winners recognized for their remarkable work in the field participated in the conferences.

Encouraging research through awards and scholarships

Every year from 1986 to 2000, a jury of specialists handed out both awards and scholarships, under the aegis of Fondation Ipsen. The awards offered recognition for outstanding contributions to Alzheimer research (K. Beyreuther, J. Hardy, J.-F. Foncin, C. Duyckaerts and A. Delacourte, to mention only a few) while the scholarships were intended to encourage the work of young researchers. More than 60 people have benefited from them in various capacities. (Fig. 3)

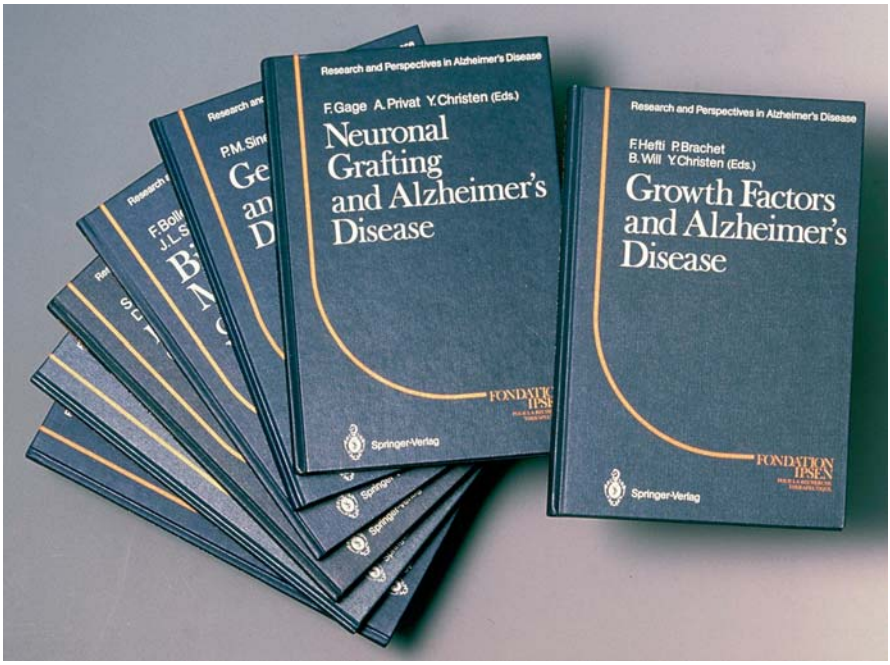


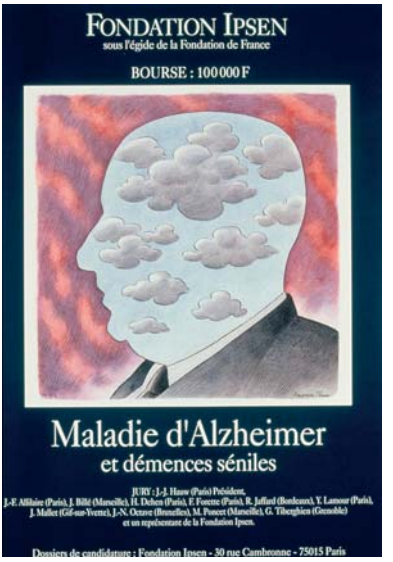
Fig. 2. The proceedings of all 20 Alzheimer meetings have been published by Springer (Heidelberg) in a Fondation Ipsen series *Research and Perspectives in Alzheimer's Disease*

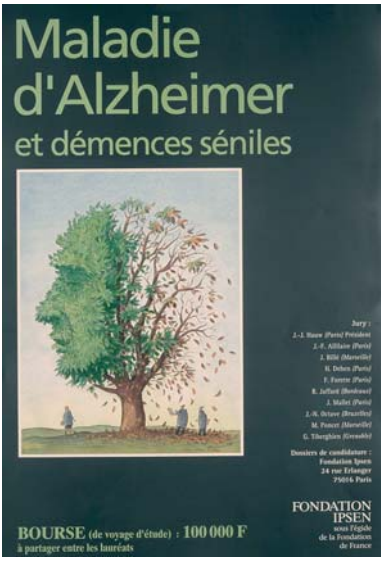
Alzheimer Actualités: 20 years and nearly 200 issues

In 1986, the French medical and scientific community's need for information about Alzheimer's disease was unmistakable: research findings from international teams were just beginning to build, yet the means of circulating them were not the same as with today's information technologies. This monthly newsletter, very widely circulated in French-speaking countries, was designed, from the outset, to highlight, summarize and, if possible, clarify research findings just as they were unveiled in the world's premier publications. For scientists eager for the latest news, reading through the monthly newsletter's concisely worded pages quickly became a habit. Today, curious minds with the leisure to do so can easily flip through each issue in the collection and see the history of Alzheimer's disease flash before their eyes, with two decades of hopes, doubts, disillusionment and, more rarely, certainties confirmed. (Fig. 4)

Serving families and caretakers

Very quickly, it became clear that the quest for information was one that also involved patients' families and the general practitioners called upon to answer their harried questions. Since 1986, a film has been available to them -*La Maladie d'Alzheimer*- in which Professor Jean-Louis Signoret (Hôpital de la Salpêtrière, Paris) and Michel





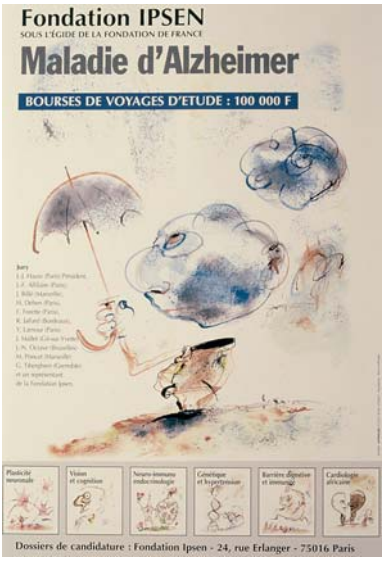


Fig. 3. Posters advertising some of the Fondation Ipsen prizes in Alzheimer's disease

Poncet (Hôpital de la Timone, Marseilles) provided a semiological description of the disease – simple, precise but still unknown to many – based on three patients' cases presented with modesty and feeling by their spouses. It has clearly been a vehicle for educating thousands of practitioners.

Fig. 4. Nearly 200 issues of the *Alzheimer Actualités* newsletter have been published since 1986

Almost at the same time, Fondation Ipsen began to widely circulate tens of thousands of copies of a translated, adapted version of *Caring* (New York City Alzheimer's Resource Center), which turned out to be virtually as successful as the original document. Later came two other brochures, also intended for families: *Pour les Aider* ("How to Help Them." 1987) and *Pour Défendre leurs Droits* ("How to Defend their Interests." 1988). The foundation also provided funding to equip a model therapeutic apartment for patients with the disease, which geriatrician Dr. Michèle Micas made widely known to her counterparts from the Toulouse area.

All of the above initiatives showed how vast the quest for information around Alzheimer's disease was at the time. Fondation Ipsen strived to provide a swift, effective and deliberately unadorned response to that need.

A place of knowledge, liberty and friendship

What lessons can we take away from these 20 years of dedication to Alzheimer's disease and collaboration with its "heroes?" I see at least three. The first has to do with the spirit of cultivating knowledge. As both specialists and non-specialists in Alzheimer's disease have always been involved in studying the disease, research has been able to benefit massively from the outside contributions. This fact can be plainly seen by anyone reading the themes and speakers' names at Fondation Ipsen's conferences. In many cases, unexpected pairings resulted from the events.

The second lesson pertains to freedom of thought. Science does not let itself be partitioned. The list of our partners provides additional proof of our desire to give a voice to proponents of all major theories, with no special consideration given to the controversies that have sometimes – and legitimately so – developed within the

scientific community. Everyone was given the opportunity to step forth, without the slightest preconception.

The third relates to loyalty. Fondation Ipsen has strived to maintain, over the last 20 years, friendly relations with those who supported and backed its activities. Many will be together next November in Tübingen to celebrate the centennial of the presentation of Auguste D's case by Alois Alzheimer. Several of them, unfortunately, are no longer with us, some departing under tragic circumstances: Yvon Lamour, Tsunao Saitoh, Jean-Louis Signoret, Henry Wisniewski, George Glenner, Nelson Butters and Luigi Amaducci. Their passing enhances – beyond the need for sometimes severe competition – the feeling of belonging to a family of sorts. Loyalty is a form of wealth. When one of her predecessors, Yvon Lamour, died on 18 July 1996 – in the crash of TWA Flight 800 between New York and Paris – Françoise Forette, gerontologist and Alzheimer specialist, called Fondation Ipsen, a "marvellous place of knowledge, liberty and caring." Our aspiration is nothing more than to be worthy of that judgment.

The *Alzheimer: 100 Years and Beyond* conference, set to open its doors in a few days in Tübingen and in whose organization our German Alzheimer counterparts have extensively involved us, will, without a doubt, be a paramount occasion for the community of researchers and clinicians involved in the exciting challenges that they will have to face for many years to come.

Fondation Ipsen, and in particular Yves Christen and I, will feel a measure of pride at having been able to make our contribution.

Table 1. *Colloques Médecine et Recherche* in the Alzheimer series *Research and Perspectives in Alzheimer's Disease* (Springer)

Immunological aspects of Alzheimer's disease and cerebral amyloidosis Angers – September 14, 1987
Scientific Committee: **Jean Emile** (*CHR Angers, Angers*), **Annick Pouplard-Barthelaix** (*CHR Angers, Angers*), **Yves Christen** (*Fondation Ipsen, Paris*).
Speakers: **Annick Pouplard-Barthelaix** (*CHR Angers, Angers*), **Tsuyoshi Ishii** (*Psychiatric Research Institute of Tokyo, Tokyo*), **Taihei Miyakawa** (*Kumamoto University Medical School, Kumamoto*), **Piet Eikelenboom** (*Free University, Amsterdam*), **André Delacourte** (*Faculté de Médecine, Lille*), **George G. Glenner** (*University of California San Diego, La Jolla*), **Dennis J. Selkoe** (*Brigham & Women's Hospital, Boston*), **Colin L. Masters** (*Royal Perth Hospital, Perth*), **Konrad Beyreuther** (*University of Heidelberg, Heidelberg*), **H. Hugh Fudenberg** (*Medical University of South Carolina, Charleston*), **Henry Wisniewski** (*Institute for Basic Research on Developmental Disabilities, Staten Island*).
(Pouplard Barthelaix et al. 1988)
Genetics and Alzheimer's disease Paris – March 25, 1988
Scientific Committee: **Yvon Lamour** (*Inserm U161, Paris*), **Pierre-Marie Sinet** (*Hôpital Necker, Paris*), **Yves Christen** (*Fondation Ipsen, Paris*).
Speakers: **Annick Alperovitch** (*Institut Gustave Roussy, Villejuif*), **Brian H. Anderton** (*Hospital Medical School, London*), **J.-M. Delabar** (*Hôpital Necker, Paris*), **Charles J. Epstein** (*University of California San Francisco, San Francisco*), **Marshall F. Folstein** (*The Johns Hopkins University,*

Baltimore), **Jean-François Foncin** (*Hôpital de la Salpêtrière, Paris*), **Carleton Gajdusek** (*National Institute of Health, Bethesda*), **Denis Gauvreau** (*Institut National de la Recherche Scientifique, Pointe Claire*), **Dmitry Goldgaber** (*National Institutes of Health, Bethesda*), **Kenneth S. Kosik** (*Brigham and Women's Hospital, Boston*), **Yvon Lamour** (*Inserm U161, Paris*), **Jacques Mallet** (*CNRS, Gif-sur-Yvette*), **Colin L. Masters** (*Royal Perth Hospital, Perth*), **Jean-Noël Octave** (*Université Catholique de Louvain, Bruxelles*), **Stanley I. Rapoport** (*National Institutes of Health, Bethesda*), **Allen D. Roses** (*Duke University, Durham*), **Pierre-Marie Sinet** (*Hôpital Necker, Paris*), **Peter St George-Hyslop** (*Massachusetts General Hospital, Boston*), **Christine Van Broeckhoven** (*University of Antwerp, Antwerp*), **Henry Wisniewski** (*Institute for Basic Research on Developmental Disabilities, Staten Island*), **Alison Goate** (*St Mary's Hospital, London*).

(Sinet et al. 1988)

Neuronal grafting and Alzheimer's disease: future perspectives
Montpellier – September 19, 1988

Scientific Committee: **Fred H. Gage** (*University of California San Diego, La Jolla*), **Alain Privat** (*Institut de Biologie, Montpellier*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Anders Björklund** (*University of Lund, Lund*), **Gyorgy Buzsaki** (*University of California San Diego, La Jolla*), **Carl W. Cotman** (*University of California Irvine, Irvine*), **Stephen B. Dunnett** (*University of Cambridge, Cambridge UK*), **Fred H. Gage** (*University of California San Diego, La Jolla*), **Madeleine Gumpel** (*Hôpital de la Salpêtrière, Paris*), **Jean-Paul Herman** (*Inserm U259, Bordeaux*), **Barry J. Hoffer** (*University of Colorado Health Science Center, Denver*), **Raymond D. Lund** (*University of Pittsburgh, Pittsburgh*), **Lars Olson** (*Karolinska Institut, Stockholm*), **Marc Peschanski** (*Inserm U161, Paris*), **Alain Privat** (*Institut de Biologie, Montpellier*), **Allen D. Roses** (*Duke University, Durham*), **Menahem Segal** (*The Weizmann Institute of Science, Rehovot*), **John R. Sladek** (*University Rochester Medical School, Rochester*), **Constantino Sotelo** (*Hôpital de la Salpêtrière, Paris*), **Harry W.M. Steinbusch** (*Free University, Amsterdam*), **Bruno Will** (*Inserm U44, Strasbourg*), **Joseph Yanai** (*Hebrew University Hadassah Medical School, Jerusalem*).

(Gage et al. 1989)

Biological markers of Alzheimer's disease
Toulouse – April 24, 1989

Scientific Committee: **François Boller** (*Inserm U324, Paris*), **Robert Katzman** (*La Jolla*), **André Rascol** (*Hôpital Purpan, Toulouse*), **Jean-Louis Signoret** (*Hôpital de la Salpêtrière, Paris*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **John R. Atack** (*National Institutes of Health, Bethesda*), **Konrad Beyreuther** (*University of Heidelberg, Heidelberg*), **John P. Blass** (*Burke Rehabilitation Center, New-York*), **Peter Davies** (*Albert Einstein College of Medicine, Bronx*), **André Delacourte** (*Inserm U16, Lille*), **Andrew R. Haynes** (*Saint Mary's Hospital School, London*), **Kenneth S. Kosik** (*Brigham and Women's Hospital, Boston*), **Jay W. Pettegrew** (*University of Pittsburgh, Pittsburgh*), **Tsunao Saitoh** (*University of California San Diego, La Jolla*), **Michael L. Shelanski** (*Columbia University, New York*), **Rudolph E. Tanzi** (*Massachusetts General Hospital, Boston*), **Henry M. Wisniewski** (*Institute for Basic Research in Developmental Disabilities, Staten Island*).

(Boller et al. 1989)

Imaging, cerebral topography and Alzheimer's disease
Lille – October 16, 1989

Scientific Committee: **Didier Leys** (*C.H.R. Lille, Lille*), **Henri Petit** (*CHR Lille, Lille*), **Stanley I. Rapoport** (*National Institutes of Health, Bethesda*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Jean-Claude Baron** (*Inserm U320, Caen*), **David M. Bowen** (*University of London, London*), **Pierre Celsis** (*CHU Purpan, Toulouse*), **Helen Creasey** (*University of Sydney, Concord*), **Jean-Jacques Hauw** (*Hôpital de la Salpêtrière, Paris*), **James V. Haxby** (*National Institutes of Health, Bethesda*), **William Klunk** (*University of Pittsburgh, Pittsburgh*), **Didier Leys** (*CHU Lille, Lille*), **John H. Morrisson** (*Mt Sinaï Neurobiology School of Medicine, NewYork*), **Olaf B. Paulson** (*Rigshospitalet, Copenhagen*), **Stanley I. Rapoport** (*National Institutes of Health, Bethesda*), **Martin N. Rossor** (*Saint Mary's Hospital, London*), **Mark D. Shapiro** (*National Institute on Aging, Bethesda*), **André Syrota** (*CH Orsay, Orsay*).

(Rapoport et al. 1990)

Growth factors and Alzheimer's disease
Strasbourg – April 25, 1990

Scientific Committee: **Philippe Brachet** (*C.H.R.U., Angers*), **Franz Hefti** (*University of Southern California, Los Angeles*), **Bruno Will** (*Centre National de la Recherche Scientifique, Strasbourg*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Albert J. Aguayo** (*Montreal General Hospital, Montreal*), **Ira B. Black** (*The New York Hospital Cornell Medical Center, New York*), **Philippe Brachet** (*C.H.R.U., Angers*), **Moses V. Chao** (*Cornell University Medical College, New York*), **Carl W. Cotman** (*University of California Irvine, Irvine*), **Fred H. Gage** (*University of California San Diego, La Jolla*), **Franz Hefti** (*University of Southern California, Los Angeles*), **Kenneth S. Kosik** (*Brigham & Women's Hospital, Boston*), **Dan Lindholm** (*Max-Planck Institute, Planegg-Martinsriedgg*), **William Mobley** (*University of California San Francisco, San Francisco*), **Lars Olson** (*Karolinska Institute, Stockholm*), **Nikolaos K. Robakis** (*Mt. Sinai School of Medicine, New York*), **Patricia A. Walicke** (*University of California San Diego, La Jolla*), **Bruno Will** (*Centre National de la Recherche Scientifique, Strasbourg*).

(Brachet et al. 1991)

Neurophilosophy and Alzheimer's disease
La Jolla – January 11, 1991

Scientific Committee: **Patricia S. Churchland** (*University of California San Diego, La Jolla*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Nelson Butters** (*Veterans Administration Medical Center, La Jolla*), **Jean-Pierre Changeux** (*Collège de France, Institut Pasteur, Paris*), **Paul Churchland** (*University of California San Diego, La Jolla*), **Patricia Churchland** (*University of California San Diego, La Jolla*), **Antonio R. Damasio** (*University of Iowa College of Medicine, Iowa City*), **Daniel C. Dennett** (*Tufts University, Medford*), **Michael S. Gazzaniga** (*Darmouth Medical School, Hanover*), **Stephen M. Kosslyn** (*Harvard University, Cambridge, USA*), **Mony J. de Leon** (*New York University Medical Center, New York*), **Stanley I. Rapoport** (*National Institutes of Health, Bethesda*), **Sue Savage-Rumbaugh** (*Emory University, Atlanta*), **Jean-Louis Signoret** (*Hôpital de la Salpêtrière, Paris*), **Robert D. Terry** (*University of California San Diego, La Jolla*).

(Christen et al. 1992)

Heterogeneity of Alzheimer's disease
Marseille – April 6, 1992

Scientific Committee: **François Boller** (*Centre Paul Broca, Paris*), **Françoise Forette** (*Hôpital Broca, Paris*), **Zaven S. Khachaturian** (*National Institute on Aging, Bethesda*), **Michel Poncet** (*C.H.U. Timone, Marseille*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Luigi Amaducci** (*University of Florence, Florence*), **Thomas D. Bird** (*Veterans Administration Medical Center, Seattle*), **Helena C. Chui** (*University of Southern California School of*

Medicine, Los Angeles), **Dennis W. Dickson** (*Albert Einstein College of Medicine, New York*), **Charles Duyckaerts** (*Hôpital de la Salpêtrière, Paris*), **Françoise Forette** (*Hôpital Broca, Paris*), **Samuel E. Gandy** (*The Rockefeller University, New York*), **Jordan Grafman** (*National Institutes of Health, Bethesda*), **John Hardy** (*St Mary's Hospital Medical School, London*), **Richard Mayeux** (*Columbia University, New York*), **M.-Marsel Mesulam** (*Beth Israël Hospital, Boston*), **Allen D. Roses** (*Duke University, Durham*), **Martin N. Rossor** (*St Mary's Hospital, London*), **Peter St. George-Hyslop** (*University of Toronto, Toronto*).

(Boller et al. 1992)

The β-amyloid protein precursors in development, aging and Alzheimer's disease
Lyon – June 21, 1993

Scientific Committee: **Konrad Beyreuther** (*University of Heidelberg, Heidelberg*), **Colin Masters** (*University of Melbourne, Melbourne*), **Marc Trillet** (*Hôpital Neurologique, Lyon*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Flint M. Beal** (*Massachusetts General Hospital, Boston*), **Konrad Beyreuther** (*University of Heidelberg, Heidelberg*), **Ashley I. Bush** (*Massachusetts General Hospital, Boston*), **Marie-Christine Chartier-Harlin** (*Inserm U156, Lille*), **Barbara Cordell** (*California Biotechnology Inc., Mountain View*), **Samuel E. Gandy** (*The Rockefeller University, New York*), **Caroline Hilbich** (*Center for Molecular Biology, Heidelberg*), **Gerd Multhaup** (*University of Heidelberg, Heidelberg*), **Donald L. Price** (*The Johns Hopkins University School of Medicine, Baltimore*), **Stanley B. Prusiner** (*University of California San Francisco, San Francisco*), **Tsunao Saitoh** (*Department of Neuroscience School of Medicine, La Jolla*), **Sangram S. Sisodia** (*The Johns Hopkins University School of Medicine, Baltimore*), **David H. Small** (*University of Melbourne, Melbourne*), **Rudolph E. Tanzi** (*Massachusetts General Hospital, Boston*), **Christine Van Broeckhoven** (*University of Antwerp, Antwerp*), **Kalpana White** (*Brandeis University, Waltham*), **Steven G. Younkin** (*Case Western Reserve University, Cleveland*).

(Masters et al. 1993)

Alzheimer's disease: lessons from cell biology
Paris – April 25, 1994

Scientific Committee: **Kenneth S. Kosik** (*Brigham & Women's Hospital, Boston*), **Dennis J. Selkoe** (*Brigham & Women's Hospital, Boston*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Melanie H. Cobb** (*University of Texas Southwestern Medical Center, Dallas*), **Don W. Cleveland** (*Johns Hopkins University School of Medicine, Baltimore*), **Nathaniel Heintz** (*The Rockefeller University, New York*), **Yasuo Ihara** (*University of Tokyo, Tokyo*), **Regis B. Kelly** (*University of California San Francisco, San Francisco*), **Randall L. Kincaid** (*Human Genome Sciences Inc., Rockville*), **Kenneth S. Kosik** (*Brigham & Women's Hospital, Boston*), **Eckhard-M. Mandelkow** (*Max-Planck Gesellschaft, Hamburg*), **Jean Mariani** (*Université Pierre & Marie Curie, Paris*), **Ira Mellman** (*Yale University School of Medicine, New Haven*), **Suzannne R. Pfeffer** (*Stanford University School of Medicine, Stanford*), **Dennis J. Selkoe** (*Brigham and Women's Hospital, Boston*).

(Kosik et al. 1994)

Apolipoprotein E and Alzheimer's disease
Paris – May 29, 1995

Scientific Committee: **Allen D. Roses** (*Duke University, Durham*), **Karl Weisgraber** (*University of California San Francisco, San Francisco*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Blas Frangione** (*New York University Medical Center, New York*), **Michel Goedert** (*MRC Laboratory of Molecular Biology, Cambridge, U.K.*), **Bradley Hyman** (*Massachusetts General Hospital, Boston*), **Kazuhiko Ikeda** (*Tokyo Institute of Psychiatry, Tokyo*), **Robert Malhey** (*University*

of California San Francisco, San Francisco), **Eckhard-M. Mandelkow** (*Max-Planck Gesellschaft, Hamburg*), **Eliezer Masliah** (*University of California San Diego School of Medicine, La Jolla*), **Richard Mayeux** (*Neurological Institute, New York*), **Margareth Pericak-Vance** (*Duke University, Durham*), **Judes Poirier** (*McGill University, Montréal*), **Allen D. Roses** (*Duke University, Durham*), **Annie Saunders** (*Duke University, Durham*), **Donald Schmechel** (*Duke University, Durham*), **Gérard Siest** (*Centre de Médecine Préventive, Nancy*), **Warren Strittmatter** (*Duke University, Durham*), **Karl Weisgraber** (*University of California San Francisco, San Francisco*).

(Roses et al. 1995)

Connections, cognition and Alzheimer's disease
Paris – May 20, 1996

Scientific Committee: **Charles Duyckaerts** (*Hôpital de la Salpêtrière, Paris*), **Bradley Hyman** (*Massachusetts General Hospital, Boston*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Heiko Braak** (*JW Goethe University, Frankfort*), **Antonio R. Damasio** (*University of Iowa College of Medicine, Iowa City*), **Charles Duyckaerts** (*Hôpital de la Salpêtrière, Paris*), **Dora Games** (*Athena Neuroscience Inc., South San Francisco*), **James Haxby** (*National Institutes of Health, Bethesda*), **Patrick Hof** (*Mt. Sinai School of Medicine, New York*), **Bradley Hyman** (*Massachusetts General Hospital, Boston*), **Eliezer Masliah** (*University of California San Diego School of Medicine, La Jolla*), **Dick Swaab** (*Netherlands Institute for Brain Research, Amsterdam*), **Gary Van Hoesen** (*University of Iowa, Iowa City*), **Patrick Vermersch** (*C.H.R.U., Lille*), **Mark West** (*University of Aarhus, Aarhus*).

(Hyman et al. 1997)

Presenilins and Alzheimer's disease
Paris – April 28, 1997

Scientific Committee: **Rudolph Tanzi** (*Massachusetts General Hospital, Charlestown*), **Steven Younkin** (*Mayo Clinic, Jacksonville*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Konrad Beyreuther** (*University of Heidelberg, Heidelberg*), **Karen E.K. Duff** (*Mayo Clinic, Jacksonville*), **Christian Haass** (*Central Institute for Mental Health, Mannheim*), **John Hardy** (*Mayo Clinic, Jacksonville*), **Roger Nitsch** (*University of Hamburg*), **Dennis J. Selkoe** (*Center for Neurological Diseases, Boston*), **Sangram Sisodia** (*The Johns Hopkins University School of Medicine, Baltimore*), **Peter St George-Hyslop** (*University of Toronto*), **Rudolph Tanzi** (*Massachusetts General Hospital, Charlestown*), **Wilma Wasco** (*Massachusetts General Hospital, Charlestown*), **Bruce Yankner** (*Childrens Hospital, Boston*), **Steven Younkin** (*Mayo Clinic, Jacksonville*).

(Tanzi et al. 1998)

Epidemiology of Alzheimer's disease: from gene to prevention
Paris – May 25, 1998

Scientific Committee: **Richard Mayeux** (*Columbia University, New York*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Carol Brayne** (*University of Cambridge*), **Monique Breteler** (*Erasmus University Medical School, Rotterdam*), **Jean-François Dartigues** (*Université V. Segalen Bordeaux II*), **Denis A. Evans** (*Rush-Presbyterian St Lukes Medical Center, Chicago*), **Victor Henderson** (*University Southern California School of Medicine, Los Angeles*), **Hugh Hendrie** (*Indiana University, Indianapolis*), **Albert Hofman** (*Erasmus University Medical School, Rotterdam*), **Robert Katzman** (*University of California San Francisco, La Jolla*), **Claudia Kawas** (*The Johns Hopkins School of Medicine, Baltimore*), **Richard Mayeux** (*Columbia University , New York*), **Terry Radebaugh** (*Khachaturian,*

Radebaugh & Associates Inc., Potomac), **Ingmar Skoog** (*Sahlgrenska Hospital, Gothenburg*), **Cornelia M. Van Duijn** (*Erasmus University Medical School, Rotterdam*).

(Mayeux et al. 1999)

Fatal attractions within neurons: intracytoplasmic protein aggregates in Alzheimer's disease and related neurodegenerative diseases
Paris – April 12, 1999

Scientific Committee: **Luc Buée** (*Inserm U422, Lille*), **Virginia M.Y. Lee** (*University of Pennsylvania, Philadelphia*), **John Trojanowski** (*University of Pennsylvania, Philadelphia*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Konrad Beyreuther** (*University of Heidelberg, Heidelberg*), **Luc Buée** (*Inserm U422, Lille*), **Michel Goedert** (*MRC Laboratory of Molecular Biology, Cambridge*), **Mike Hutton** (*Mayo Clinic, Jacksonville*), **Takeshi Iwatsubo** (*University of Tokyo*), **Peter Lansbury** (*Brigham and Women's Hospital, Boston*), **Virginia M.Y. Lee** (*University of Pennsylvania, Philadelphia*), **Eva-M. Mandelkow** (*Max-Planck-Gesellschaft, Hamburg*), **Robert Nussbaum** (*National Insitutes of Health, Bethesda*), **Gerard D. Schellenberg** (*Puget Sound Health Care System, Seattle*), **John Trojanowski** (*University of Pennsylvania, Philadelphia*), **Kirk Wilhelmsen** (*The Gallo Clinic and Research Center, San Francisco*).

(Lee et al. 2000)

Neurodegenerative diseases: loss of function through gain of function
Paris – February 28, 2000

Scientific Committee: **Konrad Beyreuther** (*University of Heidelberg, Heidelberg*), **Colin L. Masters** (*University of Melbourne, Melbourne*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Flint M. Beal** (*Cornell University Medical College, Boston*), **Konrad Beyreuther** (*University of Heidelberg, Heidelberg*), **Don Cleveland** (*University of California, San Diego*), **Bart De Strooper** (*Center for Human Genetics, Leuven*), **Joachin Herz** (*University of Texas Southwestern Medical Center, Dallas*), **Mathias Jucker** (*University of Basel, Basel*), **Raphael Kopan** (*Washington University, St Louis*), **Vishwanath Lingappa** (*University of California, San Francisco*), **Jean-Louis Mandel** (*I.G.B.M.C., Illkirch*), **Colin L. Masters** (*University of Melbourne, Melbourne*), **Donald L. Price** (*Johns Hopkins University, Baltimore*), **Alain Prochiantz** (*UMR 8542, Paris*), **Stanley B. Prusiner** (*University of California, San Francisco*), **Kalpana White** (*Brandeis University, Waltham*).

(Beyreuther et al. 2001)

Notch from neurodevelopment to neurodegeneration: keeping the fate
Paris – March 19, 2001

Scientific Committee: **Frédéric Checler** (*URA 411, Valbonne*), **Bart De Strooper** (*Center for Human Genetics, Leuven*), **Alain Israël** (*Institut Pasteur, Paris*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Wim Annaert** (*Center for Human Genetics, Leuven*), **Frédéric Checler** (*URA 411, Valbonne*), **Luciano D'Adamio** (*Albert Einstein College in Medicine, Bronx*), **Bart De Strooper** (*Center for Human Genetics, Leuven*), **Christian Haass** (*Ludwig Maximilians University, Munich*), **Alain Israël** (*Institut Pasteur, Paris*), **Anne Joutel** (*Hôpital Lariboisière, Paris*), **Edward Koo** (*University of California, La Jolla*), **Kenneth S. Kosik** (*Brigham and Women's Hospital, Boston*), **Pasko Rakic** (*Yale University School of Medicine, New Haven*), **Robert B. Rawson** (*University of Texas Southwestern Medical Center, Dallas*), **Peter St George Hyslop** (*Center for Research in Neurodegenerative Diseases, Toronto*), **Elisabeth Tournier-Lasserve** (*Hôpital Lariboisière, Paris*), **Michael**

S. Wolfe (*Brigham and Women's Hospital, Boston*).

(Checler et al. 2002)

Immunization against Alzheimer's disease and other neurodegenerative disorders
Paris – March 13, 2002

Scientific Committee: **Dennis J. Selkoe** (*Brigham and Women's Hospital, Boston*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Fredrika Bard** (*Elan Pharmaceuticals, South San Francisco*), **David Holtzman** (*Washington University Medical Center, Saint Louis*), **Bradley Hyman** (*Massachusetts General Hospital, Boston*), **Cynthia Lemere** (*Brigham and Women's Hospital, Boston*), **David Peretz** (*Institute of Neurodegenerative Diseases, San Francisco*), **Dale Schenk** (*Elan Pharmaceuticals, South San Francisco*), **Dennis J. Selkoe** (*Brigham and Women's Hospital, Boston*), **Bekka Solomon** (*Tel-Aviv University, Tel-Aviv*), **Kenneth E. Ugen** (*University of South Florida, Tampa*), **Howard Weiner** (*Brigham and Women's Hospital, Boston*), **David Westaway** (*Center for Research in Neurodegenerative Diseases, Toronto*).

(Selkoe and Christen 2003)

The living brain and Alzheimer's disease
Paris – March 17, 2003

Scientific Committee: **Bradley Hyman** (*Massachusetts General Hospital, Boston*), **Jean-François Demonet** (*Hôpital Purpan, Toulouse*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Brian Bacskai** (*Massachusetts General Hospital, Charlestown*), **Jean-Claude Baron** (*University of Cambridge, Cambridge, UK – Inserm, Caen*), **Charles Duyckaerts** (*Hôpital de la Salpêtrière, Paris*), **Henry Engler** (*Uppsala University, PET Centre, Uppsala*), **Nick Fox** (*National Hospital for Neurology/Neurosurgery, London*), **Bradley Hyman** (*Massachusetts General Hospital, Boston*), **William Klunk** (*University of Pittsburgh, Pittsburgh*), **Andreas Papassotiropoulos** (*University of Zurich, Zurich*), **Christoph Hock** (*University of Zurich, Zurich*), **Eric Reiman** (*University of Arizona, Phoenix*), **Paul Thompson** (*University of California Los Angeles School of Medicine, Los Angeles*), **Thomas Wisniewski** (*NYU School of Medicine, New York*).

(Hyman et al. 2004)

Genotype – Proteotype – Phenotype correlations in dementia
Paris, September 13, 2004

Scientific Committee: **Jeffrey Cummings** (*University of California, Los Angeles*), **John Hardy** (*National Institutes of Health, Bethesda*), **Michel Poncet** (*CHU de Marseille*), **Yves Christen** (*Fondation Ipsen, Paris*).

Speakers: **Alexis Brice** (*Hôpital de la Salpêtrière, Paris*), **John Collinge** (*The National Hospital for Neurology and Neurosurgery, London*), **Jeffrey Cummings** (*University of California, Los Angeles*), **Norman Foster** (*University of Michigan, Ann Harbor*), **Doug Galasko** (*University of California San Diego, San Diego*), **Alison Goate** (*Washington University School of Medicine, Saint Louis*), **Neill Graff-Radford** (*Mayo Clinic, Jacksonville*), **Katrina Gwinn-Hardy** (*National Institutes of Health, Bethesda*), **John Hardy** (*National Institutes of Health, Bethesda*), **Virginia Lee** (*Center for Neurodegenerative Disease Research, Philadelphia*), **Domenico Pratico** (*University of Pennsylvania, Philadelphia*), **Peter Schofield** (*Garvan Institute of Medical Research, Sydney*), **Christine Van Broeckhoven** (*University of Antwerp, Antwerp*).

(Cummings et al. 2005)

June Kinoshita

Gabrielle Strobel

Alzheimer Research Forum: A Knowledge Base and e-Community for AD Research

June Kinoshita[1] and *Gabrielle Strobel*[1]

Introduction

This centenary year of Alois Alzheimer's seminal study also marks the tenth anniversary of the Alzheimer Research Forum website (www.alzforum.org), known popularly as "Alzforum" by Alzheimer's Disease (AD) researchers around the world. The Alzforum is an independent, not-for-profit resource dedicated to Alzheimer's disease and related disorders. Founded in 1996, the website has become a true global online community, with approximately 30–50% of all active Alzheimer researchers visiting it regularly.

From its humble beginnings as a small set of HTML pages featuring manually curated lists of articles, the Alzforum has grown tremendously. Today, the website contains more than 40,000 literature citations, 1,500 research news articles, 4,000 comments, 10,000 antibodies, 200 research models, 350 genes from published association studies of late-onset AD, as well as all known mutations causing familial AD and frontotemporal dementia with parkinsonism (FTDP-17). The site receives over one million visits per year. More than 3,400 people have registered as members of Alzforum, representing a significant portion of AD researchers worldwide.

Although it is difficult to gauge the precise impact of Alzforum, many researchers have told us that we have succeeded to a substantial degree in opening our readers' minds to more diverse areas of research, fostering healthy discussion of novel and controversial topics, and serving as a central repository for core data sets such as genetic association studies, research models, clinical trials and antibodies.

While the Alzforum today enjoys a high profile in the Alzheimer field, few people are familiar with the website's background or inner workings. On the occasion of the Alzheimer 100 conference, we would like to share some stories about how the Alzforum began and developed over the years and to present our vision of some future initiatives.

Origins of the Alzforum

The idea for the Alzheimer Research Forum website was spawned over a lunch conversation between one of the authors (Kinoshita), a philanthropist, and a foundation leader who were concerned about barriers to progress in Alzheimer research. The time was late 1995, and the AD field was caricatured as a battle zone between "BAPtists" and "TAUists." At the same time, research was exploding with new findings driven by the landmark discoveries a few years earlier of familial Alzheimer genes (Goate et

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Jucker et al.
Alzheimer: 100 Years and Beyond

al. 1991, Schellenberg et al. 1992, St. George-Hyslop et al., 1992) and the association between late-onset AD and the epsilon4 allele of apolipoprotein E (Strittmatter et al. 1993). It seemed that AD research might benefit from more open dialogue and better management of the diverse streams of data pouring out of laboratories.

The Internet was not yet a household word, but the philanthropist had seen this technology begin to transform business communications and was convinced that the World Wide Web would quickly become a ubiquitous tool for scientific and medical research. The participants in the lunch believed that a Web-based information source, with expert editorial moderation and professional management, would fill a real need in the community. An anonymous foundation stepped forward to fund the concept and assemble the project team, and in July of 1996, the website made its debut at the International Conference on Alzheimer's Disease and Related Disorders in Osaka, Japan.

During the planning phase, the team considered whether the website should be hosted at an academic institution, but initial research indicated that individual and institutional rivalries could undermine the goal of creating an open community resource. The Alzforum was therefore established as an independent, not-for-profit entity.

Without an institutional imprimatur, the Alzforum faced the challenge of establishing its credibility within the scientific community. The editor invited prominent leaders with diverse scientific backgrounds to join the scientific advisory board. Happily, most agreed at once. (The founding members were Eva Braak, Joseph Coyle, Peter Davies, Bradley Hyman, Gerald Fischbach, Zaven Khachaturian, Kenneth Kosik, Virginia Lee, Elliott Mufson, Donald Price, John Olney, and Robert Terry.) The composition of the board was intended to signal that the site is dedicated to high scientific standards, that it is neutral with regard to hypothesis or dogma, and that perspectives from multiple disciplines are welcome.

At its launch, the Alzforum featured a "Papers of the Week" list of peer-reviewed publications in the AD field, virtual seminars (slide and audio), and a Milestone Papers list of seminal publications dating back to Alois Alzheimer's original paper, including an English translation by Katherine Bick, describing the case of Auguste Deter (Alzheimer 1907). Although the initial Alzforum offerings seem modest in retrospect, the feedback was positive. By the end of its first year, the site had 1,200 registered members.

A New Niche for Scientific Discourse

Having established a foothold in cyberspace, the challenge for Alzforum was and continues to be to define new types of scientific publishing that take advantage of the speed and wide distribution of the Web and to curate and add value to information available from other public sources. This is a perennial challenge, thanks to the rapid advances in biomedical resources on the Web.

This uphill struggle, however, seems less strenuous when we compare the current situation with the "old days." Recall that in 1996, PubMed did not exist! (PubMed was launched in June of 1997.) Medical institutions had access to Medline, but in order for Alzforum to produce its Papers of the Week listings, the editor had to ask the Countway Medical Library at Harvard Medical School to provide weekly text files listing newly indexed AD papers. The Alzforum hired a curator to paraphrase each

abstract so that this information could be posted without violating journal copyrights. These documents were manually edited, sent out in a weekly email to the advisors for comments, and compiled into a static HTML page. Looking back, we can see that the entire process seems as antiquated as the hand-copying of manuscripts in the Middle Ages.

Soon after launching Alzforum, we found that scientists were eager to experiment with the informal, rapid communication made possible by the Web. We began to host live chats and post comments on recently published papers. We were also seeking a project to demonstrate how the Alzforum could serve as a community repository.

The first opportunity came in 1997 when geneticists John Hardy and Richard Crook, both then at the Mayo Clinic in Jacksonville, Florida, approached Alzforum about posting a comprehensive list of familial AD mutations in the genes for amyloid precursor protein (APP), presenilin 1 and presenilin 2 (Hardy and Crook updated 2001, Alzform updated 2006). Jennifer Kwon at Washington University in St. Louis volunteered to curate a list of FTDP-17 tau mutations and Michael Hutton of the Mayo Clinic Jacksonville helped to edit and update this list (Kwon updated 2006). These resources were well received. Scientists contacted Alzforum for permission to reproduce the diagrams in their lectures and papers. "These tables and reviews are incredibly useful for people like me, who don't closely follow the genetics field," observed one well-known AD biochemist.

Another way that the Alzforum adds value is by integrating information. Editors link primary research articles to related news, papers, databases, discussions and so on. In the early years, we were severely limited in this regard, because we had to program each link by hand. This changed dramatically in the year 2000, when the Alzforum converted to a data-driven, dynamic system. The new system automatically searches and downloads PubMed citations into a database every night and provides tools to let editors post news and comments and crosslink them to related material.

In addition, we made sure that the new server could be maintained entirely by Alzforum editors and programmers, without an intermediary company to make changes. This flexibility is essential to the Alzforum's ability to develop new ways of providing value-added services and to keep up with the rapidly evolving landscape of Web resources for scientific research.

Most importantly, perhaps, we overhauled the look and feel of the site, from a staid (some said funereal) look to a bright, colorful style that highlights the richness of content and provides modern navigational tools to help readers wend their way through the ever-expanding menu of offerings.

The Alzforum in 2006

A guiding principle of the Alzforum homepage is that the site should be "the daily tabloid for AD research," one that AD researchers would want as their personal homepage. To keep our readers coming back, we work hard to keep the homepage dynamic, useful and entertaining. Almost daily, the readers will find something of interest: the latest news, a live discussion, conference reports, commentaries, grant and job postings, new genes and mouse models, and so on.

The Alzforum team strives to make the website an essential resource for scientists by adding value to information that is already available in the public domain. How we pursue this goal is illustrated by specific examples of our major content areas.

Papers of the Week

Many AD researchers keep up with the literature by browsing the Papers of the Week, because it provides a high-quality list of articles about AD, related disorders, key genes, relevant developments from broad areas of basic research, and advances in technology – a list that would require multiple searches on PubMed. The "POW" citations are enriched with news stories and commentaries, as well as links to related articles. High-impact articles are designated as "ARF Recommended" papers and "Milestones." For many scientists, the real-time reaction by peers supplies context that is missing from traditional journal publications.

Papers of the Week is essential for the knowledge management role of the Alzforum, and it drives much of the content development on the site. Editors screen the citations for news and for data to send to curators of the AlzGene database, Telemakus AD biomarkers database, mutations directory, research models database, antibody database, and so forth. The scientific advisory board annotates new citations on a weekly cycle. Thus, the "firehose" of PubMed citations is channeled into multiple streams and helps ensure that the Alzforum's information resources are up-to-date.

Research News

Our news operation has been directed by Gabrielle Strobel since 2001. It has become one of the most important ways in which Alzforum delivers value, by providing reporting and analysis of news of broad relevance to AD research composed by journalists with extensive knowledge of the field. Our writers aim to place new findings in the context of other research. With their detailed conference coverage, they have mastered the art of informing the field of new developments many months ahead of formal publication to accelerate the spread of new ideas, without interfering with formal publication. They also scout for discoveries and methods from other fields that could be useful to AD research, conduct interviews with thought leaders, and prepare the background texts for discussion forums.

Commentaries and Discussion Forums

The Alzforum provides scientists with a forum to respond quickly and publicly to new findings. Readers can post commentaries on any Papers of the Week citation or news story via a "Vote/Submit Comment" text-entry box. Every week, Alzforum editors invite individual scientists to comment on news or journal articles. At 75% or better, the response rate is high, and scientists pay close attention to what is being discussed (especially about their own work!).

Over the years, many scientists have remarked on how effective the Alzforum has been in nurturing productive discussion of their ideas and findings. For example, in

2005 Vincent Marchesi, a cell biologist at Yale University, published an alternative interpretation of the amyloid hypothesis that might ordinarily have been quietly ignored by most AD researchers (Marchesi 2005). Instead, when Alzforum featured the paper, 17 scientists posted lengthy, detailed and productive commentaries. "The postings on the Alzforum site regarding my PNAS paper have been incredibly rewarding for me, and I suspect, for many of the others that participated," wrote Marchesi. "I don't see how so many candid exchanges could have taken place any other way." In the spring of 2006, the Alzforum invited public discussion on the difficult question of how presenilin mutations cause Alzheimer's disease a key issue not only in understanding pathogenesis but also in drug development. Challenging a comfortable but simplistic dogma, the debate drew 24 thoughtful comments from highly regarded scientists that together laid out the subtleties of the current state of knowledge.

Compendia and Databases

Data about key findings and reagents are curated into databases designed by the Alzforum. These data are published in disparate articles and formats, and scientists expend much time and effort to keep up-to-date. Because there is little incentive for individuals to carry out and share this task on behalf of the scientific community, the Alzforum considers the development and upkeep of open databases to be a high priority. Data sets on the Alzforum include the following:

- Familial AD mutations. All published mutations in the amyloid precursor protein (APP), presenilin-1 and presenilin-2, as well as tau mutations that cause frontotemporal dementia with parkinsonism (FTDP-17). Individual mutations are displayed in a table along with clinical, pathology and biochemical data and primary publications. (http://www.alzforum.org/res/com/mut/default.asp)
- AlzGene. All published genetic association studies for late-onset AD, conceived and curated by Lars Bertram and his colleagues at Massachusetts General Hospital. The database can be browsed by chromosome or searched by gene, polymorphism, protein, keyword or author. Each gene is summarized in a table listing details of all published studies, and a meta-analysis of the findings can be calculated with a single click. (http://www.alzforum.org/res/com/gen/alzgene/default.asp)
- Antibodies. More than 10,000 antibodies to proteins that are widely studied by AD researchers have been entered into this database. The database includes noncommercial and commercial antibodies, and displays the data in a table summarizing points of interest to researchers. (http://www.alzforum.org/res/com/ant/default.asp)
- Drugs in Clinical Trials. This database contains all drugs that we have confirmed to have entered Phase 2 clinical trials and beyond, including drugs that were discontinued following clinical trials. We are planning to re-design this database to include preclinical compounds and additional data of value to preclinical researchers. (http://www.alzforum.org/drg/drc/default.asp)

Who Are Our Members, and What Are They Saying?

The Alzforum is freely accessible to the public, so we do not have statistics on our entire user population. However, more than 3,400 individuals have registered as members,

of whom more than 2,000 have also filled out the "researcher profile" form. Thus, we assume a lower limit of 2,000 on scientists and clinicians who use the site and estimate that around the same number are using the site without registering. This implies that 30–50% of the global community of AD researchers are regular visitors.

Feedback has been strongly positive. Many of our scientific advisory board members (all very busy researchers and clinicians) visit the website around one to three times per week. "[Alzforum] is the local newspaper for Alzheimer research," writes John Hardy, Director of the Laboratory of Neurogenetics at the National Institutes of Aging. "I visit it one to two times a week just to see what's going on … to check up on recent papers, … to see who's hiring people and so on. I read people's comments on papers, and I go from there to PubMed for anything I've missed. I think pretty much everyone in the field uses it in the same way, and I have often seen my informal reviews on the site cited."

Scientists mention a variety of reasons why they find the Alzforum valuable. One is that the Alzforum enriches published papers with news analysis and rapid peer commentary. "This is the major e-forum for AD ideas," observes Jeffrey Cummings, of the University of California in Los Angeles. "The discussion forums have shaped and sharpened my ideas. It's a great way to get a grasp of the literature and to follow emerging events in real time."

Many researchers value the breadth of the Alzforum's coverage, which is intended to communicate diverse developments with which no specialist could possibly keep up. "Instead of relying only on published papers and meetings, you provide rapid insights into new developments and introduce us to areas that are related to our work but yet we fail to notice were it not for you," writes Gunnar Gouras of Weill Medical College of Cornell University. The databases also are frequently mentioned as resources that help scientists stay abreast of advances in fields outside their own.

Another important aspect of Alzforum is its community-building function. Through commentaries and live discussion forums, the website provides a neutral ground for scientists to get comfortable with one another. Scientists are directly involved in creating resources on Alzforum, volunteering significant time to report on meetings, propose and lead discussions, consult on databases and offer unvarnished feedback. To all of them, we are deeply indebted.

Future Directions

A constant challenge for the Alzforum is to find new ways to apply information technology to significant problems in AD research. The most ambitious effort to date is the SWAN (Semantic Web Applications in Neuromedicine), a collaboration between Alzforum and Massachusetts General Hospital (Gao et al. 2006).

One of the key drivers behind SWAN is the realization that the Alzforum, for all its content and community activity, still is little more than collections of documents and data with some links. This information is not embedded in a knowledge model. Rather, the human user carries the knowledge model in his or her head. When a person reads a paper or follows a link, he or she fills in the contextual blanks, such as "this paper challenges hypothesis X," or "So-and-so draws the opposite conclusion from this data." With SWAN, we will provide scientists with a tool to embed their documents, data and

other digital materials in a knowledge model and then to share the entire model with other scientists and communities, who can then build upon it.

Another key concept informing the design of SWAN is that scientific ideas, documents, data and other materials evolve within a "scientific ecosystem." SWAN will incorporate the full biomedical research life cycle in its ontological model, including support for personal data organization, hypothesis generation, experimentation, lab data organization, and digital pre-publication collaboration. Community, laboratory, and personal digital resources may all be organized, interconnected and shared using SWAN's common semantic framework.

Individuals will use a version called "MySWAN" as a personal tool to find and organize information, extend their knowledge, motivate discoveries and form and test hypotheses. At the community level, the same software and the same ontological framework can be used to organize and curate the research of a laboratory or of an entire research community (such as the Alzforum). Therefore, elements of the personal SWAN can be shared with the community at a low incremental effort in curation. What's more, community SWAN contents may be shared back with individuals and re-used in new contexts.

The SWAN content will develop through the type of partnership that already exists between the Alzforum editors and the AD community, with editors laying the groundwork and inviting community members to contribute. We look forward to rolling out this next generation of Web technology for AD research in the coming years.

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The Potamkin prize for research in Pick's, Alzheimer's and related diseases

According to the American Academy of Neurology, the Potamkin Prize is regarded by many as the "Nobel Prize of dementia research". It has become the bellwether for progress in international research into Alzheimer's and related diseases. The Potamkin family worked with leading neurologists to create this $100 000 annual award sponsored by the American Academy Neurology. The first Potamkin prize was established in 1988 at a time when very little was known about what happened in the brain during the course of Alzheimer's disease, not to mention its causes and cure.

Since them, many prominent scientists have been awarded this prestigious annual prize in recognition of their contribution to the understanding of the disease.

Year	Recipient name, recipient institution
1988	Robert D. Terry, MD (University of California, San Diego, CA)
1989	Dennis Selkoe, MD (Harvard Medical School, Boston, MA)
	George G. Glenner MD (University of California, San Diego, CA)
1990	Colin Masters, MD (University of Melbourne, Australia)
	Konrad Beyreuther, PhD (University of Heidelberg, Germany)
1991	Stanley Prusiner, MD (University of California, San Francisco, CA)
1992	Donald L. Price, MD (Johns Hopkins University School of Medicine, Baltimore, MD)
	Robert Katzman, MD (University of California, San Diego, CA)
1993	Blas Frangione, MD, PhD (New York University Medical Center, New York, NY)
	Alison Goate, PhD (Washington University School of Medicine, St. Louis, MO)
	John Hardy, PhD (University of South Florida, Tampa, FL)
	Christine Van Broeckhoven, PhD (University of Antwerp - Belgium, Germany)
1994	Allen D. Roses, MD (Duke University Medical Center, Durham, NC)
	Gerard D. Schellenberg, PhD (University of Washington, Seattle, WA)
1995	Steven G. Younkin, MD, PhD (Case Western Reserve Univ. School of Med., Cleveland, OH)
	Khalid Iqbal, PhD (Inst. for Basic Research in Develop. Disabil., Staten Island, NY)
	Yasuo Ihara, MD (Univ of Tokyo School of Medicine, Tokyo, Japan)
1996	Rudolph Tanzi, PhD (Massachusetts General Hospital, Charlestown, MA)
	Peter St. George-Hyslop, MD (University of Toronto, Toronto, Ontario, Canada)
1997	Sangram S. Sisodia, PhD (The Johns Hopkins University School of Medicine, Baltimore, MD)
	Elio Lugaresi, MD (Bologna, Italy)

Pierluigi Gambetti, MD (Institute of Pathology, Cleveland, OH)

1998 Michel Goedert, PhD (Laboratory of Molecular Biology Hills Road, Cambridge, England)
Virginia Man-Yee Lee, PhD (University of Pennsylvania School of Medicine, Philadelphia, PA)
John Q. Trojanowski, MD, PhD (University of Pennsylvania School of Medicine, Philadelphia, PA)

1999 Arne Brun, MD, PhD (Dept. of Pathology, University Hospital, Lund, Sweden)
Kirk Wilhelmsen, MD, PhD (Dept. of Neurology, University of California, San Francisco, CA)
Bernardino Ghetti, MD (Dept. of Pathology & Lab Medicine, Indiana; University School of Medicine, Indianapolis, IN)

2000 Maria Grazia Spillantini, PhD (University of Cambridge, Cambridge, UK)
Michael Hutton, PhD (Mayo Clinic, Jacksonville, FL)

2001 Dale Schenk, PhD (Elan Pharmaceuticals, San Francisco, CA)

2002 Christian Haass, PhD (Adolf Butenandt Institute, Department of Biochemistry, Ludwig Maximilians University, Munich, Germany)
Bart De Strooper, MD, PhD (K.U. Leuven and Flanders Interuniversity Institute for Biotechnology, Leuven, Belgium)

2003 David M. Holtzman, MD (Washington University School of Medicine, St. Louis, MO)
Ashley I. Bush, MD, PhD (Harvard Medical School, Boston, MA)

2004 Leon J. Thal, MD (University of California San Diego School of Medicine, La Jolla, USA)
Roger M. Nitsch, MD (University of Zurich, Zurich, Switzerland)

2005 John C. Morris, MD (Washington University School of Medicine, Saint Louis, MO)
Ronald C. Petersen, PhD, MD (Mayo Alzheimer's Disease Research Center, Rochester, MN)

2006 Bradley Hyman, MD, PhD (Massachusetts General Hospital, Boston, MA)
Karen Ashe, MD, PhD (University of Minnesota Medical School, Minneapolis, MN)
Karen Duff, PhD (New York University, New York, NY)

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