

BRAIN DISEASE

6. D. G. Glaze, J. D. Frost Jr., H. Y. Zoghbi, A. K. Percy, *Arch. Neurol.* **44**, 1053 (1987).
7. A. L. Reiss et al., *Ann. Neurol.* **34**, 227 (1993).
8. J. B. Nielsen, L. Friberg, H. Lou, N. A. Lassen, I. L. Sam, *Arch. Neurol.* **47**, 982 (1990).
9. M. L. Bauman, T. L. Kemper, D. M. Arin, *Neuropediatrics* **26**, 105 (1995).
10. J. Armstrong, M. Pineda, E. Aibar, E. Gean, E. Monros, *Ann. Neurol.* **50**, 692 (2001).
11. P. V. Belichenko, A. Oldfors, B. Hagberg, A. Dahlstrom, *Neuroreport* **5**, 1509 (1994).
12. K. Jellinger, F. Seitelberger, *Am. J. Med. Genet. Suppl.* **1**, 259 (1986).
13. D. D. Armstrong et al., *Pediatr. Neurol.* **20**, 125 (1999).
14. R. E. Amir et al., *Nature Genet.* **23**, 185 (1999).
15. M. Wan et al., *Am. J. Hum. Genet.* **65**, 1520 (1999).
16. R. E. Amir et al., *Ann. Neurol.* **47**, 670 (2000).
17. T. Biennu et al., *Hum. Mol. Genet.* **9**, 1377 (2000).
18. L. Villard et al., *Neurology* **55**, 1188 (2000).
19. K. Hoffbuhr et al., *Neurology* **56**, 1486 (2001).
20. R. M. Carney et al., *Pediatr. Neurol.* **28**, 205 (2003).
21. A. Orrico et al., *FEBS Lett.* **481**, 285 (2000).
22. I. Meloni et al., *Am. J. Hum. Genet.* **67**, 982 (2000).
23. P. Couvert et al., *Hum. Mol. Genet.* **10**, 941 (2001).
24. S. M. Klauk et al., *Am. J. Hum. Genet.* **70**, 1034 (2002).
25. B. Winnepenninckx, V. Errijgers, F. Hayez-Delatte, E. Reyniers, R. Frank Kooy, *Hum. Mutat.* **20**, 249 (2002).
26. D. Cohen et al., *Am. J. Psychiatry* **159**, 148 (2002).
27. B. Hendrich, S. Tweedie, *Trends Genet.* **19**, 269 (2003).
28. A. Bird, *Genes Dev.* **16**, 6 (2002).
29. F. Fuks et al., *J. Biol. Chem.* **278**, 4035 (2003).
30. J. M. LaSalle, J. Goldstine, D. Balmer, C. M. Greco, *Hum. Mol. Genet.* **10**, 1729 (2001).
31. M. D. Shahbazian, B. Antalffy, D. L. Armstrong, H. Y. Zoghbi, *Hum. Mol. Genet.* **11**, 115 (2002).
32. S. Akbarian et al., *Neurobiol. Dis.* **8**, 784 (2001).
33. D. Balmer, J. Goldstine, Y. M. Rao, J. M. LaSalle, *J. Mol. Med.* **81**, 61 (2003).
34. B. P. Jung et al., *J. Neurobiol.* **55**, 86 (2003).
35. C. Colantuoni et al., *Neurobiol. Dis.* **8**, 847 (2001).
36. R. Z. Chen, S. Akbarian, M. Tudor, R. Jaenisch, *Nature Genet.* **27**, 327 (2001).
37. J. Guy, B. Hendrich, M. Holmes, J. E. Martin, A. Bird, *Nature Genet.* **27**, 322 (2001).
38. M. Tudor, S. Akbarian, R. Z. Chen, R. Jaenisch, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 15536 (2002).
39. M. Shahbazian et al., *Neuron* **35**, 243 (2002).
40. I. Stancheva, A. L. Collins, I. B. Van den Veyver, H. Y. Zoghbi, R. R. Meehan, *Mol. Cell.* **2**, 425 (2003).
41. L. Kanner, *Nervous Child* **2**, 217 (1943).
42. A. Bailey et al., *Lancet* **341**, 1225 (1993).
43. A. Y. Hardan, N. J. Minshew, M. Mallikarjunn, M. S. Keshavan, *J. Child Neurol.* **16**, 421 (2001).
44. M. R. Herbert et al., *Brain* **126**, 1182 (2003).
45. E. H. Aylward et al., *Neurology* **53**, 2145 (1999).
46. E. Courchesne, R. Carper, N. Akshoomoff, *JAMA* **290**, 337 (2003).
47. B. Luna et al., *Neurology* **59**, 834 (2002).
48. U. Frith, *Neuron* **32**, 969 (2001).
49. T. L. Kemper, M. Bauman, *J. Neuropathol. Exp. Neurol.* **57**, 645 (1998).
50. G. V. Raymond, M. L. Bauman, T. L. Kemper, *Acta Neuropathol. (Berlin)* **91**, 117 (1996).
51. A. L. Beaudet, *Genet. Med.* **4**, 399 (2002).
52. S. Folstein, M. Rutter, *J. Child Psychol. Psych.* **18**, 297 (1977).
53. A. Bailey et al., *Psychol. Med.* **25**, 63 (1995).
54. S. Steffenburg et al., *J. Child Psychol. Psych.* **30**, 405 (1989).
55. S. E. Folstein, B. Rosen-Sheidley, *Nature Rev. Genet.* **2**, 943 (2001).
56. A. Pickles et al., *Am. J. Hum. Genet.* **57**, 717 (1995).
57. P. Baker, J. Piven, S. Schwartz, S. Patil, *J. Autism Dev. Disord.* **24**, 529 (1994).
58. S. D. Cheng, N. B. Spinner, E. H. Zackai, J. H. Knoll, *Am. J. Hum. Genet.* **55**, 753 (1994).
59. M. Hotopf, P. Bolton, *J. Autism Dev. Disord.* **25**, 41 (1995).
60. E. H. Cook Jr. et al., *Am. J. Hum. Genet.* **60**, 928 (1997).
61. R. J. Schroer et al., *Am. J. Med. Genet.* **76**, 327 (1998).
62. F. Gurrieri et al., *Neurology* **52**, 1694 (1999).
63. M. M. Konstantareas, S. Homatidis, *J. Autism Dev. Disord.* **29**, 275 (1999).
64. C. M. Wolpert et al., *Am. J. Med. Genet.* **96**, 365 (2000).
65. P. F. Bolton et al., *Am. J. Med. Genet.* **105**, 675 (2001).
66. F. Z. Boyar et al., *Clin. Genet.* **60**, 421 (2001).
67. A. E. Silva, S. A. Vayego-Lourenco, A. C. Fett-Conte, E. M. Goloni-Bertollo, M. Varella-Garcia, *Arq. Neuropsiquiatr.* **60**, 290 (2002).
68. J. A. Thomas et al., *Am. J. Med. Genet.* **119A**, 1110 (2003).
69. A. Kashiwagi et al., *J. Hum. Genet.* **48**, 194 (2003).
70. S. Jamain et al., *Nature Genet.* **34**, 27 (2003).
71. *International Statistical Classification of Diseases and Related Health Problems, 1989 revision* (World Health Organization, Geneva, ed. 10, 1992) (ICD-10).
72. D. R. Cohen et al., *Mol. Cell Neurosci.* **22**, 417 (2003).
73. P. Scheiffele, J. Fan, J. Choih, R. Fetter, T. Serafini, *Cell* **101**, 657 (2000).
74. C. Dean et al., *Nature Neurosci.* **6**, 708 (2003).
75. R. Klein et al., *Cell* **75**, 113 (1993).
76. K. Tabuchi, T. C. Sudhof, *Genomics* **79**, 849 (2002).
77. M. Missler et al., *Nature* **423**, 939 (2003).
78. S. Steffenburg, C. L. Gillberg, U. Steffenburg, M. Kyllerman, *Pediatr. Neurol.* **14**, 131 (1996).
79. L. A. Laan, A. A. Vein, *Epilepsia* **43**, 1590 (2002).
80. P. Watson et al., *J. Med. Genet.* **38**, 224 (2001).
81. D. Armstrong, J. K. Dunn, B. Antalffy, R. Trivedi, *J. Neuropathol. Exp. Neurol.* **54**, 195 (1995).
82. Supported by HHMI and NIH (HD 40301). I thank D. Sweatt, J. Neul, and J. Young for comments on the manuscript, V. Brandt for editorial assistance, and J. Neul for the illustrations.

REVIEW

Looking Backward to Move Forward: Early Detection of Neurodegenerative Disorders

Steven T. DeKosky^{1*} and Kenneth Marek²

Early detection of neurodegenerative disorders would provide clues to the underlying pathobiology of these diseases and would enable more effective diagnosis and treatment of patients. Recent advances in molecular neuroscience have begun to provide the tools to detect diseases like Alzheimer's disease, Parkinson's disease, and others early in their course and potentially even before the development of clinical manifestations of disease. These genetic, imaging, clinical, and biochemical tools are being validated in a number of studies. Early detection of these slowly progressive diseases offers the promise of presymptomatic diagnosis and, ultimately, of disease-modifying medications for use early in disease and during the presymptomatic period.

In the past decade, an explosion of information in molecular neuroscience has markedly enhanced our understanding of and potential therapy for neurodegenerative disorders. These diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), motor neuron disease, Huntington's disease (HD), and other neurodegenerative dementias, generally begin late in life

and slowly but inexorably cause progressive neuronal degeneration and result in disability or death. Recent studies have demonstrated that these disorders are characterized by a presymptomatic phase, likely lasting years, during which neuronal degeneration is occurring but before clinical symptoms appear. This presents both a challenge—How do we identify individuals during this preclinical period—and an opportunity: Can preventive therapy be started during the preclinical period before disease symptoms appear? Therefore, a major goal of clinical research is to improve early detection of these diseases by developing tools to move diagnosis backward in the neurodegeneration temporal course (Fig. 1).

These tools would enable us to (i) identify at-risk groups both for disease onset and progression during the preclinical period; (ii) accelerate and enhance the accuracy of diagnosis in the early clinical phase to ensure appropriate treatment; and (iii) speed the development of drugs that might modify disease progression during the (earlier) preclinical and clinical periods and, ultimately, enable these therapies to be directed at individuals in the preclinical phase of illness to prevent or slow the onset of clinical manifestations of disease. Strategies might include therapies specific for the disease pathobiology, such as anti-amyloid medications for AD, or interventions that address nonspecific disease mechanisms, such as inflammation or oxidative stress. In the case of AD, a delay in onset by 5 years could translate into a 50% decrease in disease prevalence (1). A delay of 10 years would result in virtual disappearance of the disease.

These tools emerging from many new technologies for neurodegeneration and/or for clinical manifestations of disease. Biomarkers are broadly defined as characteristics that can be objectively measured and evaluated

¹Department of Neurology and Alzheimer Disease Research Center, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ²Institute for Neurodegenerative Disorders, New Haven, CT, USA.

*To whom correspondence should be addressed. E-mail: DeKoskyST@upmc.edu

as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (2). Given the likely multiple etiologies and subsequent pathological processes (e.g., oxidative stress and inflammation) of the neurodegenerative disorders and the heterogeneity in the expression and progression of the clinical manifestations of these diseases, several biomarkers will probably be necessary to ensure the early detection of preclinical and clinical disease.

Although biomarkers may be extraordinarily useful and new technologies very seductive, a number of caveats must be considered. First, the performance characteristics of the marker must be established in the subject population under study. Second, the marker should be meaningful or relevant to the disease process. Third, it should be clear to what extent the marker is generalizable beyond the study population. Finally, the effect of factors like age, medications, and environment on the biomarker must be clarified. It will take time to prove the accuracy and positive predictive value of such markers. However, even as identification and validation of these tools for early detection of neurodegenerative disorders is ongoing, biomarkers are rapidly changing our understanding of disease and our approach to the development of neurotherapeutics.

Biomarkers for Early Disease Detection

Biomarkers for early detection of neurodegenerative disorders may be divided into four main categories: genetic, neuroimaging, clinical, and biochemical.

Genetic markers. Molecular genetic studies of neurodegenerative disorders have been useful both in identifying genes that may be causative or associated with specific diseases and in uncovering functional mechanisms subserved by products of those genes that may be markers of early detection. Genetics provides the best assessment of who is at risk, but except in the case of rare genetic mutations, it does not provide complete information about when or if those at risk may expect the onset of neurodegeneration or disease. For example, in disorders like HD, a dominantly inherited disorder caused by a mutation in chromosome 4 that results in an expanded allele due to a trinucleotide repeat, the at-risk group is defined by their genetics. However,

although the size of the expanded allele is useful in predicting the onset of disease (3), more specific markers of disease phenotype will be required to better detect preclinical or early clinical manifestations of HD (4). The Huntington Study Group is conducting a large observational study (PREDICT-HD: Neurobiological Predictors of Huntington's Disease) to identify neurobiological predictors of HD, such as imaging and clinical biomarkers, for early HD detection in at-risk but clinically unaffected individuals.

In disorders such as AD and PD, mutations in several genes are now known to cause these disorders in families with an autosomal dominant pattern of inheritance. Identification of the mutations in these genes can identify at-risk family members, but it is not generalizable to most sporadic AD or PD (5, 6). The relative contribution of genetics in sporadic PD has been questioned in recent twin studies and in epide-

nization or infusion, or other disruption of pathobiological cascades (12, 13). In the case of PD, mutations in α -synuclein and Parkin have both resulted in defects in the ubiquitin-proteasome system—now thought critical to PD degeneration (14–16)—providing new targets for therapeutic intervention. Transgenic models of α -synuclein loss and homologs of ubiquitin-proteasome pathology in fruit flies have been identified that have begun to elucidate the pathogenic pathways that might lead to neurodegeneration (17–19). These studies have provided the rationale for ongoing research to evaluate serum or cerebrospinal fluid (CSF) markers of protein folding or aggregation as tools to assess early degeneration and early disease.

Other genetic studies in cohorts that exhibit sporadic disease or familial preponderance that is not autosomal dominant have focused on genetic association. The best example of a genetic

association has been the increased risk of developing late-onset AD in families that carry the ApoE4 allele (20). A number of other linkages to several other genes are under study and will likely uncover an array of susceptibility genes for AD, PD, and other disorders (21, 22). Ultimately, genetic susceptibility to identified genes may provide a template with which to characterize statistical risk and enable a probabilistic method for determining who to treat before disease manifestation. Genetics

therefore continues to provide valuable clues to who is at risk for neurodegenerative disorders. Although this may be crucial to assess who should be evaluated, effective early detection of disease may depend on combining genotype(s) with biomarkers for preclinical and clinical disease phenotype.

Neuroimaging biomarkers. During the past decade, neuroimaging has emerged as a strategy to define neurodegenerative disease phenotype during both the preclinical and early clinical disease periods. Imaging provides a window to the neuronal dysfunction in these disorders and thereby provides data complementary to the clinical evaluation. Imaging tests can also be done repeatedly, to assess temporal changes in the neuronal degeneration both before and after the development of clinical symptoms. Recent advances in radiopharmaceutical development, imaging detector technologies, and image analysis

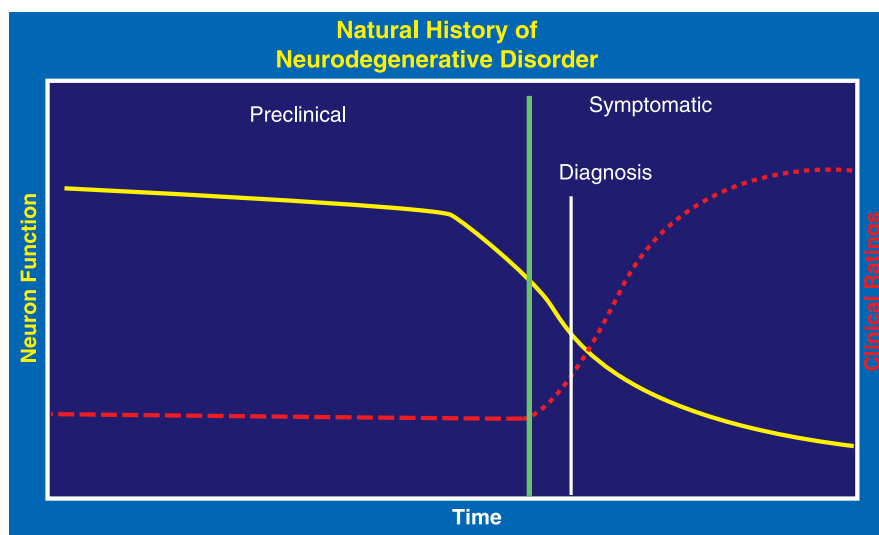


Fig. 1. Model for the progression of loss of neuronal function in neurodegenerative disorders. There is a prolonged period during which loss of neuronal function has occurred but symptoms have not yet appeared.

miologic surveys that demonstrate no significant increase in disease risk in the family members of PD patients (7–9). Perhaps the most important consequence of characterizing the genetics of autosomal dominant PD and AD families is that the identified genes and gene products have focused research on possible mechanisms of disease pathophysiology that may be critical to neurodegeneration and on targets for developing interventions. In the case of AD, the identification of β -amyloid precursor protein (APP) and presenilin 1 and 2 mutations has strengthened the amyloid hypothesis and identified potential sites for interventions. Transgenic animal models have been constructed that mimic some aspects of the pathology (10, 11), and proof-of-principle studies have been able to prevent the transgene-induced pathology with strategies that use anti-inflammatory medications, antibody stimulation by immu-

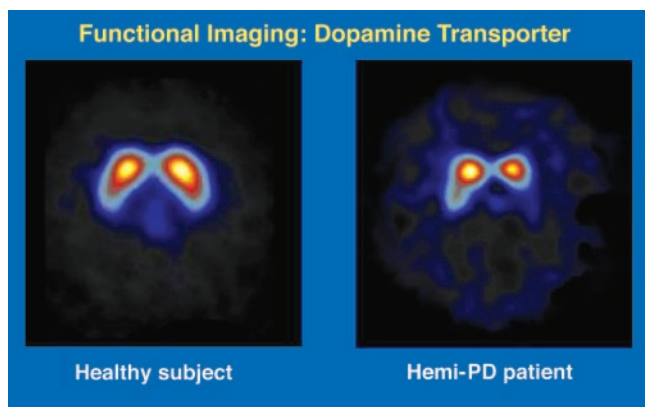


Fig. 2. SPECT 123I-(2 β -carboxymethoxy-3 β (4-iodophenyl)tropane) images of a healthy subject and an early PD patient with symptoms only on the right side. The loss of activity in the PD patient is asymmetric, but there is a bilateral reduction in activity in the basal ganglia. Levels of SPECT activity are color-encoded from low (black) to high (yellow/white). (Figure courtesy of J. Seibyl, Institute for Neurodegenerative Disorders.)

software have enhanced and accelerated the role of imaging in clinical research into PD and AD in general and into early disease detection in particular. Functional imaging using single photon emission computerized tomography (SPECT) and positron emission tomography (PET, also called dual photon emission tomography), as well as structural imaging using magnetic resonance imaging (MRI), have been useful research tools to assess early disease. The range of radioligand targets for SPECT and PET have rapidly expanded as the pathophysiology of PD, AD, and related disorders has been further elucidated. Both SPECT and PET are sensitive methods of measuring *in vivo* neurochemistry. Although in general PET cameras have better resolution than SPECT cameras, SPECT studies may be technologically and clinically more feasible, particularly for large clinical studies that require rapid patient accrual. PET studies may benefit from greater flexibility in the range of radiopharmaceuticals that can be tested, but SPECT has the advantage of radiopharmaceuticals with longer half-lives that are necessary for some studies. The choice of scintigraphic imaging modality is ultimately determined by the specific study questions and study design and is guided by the properties of the radiopharmaceuticals used to target the dopamine system in PD or amyloid or other targets in AD.

PD has served as a model neurodegenerative disorder for the use of imaging markers to assess early disease. In PD, the most mature imaging markers have targeted the hallmark pathology in the substantia nigra dopaminergic system. The two most widely used imaging biomarkers are 18F-Dopa for PET, in which conversion of 18F-Dopa to 18F-Dopamine is measured, and dopamine transporter (DAT) ligands for SPECT, where several similar ligands are available that tag the DAT and therefore measure dopamine terminal integrity. Dopamine ligands are useful

to assess PD in so far as they reflect the status of dopaminergic degeneration. In a study directly correlating changes in dopamine neuronal number and imaging outcomes, there was good correlation between dopamine neuron loss and 18F-Dopa uptake, although conclusions were limited by the very small sample size of only five subjects (23). DAT was reduced in striatum in postmortem brain from PD patients and in methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys; the loss of DAT paralleled the loss of dopamine in the striatum (24, 25). Numerous clinical imaging studies have shown reductions in 18F-Dopa and DAT ligand uptake in PD patients and in aging healthy subjects that are consistent with the expected pathology of PD and the known changes of normal aging. In PD, these imaging studies demonstrated asymmetric, putamen greater than caudate loss of dopaminergic uptake, and the imaging loss correlated with worsened clinical symptoms in cross-sectional evaluation (26, 27). In normal aging, DAT ligands also demonstrate reductions in activity (28).

Several lines of evidence suggest strongly that dopaminergic imaging can identify subjects during the preclinical phase of their disease. The most extensive preclinical data are from studies that image patients with hemi-Parkinson's disease. Typically, patients begin disease with PD symptoms on one side of the body and progress to bilateral disease within 3 to 6 years (29). In several imaging studies (Fig. 2), there was a significant reduction in putamen DAT uptake, ranging from 25 to 40% in the presymptomatic striatum (contralateral to the side without symptoms), demonstrating preclinical dopaminergic loss in these patients who are known to progress to bilateral disease (30, 31). Other attempts to identify preclinical disease with imaging methods have focused on at-risk populations such as family members or un-

affected twins of PD patients. Studies using 18F-dihydroxyphenylalanine (F-Dopa) have demonstrated that, in several well-characterized kindreds, 11 of 32 asymptomatic relatives were found to have reduced F-Dopa uptake. Three of these subjects subsequently developed symptomatic PD (32). Several asymptomatic co-twins who also showed a reduction in F-Dopa activity later developed symptoms of PD, although the concordance rate for monozygotic and dizygotic twins remains uncertain (33). In kindreds of genetically defined PD families, mild loss of imaging markers has been identified in gene-positive but symptom-negative individuals, suggesting a preclinical period of neurodegeneration (34, 35). Although these studies must be confirmed with larger sample sizes and longer follow-up, the current evidence strongly suggests that imaging can effectively identify preclinical dopaminergic degeneration.

Although dopaminergic ligands have led the way, numerous other ligands targeting the pathophysiology of neurodegenerative diseases are rapidly developing as research and possible clinical tools. Ligands targeting microglial activation, a pathological change thought to occur early in PD and AD, are currently under study (36). Several radiopharmaceuticals directed at binding to β -amyloid are being assessed as tools for early or presymptomatic detection of AD and for monitoring of disease progression and the effects of interventions (37–39) (Fig. 3). In addition, the pattern of change in FDG in early patients with AD and PD may prove useful in detecting early pathology (40, 41). Promising data have emerged from studies that combine genotyping for ApoE4 and neuroimaging with PET (42, 43). The distinctive metabolic anatomy

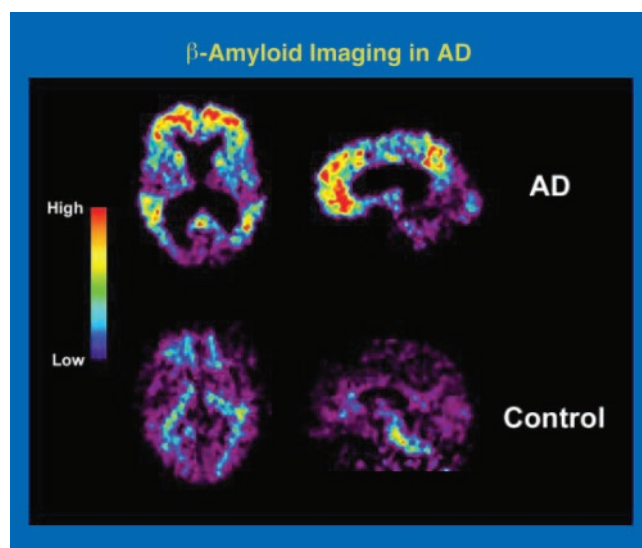


Fig. 3. PET study of amyloid ligand C11-BTA in an AD patient (upper panel) compared to a normal control subject (lower panel). Amyloid signal elevation is seen in the parietal and frontal lobes in the upper left (transverse view) and in the frontal lobe and posterior cingulate cortex (lateral view). The control scan shows no amyloid uptake. (Figure courtesy of W. E. Klunk and C. Mathis, University of Pittsburgh.)

of fluorodeoxyglucose loss in PD in particular may help distinguish early PD from related neurodegenerative disorders (44). MRI and MRS imaging have also been used to assess neurodegenerative diseases early in their course. Detection of amyloid plaques by MRI is still in the preclinical stage (45, 46). Global brain atrophy measured by volumetric MRI has been used to identify early AD and mild cognitive impairment (MCI) and to assess progression of early AD (47–49). Widespread brain atrophy also may occur early in HD, and early changes in energy metabolism in HD may be identified by magnetic resonance spectroscopy (50, 51).

Clinical biomarkers—Improved sensitivity to early disease. Early detection of neurodegenerative disorders has been transformed by more sensitive assessments of the primary clinical features of the disease(s) and by the identification of additional clinical features that may reliably predate the predominant clinical syndrome. In longitudinal epidemiologic studies of AD, reports have documented early mild onset of cognitive changes with subsequent progression to dementia, enabling researchers to pinpoint the earliest changes in serial cognitive assessments that developed before frank dementia or the ability to make a clinical diagnosis (52, 53).

Over the past several years, subjects who presented to memory disorders clinics with isolated memory loss or MCI have been followed and have subsequently progressed to dementia. As these cases came to autopsy, many were confirmed as AD (54). MCI has become a major focus of research, because of the high rates at which people who present with MCI go on to develop AD. There are several different definitions of MCI. The most studied form of MCI is isolated recent memory loss, or amnesic MCI. Criteria include subjective or objective impairment in recent memory, relatively preserved cognitive functions in other domains, and normal performance of activities of daily living (55). Subjects who present with amnesic MCI have an increased risk of developing diagnosable AD at rates of 12 to 15% per year, in contrast to 1 to 2% per year in age-matched normals. Increased risk factors for more rapid transition or “conversion” to AD include significant hippocampal atrophy on MRI, worsened performance on recent memory testing, and the presence of an apoE4 allele (56). Amnesic MCI is not the only early manifestation of impending AD; it may not even be the most common prodrome. Other patterns of MCI may occur, without isolated or marked failure of short-term memory function below some particular cutoff (usually taken as 1.5 standard deviations below age- and education-adjusted expectations). However, the (relatively) isolated loss of short-term memory is perhaps the easiest to identify, because it clearly exceeds expectations of what are “normal” age-related changes.

The nature of nonamnesic MCI, or MCI with multiple domain impairments (MCI-MCD), is just beginning to be defined.

A key issue, requiring careful longitudinal observation or new diagnostic tests, will be the differentiation of MCI, progressive or static, from the normal changes of aging (56). Another term used to describe the altered status of cognitive function in the elderly is “cognitive impairment no dementia” (CIND), for which longitudinal data defining the risk of subsequent dementia are not complete. Many patients with CIND have vascular disease, are in the early stages of neurodegenerative dementias, or have a transient process that allows cognitive performance to return to the normal range on subsequent testing, which has been seen in several epidemiological studies (57–59) and less commonly in studies from specialty clinics. Given the variability of the very early clinical signs and their overlap with the normal changes of aging, well-validated biomarkers would be of great utility in determining who to treat.

Retrospective and prospective neuroimaging and neuropathological reports have begun to define the presence of AD pathology in amnesic MCI, as defined by the CDR, or clinical dementia rating scale. Pathological changes (such as neuritic plaques and neurofibrillary tangles) enabling a diagnosis of AD or of being significantly greater than expected for age have been found in approximately 60 percent of MCI cases (defined by various criteria, but clearly mild and not meeting classic definitions of dementia of the Alzheimer’s type) that have come to autopsy (60–63). These new data prove, as long suspected, that significant pathological changes occur in the presymptomatic and very early symptomatic stages of AD and have further encouraged efforts to find early clinical or biological markers of impending symptomatic disease. Another result of studies of brains from subjects dying in earlier stages of disease has been reconsideration of the severity of pathological change needed to produce cognitive changes. The pioneering neuropathological studies that led to the findings of loss of cholinergic enzymes, cholinergic neurons (64), cortical neurons (65), and synapses (66–68) were performed for the most part on severe end-stage cases. It is now felt that, although functional decline of the cholinergic system may occur early in the course, the newer autopsy studies in earlier stages (MCI and mild AD) demonstrate preservation of both cholineacetyltransferase (ChAT) activity (60–62) and the neurons of the cholinergic basal forebrain (69). Significant loss of these markers now appears to be delayed until late-stage disease. Thus, there are new opportunities to preserve cognition, by appropriate disease-altering interventions (such as anti-amyloid, anti-neurofibrillary tangle, or anti-neuronal death strategies), and an increased urgency of developing ways to identify presymptomatic or early-stage disease by biomarkers.

As in AD, numerous studies have attempted to identify very early manifestations of motor dysfunction in PD (i.e., tremor, handwriting abnormalities, and gait disturbance) with new technologies (70, 71), but these tests have not yet been clinically useful. In addition, early detection of nonmotor findings that predate motor symptoms, most notably the loss of olfaction, has provided a potential marker of early PD (72). Combining DAT imaging and sensitive olfactory testing, individuals with olfactory dysfunction but without motor symptoms of PD were found to be more likely to have evidence on imaging of dopamine dysfunction (73).

Biochemical markers—Serum and cerebrospinal fluid. In the past several years, numerous blood and CSF tests have been proposed for early detection of AD, PD, and related disorders. Generally, these tests are based on disease pathology; for example, the measurement of serum or CSF β -amyloid and Tau or markers of oxidative stress (74). Not all results are consistent (75). Blood levels of several inflammatory markers are associated with an increased risk of developing dementia and AD (76) and may increase with severity or duration of disease (77). However, the sensitivity and specificity of these tests has been limited (78). Serum levels of β -amyloid, elevated in the blood of people with the APP or presenilin mutations, have also been reported elevated in preclinical AD and in Down Syndrome, but they are not sufficiently elevated in AD or reliably in presymptomatic AD to be useful at this time. Renal function appears to play a role in circulating levels as well, making straightforward correlations difficult (79).

More recently, newer array-based technologies including proteomics, transcriptomics, and metabolomics have offered the promise to target biochemical deficits in neurodegenerative disorders more effectively in order to identify serum and CSF markers of disease (80, 81). High-speed throughput and computerized comparison of patterns may yield profiles that will more specifically identify presymptomatic patterns.

Summary

Early detection of neurodegenerative disorders is crucial to enable us to understand and treat these disorders more effectively and to prevent or delay their emergence. The tools to assess early (and ideally preclinical) disease are developing rapidly in tandem with improved understanding of the underlying molecular pathobiology of these disorders. Early disease detection depends on combining genetic biomarkers to identify at-risk individuals with evidence of developing phenotype based on imaging, clinical, or biochemical measures. Simpler, less expensive tools are required for practical screening of individuals susceptible to and/or manifesting early degeneration. Without such tools, prevention trials such as those currently being initiated for AD will require thousands of subjects and at least 5

years' duration each to complete, because of an inability to identify people with a high probability of developing dementia.

Research to improve, expand, and better validate markers of early detection should continue to be a major focus of clinical neuroscience. A major unmet need for patients, and a clear focus of clinical therapeutics for neurodegenerative disorders, has been to identify medications that might slow or even reverse the underlying processes that result in neuronal loss. An equal goal is to initiate treatment with these or other medications during the preclinical phase before symptoms have arisen. Early detection of neurodegenerative disorders is the key to enabling early treatment and is crucial to the transformation of clinical therapeutics from symptomatic to disease-modifying therapies.

References and Notes

1. R. Brookmeyer, S. Gray, C. Kawas, *Am. J. Public Health* **88**, 1337 (1998).
2. Biomarkers Definitions Working Group, *Clin. Pharmacol. Ther.* **69**, 89 (2001).
3. L. Djousse et al., *Am. J. Med. Genet.* **119A**, 279 (2003).
4. J. S. Paulsen et al., *Neurology* **57**, 658 (2001).
5. T. Gasser, *Adv. Neurol.* **91**, 143 (2003).
6. R. E. Tanzi, L. Bertram, *Neuron* **32**, 181 (2001).
7. K. Marder et al., *Neurology* **61**, 18 (2003).
8. A. Elbaz et al., *Neurology* **61**, 11 (2003).
9. C. M. Tanner et al., *JAMA* **281**, 341 (1999).
10. D. J. Selkoe, D. Schenk, *Annu. Rev. Pharmacol. Toxicol.* **43**, 545 (2003).
11. K. Lindsten, V. Menendez-Benito, M. G. Masucci, N. P. Dantuma, *Nature Biotechnol.* **21**, 897 (2003).
12. D. Morgan et al., *Nature* **408**, 982 (2000).
13. D. Schenk et al., *Nature* **400**, 173 (1999).
14. L. Skipper, M. Farrer, *Neurotoxicology* **23**, 503 (2002).
15. K. S. McNaught, R. Belizaire, O. Isacson, P. Jenner, C. W. Olanow, *Exp. Neurol.* **179**, 38 (2003).
16. R. J. Miller, S. M. Wilson, *Trends Pharmacol. Sci.* **24**, 18 (2003).
17. M. B. Feany, W. W. Bender, *Nature* **404**, 394 (2000).
18. V. L. Dawson, *Science* **288**, 631 (2000).
19. H. Y. Zoghbi, J. Botas, *Trends Genet.* **18**, 463 (2002).
20. E. H. Corder et al., *Science* **261**, 921 (1993).
21. N. Pankratz et al., *Am. J. Hum. Genet.* **72**, 1053 (2003).
22. S. A. Oliveira et al., *Arch. Neurol.* **60**, 975 (2003).
23. B. Snow, I. Tooyama, E. McGeer, D. Calne, *Ann. Neurol.* **34**, 324 (1993).
24. E. Bezard et al., *J. Neurosci.* **21**, 6853 (2001).
25. H. B. Niznik, E. F. Fogel, F. F. Fassos, P. Seeman, *J. Neurochem.* **56**, 192 (1991).
26. D. J. Brooks, *Adv. Neurol.* **82**, 209 (2000).
27. K. Marek, *Neuroscientist* **5**, 333 (1999).
28. C. H. van Dyck et al., *Am. J. Geriatr. Psychiatry* **10**, 36 (2002).
29. M. M. Hoehn, M. D. Yahr, *Neurology* **17**, 427 (1967).
30. K. Marek et al., *Neurology* **46**, 231 (1996).
31. P. Morrish, G. Sawle, D. Brooks, *J. Neurol. Neurosurg. Psychiatry* **59**, 597 (1995).
32. P. Piccini et al., *Ann. Neurol.* **41**, 222 (1997).
33. P. Piccini, D. Burn, R. Ceravolo, D. Maraganore, D. Brooks, *Ann. Neurol.* **45**, 577 (1999).
34. M. Dekker et al., *Mov. Disord.* **18**, 751 (2003).
35. N. L. Khan et al., *Ann. Neurol.* **52**, 849 (2002).
36. A. Cagnin et al., *Lancet* **358**, 461 (2001).
37. W. E. Klunk et al., *J. Neurosci.* **23**, 2086 (2003).
38. M. P. Kung et al., *J. Mol. Neurosci.* **19**, 7 (2002).
39. K. Shoghi-Jadid et al., *Am. J. Geriatr. Psychiatry* **10**, 24 (2002).
40. D. Eidelberg et al., *Neurology* **45**, 1995 (1995).
41. R. L. Van Heertum, R. S. Tikofsky, *Semin. Nucl. Med.* **33**, 77 (2003).
42. G. W. Small et al., *JAMA* **273**, 942 (1995).
43. E. Reiman, R. Caselli, L. Yun, N. Engl. *J. Med.* **334**, 752 (1996).
44. D. Eidelberg, V. Dhawan, *Neurology* **58**, 997 (2002).
45. H. Benveniste, G. Einstein, K. R. Kim, C. Hulette, G. A. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14079 (1999).
46. J. F. Poduslo et al., *Neurobiol. Dis.* **11**, 315 (2002).
47. N. C. Fox et al., *Lancet* **358**, 201 (2001).
48. C. R. Jack Jr. et al., *Neurology* **52**, 1397 (1999).
49. M. J. de Leon et al., *Proc. Natl. Acad. Sci. U.S.A.* **98**, 10966 (2001).
50. H. D. Rosas et al., *Neurology* **60**, 1615 (2003).
51. B. G. Jenkins et al., *Neurology* **50**, 1357 (1998).
52. P. Chen et al., *Neurology* **55**, 1847 (2000).
53. P. Chen et al., *Arch. Gen. Psychiatry* **58**, 853 (2001).
54. J. C. Morris et al., *Arch. Neurol.* **58**, 397 (2001).
55. R. C. Petersen et al., *Neurology* **56**, 1133 (2001).
56. R. Petersen, *Mild Cognitive Impairment: Aging to Alzheimer's Disease* (Oxford Univ. Press, New York, 2003).
57. K. Ritchie, B. Ledesert, J. Touchon, *Compr. Psychiatry* **41**, 61 (2000).
58. H. Tuokko et al., *Arch. Neurol.* **60**, 577 (2003).
59. S. Larrieu et al., *Neurology* **59**, 1594 (2002).
60. S. T. DeKosky et al., *Ann. Neurol.* **51**, 145 (2002).
61. K. L. Davis et al., *JAMA* **281**, 1401 (1999).
62. P. Tiraboschi et al., *Neurology* **55**, 1278 (2000).
63. K. P. Riley, D. A. Snowden, W. R. Markesbery, *Ann. Neurol.* **51**, 567 (2002).
64. P. Whitehouse et al., *Science* **215**, 1237 (1982).
65. D. Neary et al., *J. Neurol. Neurosurg. Psychiatry* **49**, 229 (1986).
66. S. W. Scheff, S. T. DeKosky, D. Price, *Neurobiol. Aging* **11**, 29 (1990).
67. R. D. Terry et al., *Ann. Neurol.* **30**, 572 (1991).
68. S. T. DeKosky, S. W. Scheff, *Ann. Neurol.* **27**, 457 (1990).
69. E. J. Mufson et al., *J. Comp. Neurol.* **443**, 136 (2002).
70. S. L. Pullman, *Mov. Disord.* **13** (suppl. 3) 85 (1998).
71. E. B. Montgomery Jr. et al., *Mov. Disord.* **15**, 467 (2000).
72. R. L. Doty, S. M. Bromley, M. B. Stern, *Neurodegeneration* **4**, 93 (1995).
73. H. W. Berendse et al., *Ann. Neurol.* **50**, 34 (2001).
74. T. Sunderland et al., *JAMA* **289**, 2094 (2003).
75. T. J. Montine et al., *Ann. Neurol.* **52**, 175 (2002).
76. R. Schmidt et al., *Ann. Neurol.* **52**, 168 (2002).
77. S. T. DeKosky et al., *Ann. Neurol.* **53**, 81 (2003).
78. R. A. Frank et al., *Neurobiol. Aging* **24**, 521 (2003).
79. Z. Arvanitakis, J. A. Lucas, L. H. Younkin, S. G. Younkin, N. R. Graff-Radford, *Alzheimer Dis. Assoc. Disord.* **16**, 187 (2002).
80. W. Weckwerth, O. Fiehn, *Curr. Opin. Biotechnol.* **13**, 156 (2002).
81. M. Kanehisa, P. Bork, *Nature Genet.* **33** (suppl.) 305 (2003).
82. S.T.D. received funding from NIH that contributed to this article. K.M. received funding from NIH, the U.S. Department of Defense, the Michael J. Fox Foundation For Parkinson's Research, and the Helen I. Graham Foundation.

REVIEW

Immunotherapeutic Approaches to Alzheimer's Disease

Alon Monsonego and Howard L. Weiner

Although neurodegenerative diseases such as Alzheimer's disease are not classically considered mediated by inflammation or the immune system, in some instances the immune system may play an important role in the degenerative process. Furthermore, it has become clear that the immune system itself may have beneficial effects in nervous system diseases considered neurodegenerative. Immunotherapeutic approaches designed to induce a humoral immune response have recently been developed for the treatment of Alzheimer's disease. These studies have led to human trials that resulted in both beneficial and adverse effects. In animal models, it has also been shown that immunotherapy designed to induce a cellular immune response may be of benefit in central nervous system injury, although T cells may have either a beneficial or detrimental effect depending on the type of T cell response induced. These areas provide a new avenue for exploring immune system-based therapy of neurodegenerative diseases and will be discussed here with a primary focus on Alzheimer's disease. We will also discuss how these approaches affect microglia activation, which plays a key role in therapy of such diseases.

more highly correlated than A β 1-40 with disease and neurotoxicity. Extensive studies have demonstrated that these A β plaques are co-localized with activated microglia and astrocytes, implicating additional neurotoxicity (4-6). The accumulation of A β in the brain may be part of the aging process in all individuals, and its accumulation to toxic levels may take years. The appearance of clinical symptoms may be due to increased levels of A β above a certain threshold that is no longer controlled by endogenous clearance mechanisms. This hypothesis is supported by the observation that genetic factors such as APP

Amyloid beta-peptide (A β) is a cleavage product of neuronal amyloid precursor protein (APP) (1, 2). Cleavage can yield either A β 1-40 or A β 1-42, and accumulation and

aggregation of these products in cognitive brain regions during aging is a hallmark of Alzheimer's disease (AD) pathology (3). A β 1-42 is the more aggregated form and is

Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. E-mail: amonsonego@rics.bwh.harvard.edu (A.M.); hweiner@rics.bwh.harvard.edu (H.L.M.)

ERRATUM

post date 12 December 2003

Special Issue on Brain Disease: REVIEW: "Looking backward to move forward: Early detection of neurodegenerative disorders" by S. T. DeKosky and K. Marek (31 Oct. 2003, p. 830). Uppsala University should have been listed in the credit for Fig. 3. The corrected credit should read "Figure courtesy of the University of Pittsburgh and Uppsala University."