

Early and Differential Diagnosis of Dementia and Mild Cognitive Impairment

Design and Cohort Baseline Characteristics of the German Dementia Competence Network

Johannes Kornhuber^a Klaus Schmidtke^b Lutz Frölich^c Robert Perneczky^d Stefanie Wolf^e
Harald Hampel^f Frank Jessen^g Isabella Heuser^h Oliver Peters^h Markus Weih^a
Holger Jahnⁱ Christian Luckhaus^j Michael Hüll^b Hermann-Josef Gertz^k Johannes Schröder^l
Johannes Pantel^m Otto Rienhoff^o Susanne A. Seuchter^{a,p} Eckart Rüter^e Fritz Henn^c
Wolfgang Maier^g Jens Wiltfang^{a,n}

^aDepartment of Psychiatry and Psychotherapy, University Erlangen, Erlangen, ^bCenter for Geriatric Medicine and Gerontology, University Hospital Freiburg, Freiburg, ^cCentral Institute of Mental Health, Mannheim, ^dDepartment of Psychiatry and Psychotherapy, Technical University of Munich, Munich, Departments of Psychiatry, ^eUniversity of Göttingen, Göttingen, ^fLudwig Maximilian University Munich, Munich, ^gUniversity Bonn, Bonn, ^hCharité, Berlin, Campus Benjamin Franklin, Berlin, ⁱUniversity of Hamburg, Hamburg, ^jUniversity of Düsseldorf, Düsseldorf, ^kUniversity of Leipzig, Leipzig, ^lUniversity of Heidelberg, Heidelberg, ^mUniversity of Frankfurt, Frankfurt, and ⁿUniversity of Essen, Essen, Departments of Medical Informatics, ^oUniversity of Göttingen, Göttingen, and ^pUniversity of Erlangen, Erlangen, Germany

Key Words

Alzheimer's disease · Study design · Dementia · Galantamine · Memantine · Mild cognitive impairment · Multi-center randomized clinical trials · Neurodegenerative disease

Abstract

Background: The German Dementia Competence Network (DCN) has established procedures for standardized multi-center acquisition of clinical, biological and imaging data, for centralized data management, and for the evaluation of new treatments. **Methods:** A longitudinal cohort study was set up for patients with mild cognitive impairment (MCI), patients with mild dementia and control subjects. The aims were to establish the diagnostic, differential diagnostic and prognostic power of a range of clinical, laboratory and imaging methods. Furthermore, 2 clinical trials were conducted with patients suffering from MCI and mild to moderate Al-

zheimer's Disease (AD). These trials aimed at evaluating the efficacy and safety of the combination of galantamine and memantine versus galantamine alone. **Results:** Here, we report on the scope and projects of the DCN, the methods that were employed, the composition and flow within the diverse groups of patients and control persons and on the clinical and neuropsychological baseline characteristics of the group of 2,113 subjects who participated in the observational and clinical trials. **Conclusion:** These data have an impact on the procedures for the early and differential clinical diagnosis of dementias, the current standard treatment of AD as well as on future clinical trials in AD.

Copyright © 2009 S. Karger AG, Basel

K.S., L.F., J.W., R.P. and H.J. have received financial support and/or honoraria for oral presentations, participation in advisory boards and for conducting additional clinical trials from various pharmaceutical companies manufacturing antidementia drugs, including Janssen-Cilag and Merz Pharmaceuticals, during the last 2 years.

Introduction

Dementia is a major health problem in the elderly. All epidemiological studies have shown strong correlations between age and the incidence/prevalence of dementing diseases. During the last century alone, the German population has shown a staggering increase in its proportion of elderly citizens. While the number of persons over 65 years of age has quadrupled from 3.2 to 12.9 million, the proportion of those older than 80 years has increased even more dramatically from 300,000 to 3.2 million [1, 2]. The increasing prevalence of patients with dementia has a considerable economic impact. Forthcoming treatment interventions are most promising in modifying the disease course when they are offered at an early disease stage or as preventive treatment. This calls for improved early or even predictive diagnosis of the dementias.

The transitional stage between 'normal' functional ability and a full-blown clinical picture of dementia is described as mild cognitive impairment (MCI). The term MCI refers to some lowering of cognitive function, from a formerly normal level towards a mildly impaired level. Many different underlying reasons and etiologies may evoke this decline, including not only Alzheimer's disease (AD), but also vascular lesions, alcohol abuse, epilepsy, depression and others. Patients with AD will pass through the MCI stage before presenting with AD dementia, but not all patients presenting with clinical MCI suffer from underlying AD pathology [3]. Population studies have demonstrated that 10–25% of the over 65 year olds in western industrialized nations have MCI [4–6]. The mean prevalence of MCI is approximately 16%, and thus twice as high as the prevalence of dementia. This proportion is equivalent to more than 10 million MCI sufferers over 65 years old in the European Community.

The German Dementia Competence Network (DCN) is a research platform of university departments that teamed up to perform long-term studies on dementia, including cross-sectional and prospective longitudinal cohort studies and clinical trials. The DCN is striving for the development and application of new innovative tools in early dementia detection, early intervention, treatment and care of patients with dementia and with common preceding symptoms (MCI). We have focused on a set of diagnostic and therapeutic core questions with immediate clinical relevance: Is it possible to predict which people with MCI will develop dementia in a given time period? How can the differential diagnosis of dementia be improved in a clinical context? Can progression of MCI to dementia be prevented or delayed through administra-

tion of antidementia drugs? Can pharmacological treatment of Alzheimer's dementia be improved by combination therapy? The global objectives of the DCN were to build a horizontal network of research-oriented university-based memory clinics; to define standards for recruitment, recognition, assessment and treatment of MCI and dementia; to establish tissue and DNA banks; and to perform therapeutic studies in MCI and dementia.

In order to deal with the research questions cited above, large-scale cross-sectional as well as prospective diagnostic, treatment and observational studies were implemented, the results of which are stored in long-term data and biomaterial banks. The project has created an enormous bulk of data and biomaterials. The 7-year funding period was financed by the Federal Ministry of Research and Education. On a separate cohort of patients, another independent branch of the DCN performed epidemiological studies, which are described elsewhere [5, 7].

After screening, assessment and diagnostic classification, the patients described here were entered either into a long-term observational study (diagnostic and prognostic study, DAP study) or into 1 of 2 clinical trials: the first investigating the efficacy of galantamine/memantine combination therapy versus galantamine alone or versus placebo in MCI (MCI-COMBI study), and the second investigating the efficacy of galantamine/memantine combination therapy versus galantamine alone in AD (AD-COMBI study). In all studies, a large set of identical baseline variables were assessed. This article describes the design of the 3 studies and presents a summary of the baseline characteristics and cross-sectional results of all participants. More detailed results obtained at baseline, results obtained at follow-up and results of the clinical trials will be reported in future publications.

Materials and Methods

Participating Centers

Figures 1, 2 and table 1 give an overview of the nationwide cohort study. All subjects were investigated with standardized diagnostic and therapeutic procedures. Each of the 14 German specialist memory clinics of university hospitals (table 1) is equipped with clinical and scientific expertise in the area of dementia research, access to magnetic resonance imaging (MRI), the ability to run neuropsychological tests and to gather biological material and prepare it for shipment. All centers had significant practical experience in carrying out clinical research in this domain. Further details are available at the DCN website (www.kompetenz-netz-demenzen.de).

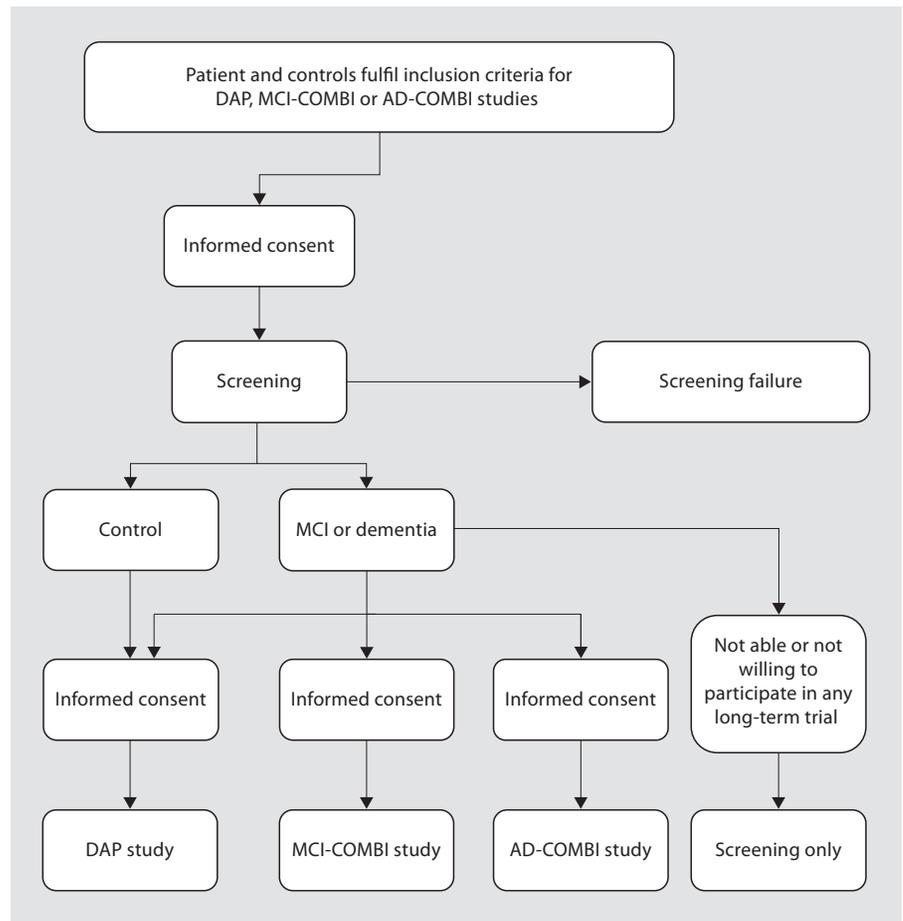


Fig. 1. Study flow chart.

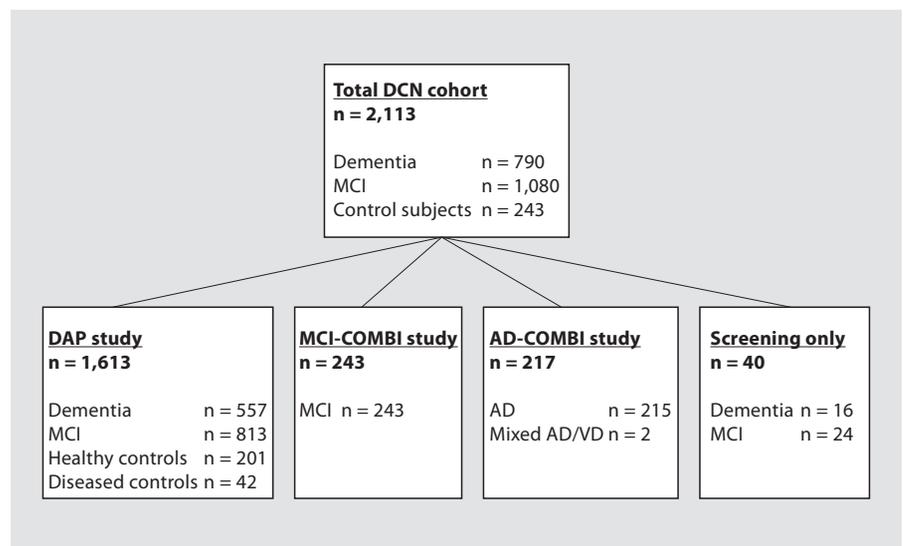


Fig. 2. Patient assignment. VD = Vascular dementia.

Table 1. Distribution of the German multicenter DCN cohort by study type and center

	DAP	MCI-COMBI	AD-COMBI	Screening only	Total
Hamburg	64	21	2	5	92
Berlin	172	28	62	2	264
Göttingen	168	9	6	0	183
Düsseldorf	143	29	6	7	185
Bonn	135	19	25	7	186
Leipzig	88	14	0	0	102
Frankfurt	94	11	6	2	113
Heidelberg	105	30	1	5	141
Homburg (Saar)	6	0	0	0	6
Mannheim	122	15	24	2	163
Erlangen	166	19	13	2	200
Freiburg	101	12	33	1	147
LMU Munich	157	22	26	0	205
TU Munich	92	14	13	7	126
Total	1,613	243	217	40	2,113

LMU = Ludwig Maximilian University; TU = Technical University.

Table 2. Diagnostic inclusion and exclusion criteria for control persons

Healthy control subjects

Inclusion criteria

- Minimum age 50 years

Exclusion criteria

- Relevant neurological diseases, e.g. Parkinson's disease, stroke, brain tumor, neuroborreliosis, epilepsy/seizures, grade II-III craniocerebral injury, normal pressure hydrocephalus, hypoxic brain damage (e.g. during surgery)
- Current alcohol abuse or known alcohol dependency or long-term intake of benzodiazepines at high doses
- Malignant disease with chemo- or radiotherapy
- Symptomatic treatment-dependent psychiatric disease (e.g. major depression)

Diseased control subjects

Inclusion criteria

- Patients who have undergone medically indicated lumbar puncture, e.g. due to headaches, intervertebral disc disease, suspected chronic CNS disease (e.g. multiple sclerosis)
- Minimum age 50 years

Exclusion criteria

- Concern about or suspicion of a neurodegenerative disease (AD, FTD, Parkinson's disease, etc.), acute inflammatory process or normal pressure hydrocephalus
- No MCI at baseline (CDR = 0), or all cognitive tests that are listed in the 'syndrome diagnosis' section are within the normal range

FTD = Frontotemporal dementia; CDR = Clinical Dementia Rating.

Screening and Selection Procedure

Patients who sought evaluation at the participating memory clinics, who were at least 50 years old and in whom organic cognitive impairment was suspected underwent a screening assessment (following informed consent) that resulted in a clinical diagnosis.

If 1 of the following clinical diagnoses was made, patients were requested to enroll in 1 of the assigned studies:

- MCI, any subtype: DAP study or MCI-COMBI study;
- AD, mild stage: DAP study or AD-COMBI trial;
- AD, moderate stage and Mini-Mental-State Examination (MMSE) score ≥ 15 : AD-COMBI trial only;
- any other dementia, mild stage only: DAP study only.

Furthermore, control subjects were recruited:

- healthy control subjects: DAP study only, with follow-up assessments;
- diseased control subjects: DAP study only, baseline assessment only.

The following exclusion criteria were applied: substance abuse or dependence, insufficient German language skills, multimorbidity, comorbid condition with excess mortality, circumstances that make regular attendance at follow-up visits questionable and lack of an informant.

Control subjects were recruited from inpatients at the participating centers (diseased control subjects) or were unpaid volunteers, e.g. spouses of patients (healthy control subjects). Inclusion and exclusion criteria are listed in table 2.

Following screening, the study criteria were checked, a second informed consent for additional diagnostic procedures or participation in treatment studies was signed and visit schedules were set up. Those patients who participated in the screening examination and received one of the relevant diagnoses, but declined participation in any study, were kept in the database (screening-only patients). Patients having none of the previously listed diagnoses were not entered into the database (screening failures). In clinical trial patients, treatment was performed according to study protocols. Standard medical treatment was initiated or recommended in the DAP study, in screening-only and in screening-failure patients.

Many, but not all, patients who participated in the 2 drug trials were requested to give written consent for the acquisition of second and third blood samples and MRI, over and above the regular follow-up examinations. This was conducted subject to the capacity of the participating centers.

Definition of Cases

The study was planned in 2001, funding was obtained for a 7-year period and consistent diagnostic criteria were applied for the total recruitment period, which lasted from 2003 to 2007. All clinical diagnoses of MCI and the different dementia subtypes were given following the published clinical research criteria at the time of study start.

The diagnoses of MCI and dementia were made on the basis of clinical and neuropsychological data. A specific MMSE threshold was not applied for diagnosis. The Clinical Dementia Rating (CDR) was applied and the overall score was determined centrally, using the Washington University CDR-assignment algorithm [8]. However, this CDR score was not used as the principle criterion for dementia. Discrepancies, therefore, exist between clinical and CDR ratings. In particular, a considerable subset of the pa-

Table 3. Diagnostic criteria for specific causes of dementia

Specific cause	Criteria proposed by	Reference No.
AD	NINCDS-ADRDA probable AD	35
AD with cerebrovascular disease	NINCDS-ADRDA possible AD	35
Cerebrovascular disease	NINDS-AIREN	36
FTD	Lund and Manchester groups	37
Lewy body disease	Consortium on DLB	38
Parkinson's disease dementia	Gelb et al., 1999	38
Huntington's disease	Vonsattel and DiFiglia, 1998	39
Progressive supranuclear palsy	Litvan, 1997	40
Corticobasal degeneration	Litvan et al., 1999	41

NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; NINDS = National Institute of Neurological Disorders and Stroke; AIREN = Association Internationale pour la Recherche et l'Enseignement en Neurosciences.

tients in the DAP study ($n = 253$), who were clinically diagnosed as having dementia, had a CDR score of only 0.5 according to the centrally determined CDR algorithm (see 'Discussion').

Diagnosis of MCI. The syndrome of MCI is heterogeneous with regard to etiology and clinical picture. Decline in memory prevails in most but not all cases. We intended to include a broad range of patients at risk of developing dementia. Therefore, instead of the classical MCI definition, which calls for a confirmed deficit in memory with otherwise essentially normal cognitive abilities, a broader definition of MCI was used, the core features being complaints of cognitive deficit in daily living and objectified decline of cognitive abilities (more than 1 SD) in at least 1 of the following domains, as evidenced by standardized neuropsychological tests: verbal learning and memory, nonverbal learning and memory, word fluency, naming, visuoconstruction, cognitive speed or executive function. Minor changes in complex activities of daily living (ADL) were tolerated, for example, handling of financial affairs and orientation in unknown surroundings [total Bayer-ADL (B-ADL) score <4].

Based on the clinical presentation and on neuropsychological test results, MCI patients received an additional clinical label of 'MCI of AD type', 'MCI of mixed-dementia type', 'MCI of frontotemporal dementia (FTD) type', 'MCI of vascular dementia (VD) type', etc., or 'unclassified', following the published research criteria for the respective dementia diagnoses (table 3), but not requiring a dementia syndrome. Clinical diagnoses and assignments of MCI subtypes were made by team conferences at the local study centers.

Inclusion in the MCI-COMBI trial was not conditional on the label of 'presumable pre-AD', but on a syndrome which comprises cognitive decline in verbal learning and memory.

Diagnosis of Mild Dementia. Decline of cognitive ability (at least 1 SD) from a previous level in at least 2 domains as evidenced by age-corrected standardized tests; impairment in ADL (B-ADL >6); changes in personality, drive, social behavior or control of emotion. These changes must have persisted for at least 3 months. No clouding of consciousness.

Diagnosis of Specific Causes of Dementia. Internationally accepted criteria were used for the diagnosis of specific causes of dementia (table 3).

Objectives and Design of the Long-Term Observational Study

The principle criterion for inclusion in the DAP study was the presence of MCI or early dementia with an MMSE score of ≥ 20 . The principle objectives of the DAP study were threefold: (1) observation of the course of a large cohort of MCI and early dementia patients, (2) examination of the utility, or combined utility, of a spectrum of methods for early diagnosis, and (3) identification of an optimal set of clinical, laboratory and imaging data for the prognosis of conversion from MCI to dementia. The principle external criterion for diagnosis and conversion will be the diagnoses that are made at follow-up.

With regard to (1), observation and multidimensional phenotyping was carried out, including demographic, medical, neuropsychological, resource utilization and other parameters. The resultant database serves to describe baseline characteristics and progression of early dementia, differences between types of dementia, rates of false and correct diagnoses, risk factors, neuropsychological profiles, etc.

With regard to (2), early diagnosis, emphasis was placed on the diagnostic power of neurochemical cerebrospinal fluid (CSF) and serum parameters, including but not limited to amyloid peptides and tau proteins, of volumetric cerebral MRI and of magnetic resonance spectroscopy (MRS). Furthermore, the diagnostic value of certain functional and behavioral inventories will be assessed.

With regard to (3), conversion rates, delay of conversion from MCI to different types of dementia, and the slope of decline will be subjected to regression analyses with relevant baseline parameters as independent variables. The AD assessment scale cognitive subscale (ADAS-cog) and the Consortium to Establish a Registry for AD neuropsychological assessment scale test series were chosen as principal measures of neuropsychological impairment, and the CDR and CDR sum-of-boxes serve as global rating scales for the severity of dementia.

Table 4. Schedule of data acquisition

Screening part 1	Screening part 2	Baseline examination
Subjective Memory Decline Scale	CDR	ADAS-cog
Baseline demographic factors	Syndrome diagnosis	Montgomery-Asberg Depression Rating Scale
Pre-existing and concomitant diseases	Etiological diagnosis	Free and Cued Selective Reminding Test
Medication/drugs	(MCI or dementia diagnosis)	Color-word test
Wechsler Memory Scale Revised (logical memory 1 and 2)	Laboratory examinations	Wisconsin Card Sorting Test
Consortium to Establish a Registry for Alzheimer's Disease – Neuropsychological Battery	Inclusion and exclusion criteria	Frontal Assessment Battery
Clock-drawing test	Informed consent	Neuropsychiatric Inventory
Trail-making test (parts A and B)	Cranial MRI	ACDS-MCI-ADL (Activities of Daily Living – MCI Version), only for patients with MCI
Assessment of spontaneous speech (Aachen Aphasia Test)		ADCS-ADL (Activities of Daily Living – AD Version), only for patients with AD
Physical examination		Risk factors
Informant Questionnaire on Cognitive Decline in the Elderly		Family history
B-ADL (activities of daily living questionnaire)		Resource Utilization in Dementia
ADL PLUS (activities of daily living questionnaire)		MRI – voxel-based morphometry
Frontal Behavioral Interview		MRS
Routine laboratory examinations		CSF for neurochemical dementia diagnosis
		Genetic testing

Objectives and Design of the Clinical Trials

The MCI-COMBI study was designed to assess the efficacy and safety of a combination of the acetylcholinesterase inhibitor galantamine with the NMDA receptor antagonist memantine [9, 10] versus galantamine alone or versus placebo in MCI. This was a 3-armed, randomized, double-blind, double-dummy, placebo-controlled, multicenter study. In total, 243 patients with MCI were randomized and treated according to the protocol. The principle inclusion criterion was the syndrome of MCI, regardless of the presumed underlying etiology. Due to suspected adverse events in 2 independent studies of galantamine in MCI [11], one sponsor (Janssen-Cilag, High Wycombe, UK) suspended delivery of medication, which led to early termination of the MCI-COMBI trial. Accordingly, the statistical analysis plan had to be amended. However, the data that were gathered were analyzed and will be published together with a detailed description of the trial. Preliminary findings, which have been presented at a conference [12], show a better outcome for the combination treatment.

The AD-COMBI study began after the termination of the MCI-COMBI-study. It assesses the 1-year efficacy and safety of a combination of galantamine and memantine versus galantamine alone. This is a 2-arm, randomized, double-blind, multicenter study. This study is still ongoing; however, recruitment is complete (last patient was in by December 2007). In total, 233 patients were randomized. As the data clearing process for the last 16 sets of baseline data is not yet complete, the baseline data of 217 patients are presented in this report.

The principle inclusion criteria for the AD-COMBI study were a clinical diagnosis of AD and an MMSE score of ≥ 15 . Of the 233 randomized patients, 2 received a diagnosis of mixed dementia,

i.e. AD plus significant cerebrovascular changes. A detailed description of the trial will be published elsewhere.

MCI patients of the DAP study who were found to have converted to AD at a follow-up visit were given the option to discontinue their enrolment in the DAP study and change to the AD-COMBI study. Three patients accepted this offer. Furthermore, 38 MCI patients from the DAP study changed to the MCI-COMBI study when this trial was started. In addition, a small number of patients ($n = 3$) who had taken part in the MCI-COMBI study, converted to AD during or after the study, and had not previously received galantamine and/or memantine were included in the AD-COMBI study.

Multidimensional Phenotyping

Neuropsychological Assessment. The tests used for patient phenotyping are given in table 4.

Routine MRI or CT Scanning. Each subject underwent an axially oriented T₁-, T₂- and FLAIR-sequence or a CT scan during the screening phase, which was clinically evaluated. In addition, semi-quantitative ratings of atrophy, infarct number and subcortical white matter changes in predefined cortical and subcortical regions of both hemispheres were performed at each center.

Structural Volumetric MRI. MRI scans were obtained on 1.5-Tesla scanners. Siemens scanners (Siemens Sonata or Siemens Magnetom Vision) were used at 8 centers and Philips scanners (Philips Gyroscan and Philips Intera) at the remaining 4 centers. The 2 centers with the lowest recruitment rate did not enroll patients into MRI scanning. For standardization of MRI acquisition across centers, acquisition parameters were provided to all centers as a guideline. The phantom test of the American College of

Radiology MRI Accreditation Program was conducted repeatedly at 11 sites of the DCN [13]. Furthermore, a single volunteer, who travelled to each of the 11 centers, was investigated at each center. The results served to further increase the comparability of the scanning results between centers. MRI scans were conducted with a sagittal magnetization-prepared rapid gradient echo sequence on Siemens scanners, and with a 3D fast T₁-weighted gradient echo sequence on Philips scanners. At all centers, the repetition time varied between 9.3 and 20 ms and echo time between 3.93 and 4.38 ms. Hippocampal and amygdalar volumes were determined for all scans [14].

Proton MRS. Four centers (Bonn, Erlangen, Hamburg and Mannheim) participated in a single-voxel ¹H-MRS study of the left medial temporal lobe using 1.5-T scanners (Philips Gyroscan Intera, Siemens Magnetom Vision, Siemens Magnetom Sonata). Multicenter reproducibility was assured by using measurements of a human volunteer [15].

Routine Clinical Chemistry Examinations. Clinical chemistry investigations were performed in the routine laboratories of the participating centers and interpreted with reference to the normal values of the respective laboratories.

Apolipoprotein E ε4 Genotyping. For DNA analysis, leukocyte DNA was isolated with the Qiagen blood isolation kit (Qiagen, Hilden, Germany). The apolipoprotein E ε4 genotype was studied as previously described [16].

Neurochemical Dementia Diagnostics. Standardized operating procedures (SOPs) for collecting, storing and shipping human body fluids were defined [17] and implemented in the participating centers. Since the biomarkers (amyloid β peptides and, to a lesser extent, tau proteins) are sensitive not only to the storage conditions, but also to the plastic material of the test tubes [18], these SOPs took several aspects of sample collection and preparation into consideration. To avoid inter-laboratory imprecision of measurements [19], all samples were measured in 1 center (Erlangen). Based on our preliminary studies on biomarkers in AD and other dementias [20–23], we performed measurements of the ‘classic’ biomarkers: Aβx–40 (The Genetics Co., Zürich, Switzerland) [24], Aβx–42 (The Genetics Co.) [24], Aβ1–42 (Innogenetics, Ghent, Belgium) [25], Tau (Innogenetics) [26] and p-Tau181 (Innogenetics) [20, 27] using established ELISAs in all available CSF samples. All samples were measured in duplicate and the means were used for further analysis.

Further Variables. In addition, a range of further demographic, historical, laboratory and psychological data were acquired at screening and at baseline, and will be presented in follow-up publications: history and medical data: schooling, professional education and achievement; medical and psychiatric history; family demographics and history of neuropsychiatric disease; medication and vitamin use; smoking and alcohol consumption; initial and current dementia and behavioral symptoms; resource utilization parameters; general medical and neurological findings. For neuropsychological and ADL variables, see table 4.

Ethical Considerations

The DAP study was approved by the Ethics Review Board of the Erlangen medical faculty (coordinating center) and by the Ethics Committees at each individual center, and was conducted in accordance with the Declaration of Helsinki. The MCI-COMBI and AD-COMBI studies were approved by the Ethics Review Board at the location of the principle investigator of the studies

(Berlin) and by the Ethics Committees at each individual center, in compliance with the regulations of the German medical drug law. Patients initially received written and verbal information regarding the study during the screening visit, for which they gave informed consent. After screening, participants were requested to sign the consent form for performing additional diagnostic procedures and for participation in the treatment studies. Patients who gave informed consent to participate were then examined in a comprehensive clinical and neuropsychological assessment. In parallel, caregivers/informants agreed to fill out a caregiver-focused questionnaire.

Data Collection

The data that were collected in the centers were transferred into a GCP-certified system (secuTrial 1.8 based on a central Oracle V9 database) via an internet-based remote data entry system. Data security and confidentiality were ensured by keeping identification data and clinical data separately, linked only by center-specific ID codes (pseudonyms). This pseudonymization was performed in the study centers, according to the TMF generic data protection concept B [28]. During the entire process of data capture, verification and analysis, clinical data were identified and linked using the pseudonyms. In addition to the data stored in the central database, each center kept a copy of its data in electronic or written form. MR images were saved in standardized format, a copy being kept both at the study center and at the DCN reference center for MR analysis. Neuropsychological tests and demographic data were stored in paper-based case report forms and case files, and were transferred from there to the electronic DCN database. Likewise, standard biochemical laboratory data were kept in a laboratory database and in a printed version at each study center, and were transferred to the DCN database.

Multimodal Biomaterial and Databank

Blood, serum and CSF samples were collected in polypropylene vials with a unique 14-digit 2-dimensional barcode. The vials were then placed and stored in a 96-well format plastic container in predefined positions. This system enabled systematic storage of several thousand aliquots collected by the participating centers, and significantly improved management of the samples.

Clinical, neurochemical and imaging data were transferred to a single databank. This enabled the linkage of different types of data from individuals or selected subject groups, as identified by unique pseudonyms. Access to these linked data is granted by the DCN steering committee.

Quality Control and Methods against Bias

Neuropsychological and related assessments were performed by experienced physicians and psychologists. Rater trainings were conducted beforehand. The clinical and neuropsychological evaluation was supported by the use of clinical DCN SOPs [29]. Furthermore, a telephone hotline was available for the center co-workers to answer ad hoc questions. Neuropsychological raters were blinded for MRI and CSF results, and vice versa. Reasons for dropout were investigated and documented. In order to minimize non-response and missing data, patients were reminded of upcoming follow-up visits, and, if necessary, called at home. SOPs for neurochemical dementia diagnostics were developed [17] and transmitted to the participating centers in written form. This secured rigorously monitored large-scale preanalytical sample han-

Table 5. Demographic and neuropsychological baseline data of main subject groups (mean values)

	All cases, MCI and dementia (n = 1,870)	MCI (n = 1,080)	All dementia cases (n = 790)	AD (n = 577)	VD (n = 35)	Mixed AD, VD (n = 72)	FTD (n = 51)	Other/undiagnosed dementias (n = 55)	Healthy control subjects (n = 201)	Diseased controls (n = 42)
Age at entry, years	68.9	66.9	71.6	71.9	72.4	75.9	64.3	69.1	66.0	64.4
Schooling, years	9.3	9.5	9.1	9.2	8.4	8.5	9.0	9.2	10.2	9.2
Male, %	48.6	53.8	41.4	37.6	51	44	51	60	44	52
CDR (sum of boxes)	3.0	1.6	4.9	4.8	4.4	5.0	5.4	5.1	0.03	0.06
MMSE score	25.4	27.1 (14)	23.1 (24)	22.9	24.2	23.4	23.7	23.5	28.5	29.0
ADAS-cog (sum)	14.8	11.5 (85)	19.4 (58)	19.3	18.5	20.3	20.9	18.4	–	–
BAYER ADL (mean)	3.3	2.3 (85)	4.6 (84)	4.5	4.9	5.0	4.9	5.0	1.1	1.1
NPI (sum)	6.3	5.0 (275)	7.8 (94)	6.8	7.3	9.8	14.6	10.3	0.5	–
MADRS (sum)	7.7	7.8 (89)	7.7 (66)	7.1	9.7	7.7	9.6	11.0	2.4	–
FBI (sum)	10.3	7.8 (61)	13.8 (78)	12.5	16.3	13.3	23.7	16.7	0.6	0.4

Figures in parentheses show the number of subjects with missing data. NPI = Neuropsychiatric Inventory; MADRS = Montgomery-Åsberg Depression Rating Scale; FBI = Frontal Behavioral Inventory.

dling. A barcode-based storage system assured fast and precise access to a required body fluid sample.

Routine MRI and research MRI data acquisition were performed in a standardized fashion [13] by the local neuroradiology departments. Multicenter reproducibility of volumetric MRI and MRS was assured by a technical phantom and a human volunteer [13, 15]. The measurements of biochemical and genetic markers, and the processing of imaging data were performed in 1 reference center to avoid center effects (neurochemical dementia markers: Erlangen; apolipoprotein E genotyping: Bonn; quantitative hippocampal and corpus callosum volumetry: LMU Munich; MRS: Bonn). As an incentive for timely and complete delivery, the participating centers obtained capitation fees for including patients and data into the study. Fees were calculated according to the completeness of clinical data, imaging studies and biological materials, i.e. only costs of data and materials that were successfully shipped to the reference centers were reimbursed.

Data handling was conducted according to the concept established by the TMF project (Telematic Platform for Medical Research Networks). Data quality in the central database was assured by several measures: (1) direct plausibility checks during data entry, where applicable, (2) queries were sent to the centers about conspicuous or incongruent data entries, as identified automatically by a set of checking rules; missing data were not replaced in the DAP study, and handling of missing data in the clinical trial will be described in the respective publication, (3) the algorithm of Morris [8] was applied centrally to determine the overall CDR score, based on the ratings of the 6 categories that were provided by the centers, and (4) volumetric MRI datasets were transferred into the central database where a rigorous completeness check was performed. Finally, the whole study was supervised by an external advisory board.

Statistical Analysis

Analyses of the baseline data set presented here were performed using descriptive statistics, 2-sided t tests, non-parametric tests and calculation of Pearson's correlations, as appropriate.

The statistical analyses were performed with SPSS for Windows (version 15.0, Chicago, Ill., USA) and SAS software (version 9.3, Cary, N.C., USA). The data presented here are based on an export of the databank from November 2007.

Results

From May 2003 until November 2007, 2,113 subjects passed the screening examinations and were assigned to 1 of the relevant diagnostic groups (fig. 2; tables 1, 5 and 6). There were 1,870 patients (790 dementia, 1,080 MCI patients) and 243 control subjects (201 healthy, 42 diseased controls). Of note, the actual number of patients is minimally lower than the number of 'cases', because a few patients who were first referred to the DAP study later switched to one of the clinical trials (MCI-COMBI or AD-COMBI). These patients were handled in the database as if they were new subjects. Upon entering one of the drug trials, patient screening was repeated and all data refreshed.

Dementia cases were further subdivided into AD (n = 577), FTD (n = 51), VD (n = 35), mixed AD/VD (n = 72), Lewy body dementia (n = 8), Parkinson dementia (n = 5), Huntington's disease and corticobasal degeneration (n = 2 each), progressive supranuclear palsy and multisystem atrophy (n = 1 each), other (n = 11) and undetermined (n = 25). Recruitment across memory clinics was uneven (table 1). Selected demographic and neuropsychological characteristics of the main groups and subgroups are shown in table 5. Additional variables not presented here

Table 6. Comparison of key demographic and key neuropsychological characteristics (mean values) of patients who participated in the observational study (DAP study) vs. those who participated in the clinical trials (MCI-COMBI study, AD-COMBI study)

	MCI all patients (n = 1,080)	MCI patients in DAP (n = 813)	MCI patients in MCI-COMBI (n = 243)	AD all patients (n = 577)	AD patients in DAP (n = 353)	AD patients in AD-COMBI (n = 215)
Age at entry, years	66.9	66.6	67.7	71.9	71.8	72.1
Schooling, years	9.5	9.5	9.2	9.2	9.2	9.0
Male, %	53.8 ¹	55.1	51.0	37.6 ²	40.8	33.0
CDR (sum of boxes)	1.6	1.7	1.4	4.8	4.7	4.9
MMSE score	27.1	27.2	27.1	22.9	23.4	22.1
ADAS-cog (sum)	11.5	11.5	11.3	19.3	19.1	19.6

¹ Significantly higher proportion of men in the group of MCI patients; p = 0.013 (χ^2 test).

² Significantly higher proportion of women in the group of AD patients; p < 0.001 (χ^2 test).

Table 7. Pearson correlations between main neuropsychological and descriptive variables in the group of all dementia patients (n = 790)

	MMSE	CDR (sum of boxes)	ADAS-cog	FBI	B-ADL
CDR (sum of boxes)	-0.33**				
ADAS-cog	-0.55**	0.27**			
FBI	-0.08	0.34**	0.11		
B-ADL	-0.28**	0.47**	0.23**	0.46**	
Age	-0.08	0.10**	0.13**	-0.04	0.15**

FBI = Frontal Behavioral Inventory. ** p < 0.01, 2-sided.

are listed in the 'Methods'. The assignment of the subjects to the 3 studies (DAP, MCI-COMBI and AD-COMBI) and the residual 'screening only' category is shown in figures 1 and 2.

Comparisons between subgroups defined by disease status show that mean age was significantly higher in AD, VD and mixed AD/VD patients compared to MCI patients (t tests, p < 0.001). Mean age was significantly lower in FTD patients compared to other dementia patients (t test, p < 0.001). Mean values for MMSE and CDR sum of boxes showed only minor variations in the 4 principle dementia subgroups (table 1). The highest achieved educational level, as determined by a 6-step ordinal scale, was significantly higher in control subjects (healthy and diseased controls) compared to dementia and MCI patients (Kruskal-Wallis test, p < 0.001, data not shown). A comparison of MCI and dementia patients showed a slightly lower mean education in dementia patients (years in school, Mann-Whitney U test, p < 0.001).

In total, 599 CSF samples were acquired (298 MCI, 259 dementia, 42 diseased controls), as well as approximately 1,700 MRI and 500 MRS data sets.

Violations of inclusion criteria were noted in a small minority of patients, i.e. 26 patients were younger than 50 years of age, 31 patients in the DAP study had MMSE scores below 20, and 4 participants of the AD-COMBI trial had MMSE scores below 15. These cases were retained in the study, and were included in the analysis following an intention-to-treat policy.

Flow Chart

The 2,113 subjects who were included in the study were assigned to 1 of 4 tracks, as shown in figure 1. Patients in the long-term observational study (DAP study) consented to participate in annual follow-up examinations. Patients in MCI-COMBI (MCI only) and AD-COMBI (AD only) were included in 2 randomized and controlled clinical trials which compared the efficacy of combined galantamine/memantine treatment with

Table 8. Comparison of large prospective multicenter studies in MCI

	DCN (MCI patients in DAP study)	Pre-AL	ACCORD	ITINAD	DESCRIPA	ADNI
Sample size (MCI subjects)	813	251	466	269	883	394
Recruitment period	2003–2007	2001–2002	1997–1999	unknown	2003–2006	2004–2007
<i>Inclusion criteria</i>						
MMSE cutoff	no	>29	no	‘abnormal’	no	<24
CDR or equivalent score = 0.5	yes	no	no	no	no	yes
Test impairment	yes	yes	no	yes	no	yes
Informant required	yes	yes	no	no	no	yes
Cutoff score for age	>50	>58	none	50–80	>55	55–90
<i>Exclusion criteria</i>						
Detailed exclusion criteria for potential causes of cognitive impairment or dementia other than neurodegenerative or vascular disease	yes	yes	no	yes	yes	yes
<i>Characteristics of the cohort</i>						
Mean age, years	66.6	72	65	70.3	70.3	74.7
Education (mean), years	9.5 (schooling years)	unknown	12.8	7.9	10.4	15.7
Females, %	45	60	55	57	57	36
Apolipoprotein E ε4 carriers, %	will be reported in future publications	unknown	40	unknown	41	unknown
<i>Biomaterials collected</i>						
Serum	yes	no	no	no	yes	yes
Volumetric MRI	yes	no	no	no	yes	yes
MRS	yes	no	no	no	no	no
CSF	yes	no	no	no	yes	yes
FDG-PET or SPECT	no	yes	no	no	no	yes

galantamine monotherapy and with placebo treatment in MCI, and galantamine/memantine treatment with galantamine monotherapy in AD.

Table 6 shows key demographic and neuropsychological characteristics of MCI and AD patients who took part in the DAP study versus those who took part in the clinical trials MCI-COMBI and AD-COMBI. In the group of MCI patients, the proportion of men was significantly higher ($p = 0.013$; χ^2 test), while there was a higher proportion of women in the group of AD patients ($p < 0.001$; χ^2 test).

Correlational Analyses

An exploratory correlation analysis between the principle neuropsychological and descriptive values was performed within the group of all dementia patients ($n = 790$; table 7).

Discussion

The DCN formed an academic research network among 14 memory clinics at German university hospitals. All patients in this study were uniformly evaluated using a defined set of clinical, neuropsychological, biochemical and imaging tests and followed up longitudinally. A longitudinal clinical database was established with the data obtained from these patients, including neuropsychological and clinical variables, a biochemical database involving blood and CSF samples, an imaging database involving a volumetric data set using a FLAIR sequence, and a DNA bank.

The largest diagnostic subgroup is represented by MCI patients. This may be partly related to an inclination of German general practitioners to refer early-stage patients with a self-reported mild cognitive decline to a university memory clinic, and may also be due to the active recruit-

ment of patients with mild cognitive disturbances. The high proportion of MCI patients, and the focus on mild-stage dementia may explain the low overall mean average age of 68.5 years.

In our cohort, the observed spectrum of dementia etiologies shows a strong preponderance of AD cases (73%). This proportion is slightly higher than the estimated percentage of AD among all causes of dementia [30]. A potential reason may be that dementia cases with early physical (neurological) symptomatology are underrepresented in the present sample, most likely due to a referral bias (i.e. most collaborating memory clinics are affiliated with psychiatric university hospitals, while dementia cases with movement disorders or vascular disorders tend to be referred to neurological departments). The second-largest subgroup of demented patients was the one including patients with FTD (6.5%).

The degree of cognitive impairment, as assessed by the MMSE score, was 4 points lower in dementia patients than in MCI patients (23.1 vs. 27.1). Mean age was 4.7 years lower in MCI than in demented patients, which may partly be explained by the delay between onset of cognitive impairment and conversion to dementia. It is also likely that some MCI patients suffered from a non-organic or non-progressive disease, which will be clarified by analyzing the follow-up data.

Participants in the AD-COMBI study had a mean MMSE score of 22.1 (inclusion criterion: MMSE score ≥ 15), while the mean MMSE score was 23.4 (inclusion criterion: MMSE score ≥ 20) for participants of the long-term observational DAP study. Otherwise, cognitive and demographic data of AD patients in the DAP and AD-COMBI studies were closely matched (table 6).

The CDR score of dementia, as determined centrally by the CDR algorithm, was 0.5 in 253 patients who received a clinical diagnosis of dementia. This discrepancy between CDR score and diagnosis of syndrome can be explained by the fact that the clinical diagnosis of dementia was made by a team of experts at each center in consideration of the whole spectrum of symptoms, which is not entirely assessed by the 6 categories of the CDR. Probably more importantly, the local investigators tended to diagnose dementia when 1 or 2 'boxes', i.e. domains, in addition to memory were rated 1. Remarkably, the Washington algorithm rates the overall CDR as '0.5' even when 3 domains including memory are rated 1, and the other 3 are 0.5. The experience that we had here is relevant to studies, e.g. drug trials that employ the CDR as the principle criterion for conversion to dementia. The investigators of this study will an-

alyze and describe the limitations of the CDR in a subsequent publication.

As expected, the analysis of correlations between key neuropsychological variables (table 7) showed a substantial inverse correlation between ADAS-cog and MMSE scores. The overall degree of dementia severity, as assessed by the CDR sum of boxes, showed a substantial correlation with the B-ADL score, but less so with ADAS-cog and MMSE scores. This is explained by the mode of CDR assessment, which is based on patients' competence in daily living. Likewise, the Frontal Behavioral Inventory score was correlated with CDR sum of boxes and B-ADL scores, but unrelated to MMSE and ADAS-cog.

For the key variables presented in table 5, the proportion of missing data was generally low and tolerable, i.e. $<2\%$ for dementia patients and 0–8% for MCI patients.

Strengths of the DCN Platform and Studies

A network of research-oriented university-based memory clinics was set up for standardized assessment of MCI, early dementia and controls in longitudinal observational studies and clinical trials. An integrated structure to capture patient data and to establish tissue and DNA banks for diagnostic and therapeutic studies in dementia and MCI were developed. Patients and controls were thoroughly phenotyped. A rigorous procedure for standardized preanalytical sample handling of biological fluid samples was implemented. This research infrastructure was supplemented by an integrated clinical databank including data from patients with dementia and MCI (covering neuropsychological, neuroimaging, neurochemistry and genetics), supported by study-specific comprehensive clinical research forms. Currently, the DCN biomaterial bank represents one of the largest prospectively collected sets of samples from subjects with MCI and early dementias. With the samples collected in the biomaterial bank, recently introduced analytical technologies (e.g. SELDI-TOF-MS, surface-enhanced laser desorption-ionization time-of-flight mass spectrometry; DIGETM, differential gel electrophoresis; and multiplexing) can be promptly tested with regard to their potential usefulness in neurochemical dementia diagnostics. A core achievement is the creation of the methods for multicenter neurochemical dementia diagnostics, volumetric MRI and MR spectroscopy. They enable the determination of the negative and positive predictive values of markers for the conversion of MCI to dementia using a multiparameter approach including clinical, neuropsychological, neurochemical and neuroimaging data.

The DCN consortium has been instrumental in improving the German dementia research infrastructure by creating a platform of centers for future studies, e.g. upcoming government-funded trials on the disease-modifying effect of simvastatin in MCI, on the effects of a behavioral cognitive therapy program for coping with dementia, and on the influence of physical activity on cognition in patients with mild Alzheimer's dementia. From a worldwide perspective, the DCN-DAP study is one of the large prospective studies on MCI and mild dementia. The SOPs that were developed in the DAP study have been adopted by other large clinical studies, e.g. the North American Alzheimer's Disease Neuroimaging Initiative (ADNI) study.

Limitations

The study was less successful than hoped in recruiting diseased control subjects, this was mainly due to the late start of recruitment within the funding period. The composition of dementia and MCI cases is not entirely representative due to the referral bias discussed above. Although every attempt was made to attain complete datasets and a rigorous data quality control process was installed, some data are missing. This is, however, mostly in the range of well below 10% per incomplete dataset. In the DAP study, data monitoring was performed centrally, without any onsite monitoring. As noted above, the MCI-COMBI study was not completed according to plan. Due to the long time lag between study initiation and completion, several advances in diagnostic criteria could not be implemented. Potential discrepancies between old and new diagnostic criteria will be analyzed post hoc and published separately, e.g. regarding criteria for vascular dementia, FTLD or MCI subtypes.

Comparison with Other Longitudinal Studies

The data on the DAP study can be compared with several other clinic-based longitudinal cohort studies, i.e. the European DESCRIPA study [31], the Italian Interdisciplinary Network on Alzheimer's disease (ITINAD) study [32], the French Pre-AL study [33], the Canadian ACCORD study [34], the AddNeuroMed (not published yet) and the (ADNI; www.adni-info.org). All of these studies are longitudinal multisite observational studies of subjects with MCI, some also include healthy elderly subjects as normal controls. These studies varied considerably with respect to sample size, inclusion and exclusion criteria and primary aims. Some of these studies, such as the European DESCRIPA study and the Pre-AL study in France, aimed to investigate markers of predementia AD,

the prospective ITINAD-study investigated MCI patients, while other studies were not designed for this purpose, e.g. the ACCORD study in Canada. The ADNI, which is the most recent of these studies, used the German DCN study as a template and is aiming primarily at: (1) collecting data to establish a brain imaging and biomarker database, (2) determining the optimum methods for acquiring and processing images for clinical trials, (3) developing 'standards' for imaging and biomarker acquisition in dementia diagnosis, and (4) 'validating' imaging and biomarker data by correlating them with behavioral and clinical data to facilitate trials on new AD therapies.

A summary of the inclusion and exclusion criteria and the patient characteristics of these studies are shown in table 8. The sample size of MCI patients recruited in these studies varied from 251 to 883, and mean age varied from 65 to 74.7 years. All earlier studies used less restrictive clinical or neuropsychological inclusion criteria than those implemented in the German DCN and the ADNI studies. The collection of biomaterials and the analysis of biomarkers were most extensive in the DCN and the ADNI studies.

It is clear from table 8 that the sample of MCI patients collected in earlier studies, i.e. Pre-AL, ACCORD and ITINAD, or the study with the broadest set of inclusion criteria, i.e. DESCRIPA, will differ strongly from the MCI patient sample in the DCN and the ADNI studies. In accordance with the primary aims of the latter 2 studies, it is likely that the DCN and the ADNI cohorts will have the highest prevalence of predementia AD patients in their patient sample, which is prerequisite to the development, evaluation and comparison of various markers. The sample size in the DCN cohort is approximately twice the size of the ADNI sample, while both have a similar CSF sampling rate.

Acknowledgements

This study has been supported by a grant from the German Federal Ministry of Education and Research (BMBF): Kompetenznetz Demenzen (01GI0420). Additional funding related to the randomized clinical trials came from Janssen-Cilag and Merz Pharmaceuticals. The latter funds were exclusively used for personnel, pharmaceuticals, blistering and shipment of medication, monitoring and as capitation fees for recruiting centers. We wish to thank all patients and their caregivers, as well as all coworkers in the cooperating centers who participated in the trial. Jack Baty (University of Washington, St. Louis, Mo., USA) provided an SAS macro and explanations for computation of the global Clinical Dementia Ratings based on the Washington University CDR-assignment algorithm.

References

- 1 Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Sczufca M: Global prevalence of dementia: a Delphi consensus study. *Lancet* 2005;366:2112–2117.
- 2 Qiu C, De Ronchi D, Fratiglioni L: The epidemiology of the dementias: an update. *Curr Opin Psychiatry* 2007;20:380–385.
- 3 Petersen RC: Mild cognitive impairment: current research and clinical implications. *Semin Neurol* 2007;27:22–31.
- 4 Panza F, D'Introno A, Colacicco AM, Capurso C, Del Parigi A, Caselli RJ, Pilotto A, Argenti G, Scapicchio PL, Scafato E, Capurso A, Solfrizzi V: Current epidemiology of mild cognitive impairment and other pre-dementia syndromes. *Am J Geriatr Psychiatry* 2005;13:633–644.
- 5 Luck T, Riedel-Heller SG, Kaduszkiewicz H, Bickel H, Jessen F, Pentzek M, Wiese B, Koelsch H, van den Bussche H, Abholz HH, Moesch E, Gorfer S, Angermeyer MC, Maier W, Weyerer S: Mild cognitive impairment in general practice: age-specific prevalence and correlate results from the German study on ageing, cognition and dementia in primary care patients (AgeCoDe). *Dement Geriatr Cogn Disord* 2007;24:307–316.
- 6 Di Carlo A, Lamassa M, Baldereschi M, Inzitari M, Scafato E, Farchi G, Inzitari D: CIND and MCI in the Italian elderly: frequency, vascular risk factors, progression to dementia. *Neurology* 2007;68:1909–1916.
- 7 Jessen F, Wiese B, Cvetanovska G, Fuchs A, Kaduszkiewicz H, Kölsch H, Luck T, Mösch E, Pentzek M, Riedel-Heller SG, Werle J, Weyerer S, Zimmermann T, Maier W, Bickel H: Patterns of subjective memory impairment in the elderly: association with memory performance. *Psychol Med* 2007;37:1753–1762.
- 8 Morris JC: The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* 1993;43:2412–2414.
- 9 Kornhuber J, Bormann J, Retz W, Hübers M, Riederer P: Memantine displaces [³H]MK-801 at therapeutic concentrations in post-mortem human frontal cortex. *Eur J Pharmacol* 1989;166:589–590.
- 10 Kornhuber J, Weller M, Schoppmeyer K, Riederer P: Amantadine and memantine are NMDA receptor antagonists with neuroprotective properties. *J Neural Transm Suppl* 1994;43:91–104.
- 11 Winblad B, Gauthier S, Scinto L, Feldman H, Wilcock GK, Truyen L, Mayorga AJ, Wang D, Brashear HR, Nye JS: Safety and efficacy of galantamine in subjects with mild cognitive impairment. *Neurology* 2008;70:2024–2035.
- 12 Peters O, Frölich L, Heuser I: Effect of combined antidementive treatment on cognitive function in MCI. *Biol Psychiatry* 2007;61: S143.
- 13 Ewers M, Teipel SJ, Dietrich O, Schönberg SO, Jessen F, Heun R, Scheltens P, van de Pol L, Freymann NR, Moeller HJ, Hampel H: Multicenter assessment of reliability of cranial MRI. *Neurobiol Aging* 2006;27:1051–1059.
- 14 Teipel SJ, Pruessner JC, Faltraco F, Born C, Rocha-Unold M, Evans A, Möller HJ, Hampel H: Comprehensive dissection of the medial temporal lobe in AD: measurement of hippocampus, amygdala, entorhinal, perirhinal and parahippocampal cortices using MRI. *J Neurol* 2006;253:794–800.
- 15 Träber F, Block W, Freymann N, Gür O, Kucinski T, Hammen T, Ende G, Pilatus U, Hampel H, Schild HH, Heun R, Jessen F: A multicenter reproducibility study of single-voxel ¹H-MRS of the medial temporal lobe. *Eur Radiol* 2006;16:1096–1103.
- 16 Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha*I. *J Lipid Res* 1990;31:545–548.
- 17 Lewczuk P, Kornhuber J, Wiltfang J: The German Competence Net Dementias: standard operating procedures for the neurochemical dementia diagnostics. *J Neural Transm* 2006;113:1075–1080.
- 18 Lewczuk P, Beck G, Esselmann H, Bruckmoser R, Zimmermann R, Fiszer M, Bibl M, Maler JM, Kornhuber J, Wiltfang J: Effect of sample collection tubes on cerebrospinal fluid concentrations of tau proteins and amyloid beta peptides. *Clin Chem* 2006;52:332–334.
- 19 Lewczuk P, Beck G, Ganslandt O, Esselmann H, Deisenhammer F, Regeniter A, Peterleit HF, Tumani H, Gerritzen A, Oschmann P, Schröder J, Schönknecht P, Zimmermann K, Hampel H, Bürger K, Otto M, Haustein S, Herzog K, Dannenberg R, Wurster U, Bibl M, Maler JM, Reubach U, Kornhuber J, Wiltfang J: International quality control survey of neurochemical dementia diagnostics. *Neurosci Lett* 2006;409:1–4.
- 20 Lewczuk P, Esselmann H, Bibl M, Beck G, Maler JM, Otto M, Kornhuber J, Wiltfang J: Tau protein phosphorylated at threonine 181 in CSF as a neurochemical biomarker in Alzheimer's disease: original data and review of the literature. *J Mol Neurosci* 2004; 23:115–122.
- 21 Lewczuk P, Esselmann H, Groemer TW, Bibl M, Maler JM, Steinacker P, Otto M, Kornhuber J, Wiltfang J: Amyloid beta peptides in cerebrospinal fluid as profiled with surface enhanced laser desorption/ionization time-of-flight mass spectrometry: evidence of novel biomarkers in Alzheimer's disease. *Biol Psychiatry* 2004;55:524–530.
- 22 Lewczuk P, Esselmann H, Otto M, Maler JM, Henkel AW, Henkel MK, Eikenberg O, Antz C, Krause WR, Reulbach U, Kornhuber J, Wiltfang J: Neurochemical diagnosis of Alzheimer's dementia by CSF Aβ₄₂, Aβ₄₂/Aβ₄₀ ratio and total tau. *Neurobiol Aging* 2004;25:273–281.
- 23 Wiltfang J, Esselmann H, Bibl M, Hüll M, Hampel H, Kessler H, Frölich L, Schröder J, Peters O, Jessen F, Luckhaus C, Perneczky R, Jahn H, Fiszer M, Maler JM, Zimmermann R, Bruckmoser R, Kornhuber J, Lewczuk P: Amyloid β peptide ratio 42/40 but not Aβ₄₂ correlates with phospho-Tau in patients with low- and high-CSF Aβ₄₀ load. *J Neurochem* 2007;101:1053–1059.
- 24 Jensen M, Hartmann T, Engvall B, Wang R, Uljon SN, Sennvik K, Näslund J, Muehlhauser F, Nordstedt C, Beyreuther K, Lannfelt L: Quantification of Alzheimer amyloid β peptides ending at residues 40 and 42 by novel ELISA systems. *Mol Med* 2000;6:291–302.
- 25 Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Buysse MA, Andreassen N, Minthon L, Wallin A, Blennow K, Vanmechelen E: Standardization of measurement of β-amyloid_(1–42) in cerebrospinal fluid and plasma. *Amyloid* 2000;7:245–258.
- 26 Hulstaert F, Blennow K, Ivanoiu A, Schoonderwaldt HC, Riemenschneider M, De Deyn PP, Bancher C, Cras P, Wiltfang J, Mehta PD, Iqbal K, Pottel H, Vanmechelen E, Vanderstichele H: Improved discrimination of Alzheimer's disease patients using the combined measure of β-amyloid_(1–42) and tau in CSF, a multicenter study. *Neurology* 1999;52:1555–1562.
- 27 Vanmechelen E, Vanderstichele H, Davidsson P, Van Kerschaver E, Van Der Perre B, Sjogren M, Andreassen N, Blennow K: Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: a sandwich ELISA with a synthetic phosphopeptide for standardization. *Neurosci Lett* 2000; 285:49–52.
- 28 Reng CM, Debold P, Specker C, Pommereining K: Generische Lösungen zum Datenschutz für die Forschungsnetze in der Medizin. Berlin, Schriftenreihe der Telematikplattform für Medizinische Forschungsnetze, 2006.
- 29 Kurz A, Perneczky R, Wolf S, Reischies F, Kornhuber J, Wiltfang J, Schmitt B, Ihl R, Hubrich P: BMBF Kompetenznetz Demenzen – Handbuch für E1 und E2. Version: 18.02. Erlangen, Berlin, 2004.
- 30 Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales: Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). *Lancet* 2001;357:169–175.

- 31 Visser PJ, Verhey F, Boada M, Bullock R, De Deyn PP, Frisoni GB, Frölich L, Hampel H, Jones R, Minthon L, Nobili F, Rikkert MO, Rigaud AS, Scheltens P, Soininen H, Spuru L, Touchon J, Tsolaki M, Vellas B, Wahlund LO, Wilcock G, Winblad B: Development of screening guidelines and clinical criteria for pre-dementia Alzheimer's disease. The DESCRIPA study. *Neuroepidemiology* 2008;30: 254–265.
- 32 Perri R, Serra L, Carlesimo GA, Caltagirone C: Preclinical dementia: an Italian multicentre study on amnesic mild cognitive impairment. *Dement Geriatr Cogn Disord* 2007;23: 289–300.
- 33 Robert PH, Berr C, Volteau M, Bertogliati C, Benoit M, Mahieux F, Legrain S, Dubois B: Neuropsychological performance in mild cognitive impairment with and without apathy. *Dement Geriatr Cogn Disord* 2006;21: 192–197.
- 34 Feldman H, Levy AR, Hsiung GY, Peters KR, Donald A, Black SE, Bouchard RW, Gauthier SG, Guzman DA, Hogan DB, Kertesz A, Rockwood K: A Canadian cohort study of cognitive impairment and related dementias (ACCORD): study methods and baseline results. *Neuroepidemiology* 2003;22:265–274.
- 35 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan E: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 1984;34:939–944.
- 36 Román GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, Amaducci L, Orgogozo JM, Brun A, Hofman A, Moody DM, O'Brien MD, Yamaguchi T, Grafman J, Drayer BP, Bennett DA, Fisher M, Ogata J, Kokmen E, Bermejo F, Wolf PA, Gorelick PB, Bick KL, Pajeau AK, Bell MA, DeCarli C, Culebras A, Korczyn AD, Bogousslavsky J, Hartmann A, Scheinberg P: Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology* 1993; 43:250–260.
- 37 Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, Freedman M, Kertesz A, Robert PH, Albert M, Boone K, Miller BL, Cummings J, Benson DF: Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 1998;51: 1546–1554.
- 38 Gelb DJ, Oliver E, Gilman S: Diagnostic criteria for Parkinson disease. *Arch Neurol* 1999;56:33–39.
- 39 Vonsattel JP, DiFiglia M: Huntington disease. *J Neuropathol Exp Neurol* 1998;57: 369–384.
- 40 Litvan I: The clinical and pathologic hallmarks of progressive supranuclear palsy. *Curr Opin Neurol* 1997;10:346–350.
- 41 Litvan I, Grimes DA, Lang AE, Jankovic J, McKee A, Verny M, Jellinger K, Chaudhuri KR, Pearce RK: Clinical features differentiating patients with postmortem confirmed progressive supranuclear palsy and corticobasal degeneration. *J Neurol* 1999;246(suppl 2):II1–II5.