Risk factors for Alzheimer's disease

A genetic-epidemiologic study

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Duijn, Cornelia Marja van
Risk factors for Alzheimer's disease:
A genetic-epidemiologic study/
Cornelia Marja van Duijn.-[S.l.:s.n.].-Ill.
Thesis Rotterdam.-With ref.-With summary in Dutch.
ISBN 90-9004811-1
Subject headings: Alzheimer's disease/epidemiology/genetics

Cover: Frans Huisman, "The Jump", 1990. Oil on canvas. 50x70 cm.

Risk factors for Alzheimer's disease

A genetic-epidemiologic study

Risicofactoren voor de ziekte van Alzheimer
Een genetisch-epidemiologisch onderzoek

PROEFSCHRIFT

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. Dr C.J. Rijnvos
en volgens besluit van het College van Dekanen.
De openbare verdediging zal plaatsvinden op
woensdag 29 januari 1992 des namiddags
te 13.45 uur

DOOR

Cornelia Marja van Duijn

geboren te Katwijk aan Zee

PROMOTIECOMMISSIE

Promotoren:	Prof. Dr A. Hofman
	Prof. Dr M.F. Niermeijer
Overige leden:	D.G. Clayton
	Prof. Dr D.F. Swaab

Voor mijn ouders
Voor Henk

Acknowledgements

The work presented in this thesis was made possible by a grant from The Netherlands Organisation for Scientific Research (NWO). The Dutch case-control study of risk factors for Alzheimer's disease was supported by a grant from the SOOM foundation, financed by the Ministry of Health and Welfare. The collaborative re-analysis of case-control studies was supported by the Commission of the European Community for the EURODEM concerted action on the epidemiology of dementia and was conducted in collaboration with the National Institute on Aging of the US National Institutes of Health and with the World Health Organization.

The author gratefully acknowledges the collaboration with the Neurogenetics Lab of the Born Bunge Foundation, University of Antwerp (C. Van Broeckhoven, L. Hendriks), the Department of Biochemistry, St Mary's Hospital London (J.A. Hardy, M.J. Mullan), the Department of Neurology, Boston University (L.A. Farrer), the TNO Institute for Aging and Vascular Research Leiden (M.E.T.I. Boerrigter, L. Nagelkerken, E. Nijhuis, J. Vijg), The Netherlands Brain Bank (D.F. Swaab, R. Ravid), the Department of Neurology, Erasmus University Rotterdam (F. van Harskamp), The Netherlands Working Group on Dementia EPIDEM (R. Haaxma, A.J. Lameris, V. Otten, R.J. Saan, W. Schulte, T.A. Tanja), The Netherlands Alzheimer Foundation Bunnik (H.W. ter Haar) and the EURODEM Risk Factors Research Group (L.A. Amaducci, V. Chandra, D.G. Clayton, L. Fratiglioni, A.B. Graves, A. Heyman, A. Hofman, A.F. Jorm, E. Kokmen, K. Kondo, J.A. Mortimer, W.A. Rocca, S.L. Shalat, H. Soininen).

Financial support for the publication of this thesis by Eli Lilly Nederland B.V., Glaxo B.V., The Netherlands Alzheimer Foundation Bunnik, The Netherlands Organisation for Scientific Research (NWO), the Rotterdam Medical Research Foundation and Schering Nederland B.V. is gratefully acknowledged.

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Publications and manuscripts based on the studies described in this thesis

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Van Duijn CM, Stijnen T, Hofman A. Risk factors for Alzheimer's disease: Overview of the EURODEM collaborative re-analysis of case-control studies. International Journal of Epidemiology 1991; 20 (suppl 2): S4-S12.

Chapter 3.2

Van Duijn CM, Clayton D, Chandra V, Fratiglioni L, Graves AB, Heyman A, Jorm AF, Kokmen E, Kondo K, Mortimer JA, Rocca WA, Shalat SL, Soininen H, Hofman A. Familial aggregation of Alzheimer's disease and related disorders: A collaborative re-analysis of case-control studies. International Journal of Epidemiology 1991; 20 (suppl 2): S13-S20.

Chapter 3.3

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Chapter 4.2
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Chapter 5.4
Van Duijn CM, Clayton D, Chandra V, Fratiglioni L, Graves AB, Heyman A, Jorm AF, Kokmen E, Kondo K, Mortimer JA, Rocca WA, Shalat SL, Soininen H, Hofman A. Interaction between genetic and environmental risk factors for Alzheimer's disease: a re-analysis of case-control studies. Submitted.

Chapter 1

Introduction

Chapter 1

Introduction

In 1907, Aloïs Alzheimer wrote on a disorder of the brain we now call Alzheimer's disease: "On the whole, it is evident that we are dealing with a peculiar, little-known disease process. In recent years these particular disease processes have been detected in great numbers. This fact should stimulate us to further study and analysis of this particular disease".[1] Despite the extensive search for clues on the cause of Alzheimer's disease by scientists for over eighty years, the words "peculiar" and "little-known" still apply to our current knowledge of the disease process. At present, Alzheimer's disease is already a major cause of disability in western populations and given the increase of the proportion of elderly people the number of patients is likely to rise in most populations.[2] Yet, remarkably little epidemiologic research into the etiology of the disease has been performed to date.[3]

The work presented in this thesis has been motivated by the lack of knowledge of risk factors for Alzheimer's disease. It has been long recognised that genetic factors are implicated, in particular in early-onset Alzheimer's disease.[4] But to what extent are genetic factors involved? Are all cases with Alzheimer's disease of genetic origin or is the disease in some cases primarily of environmental origin? If the latter is true, which environmental factors may lead to Alzheimer's disease and how do these factors interact with the genetic component? The research described in this thesis aimed at resolving these questions.

The studies of Alzheimer's disease presented here have started very much from an epidemiologic point of view. Yet, the issues addressed in this thesis required methodologic and analytic techniques of the field of genetics. The schools of thought of both epidemiology and genetics are also reflected in the design of the various studies on which this thesis is based. Some studies follow the traditional epidemiologic design as they deal with comparisons of cases and controls. Other are more compatible with genetic studies as relatives of cases and controls are the subject of investigation.

In *chapter 2* an overview is presented of the design of the studies reported. In *chapter 3* the role of genetics in Alzheimer's disease is studied. *Chapter 4* gives the evidence for

the involvement of environmental risk factors and *chapter 5* deals with gene-environment interaction. Finally, in *chapter 6* the limitations of the studies are reviewed and the findings are discussed in the context of the present knowledge of the etiology of Alzheimer's disease and related disorders.

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Chapter 2

Methods

Genetic-epidemiologic study of early-onset Alzheimer's disease

In the period from 1982 to 1987 a study of risk factors for early-onset Alzheimer's disease was carried out.* The aim of the study was to investigate genetic and environmental determinants of Alzheimer's disease. Initially, the study was designed as a case-control study. In the second phase, the investigation was extended to first degree relatives of cases and controls. The study was performed in the four northern provinces of the Netherlands and the region of Rotterdam. Here we describe the ascertainment procedure of cases and controls, the data collection and some characteristics of the population. Details on the study design have been published earlier.[1]

Ascertainment of subjects

Cases. For this study, patients were eligible in whom the diagnosis of Alzheimer's disease was made before the age of 70 years in the period of January 1980 to July 1987. The study was population-based and aimed at a complete ascertainment of cases with early-onset Alzheimer's disease in two areas of the Netherlands, i.e., the four northern provinces (Groningen, Friesland, Drenthe and Overijssel), and the region of the city of Rotterdam. To obtain a full ascertainment of patients, all nursing homes, psychiatric institutions, social-geriatric services, neurologists and facilities for computed tomography in the specified areas were asked for patients with dementia. The patients were then seen by Drs Tanja, Haaxma, or Schulte who independently confirmed the diagnosis of probable Alzheimer's disease using a standard protocol similar to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the

*The study was initiated by the Working Group Epidemiology of Dementia. At that time, members of this working group were: Drs R Haaxma, A Hofman, AJ Lameris, RJ Saan, W Schulte, TA Tanja, with the support of Mrs V Otten.

Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA).[2] Dementias other than Alzheimer's disease (e.g., multi-infarct dementia and dementia secondary to alcoholism, depression, metabolic disorders, and other conditions) were excluded on the basis of the clinical history, neurologic examination, and neuropsychologic and laboratory tests. Patients with symptoms of Parkinson's disease before the onset of Alzheimer's disease were also excluded.

The inclusion criteria for patients were: a typically slow progressive decline of intellectual function,[2] a score on the Clinical Dementia Rating scale of more than 0.5,[3] a score on the Short Portable Mental Status Questionnaire (SPMSQ) of less than 20 (out of 30),[4] a score of seven or less on the Hachinski-scale,[5] no evidence of abnormalities on computed tomography other than cerebral atrophy, nor of focal dysfunction on electro-encephalography. Of the 278 patients brought to our attention, 201 satisfied these criteria. The family of one patient refused cooperation and for two others no informant could be found. In 198 (98%) patients, data on risk factors were obtained along with a serum sample. In all ten cases, who had an autopsy, the diagnosis was confirmed.[1]

Controls. For each patient a reference subject was selected, matched for age (within five years), gender and place of residence. These controls were drawn randomly from the population register of the municipality of the patient at the time of diagnosis. All control subjects had a SPMSQ score of 20 or over. For controls, the first person asked consented in 103 cases (52%), in 68 (34%) it was the second selected person, in 23 (12%) the third, and in four (2%) the fourth.

Data collection

Case-control study. Informed consent was obtained from the responsible family member for cases and directly from the control subjects. Data on putative risk factors for Alzheimer's disease were assessed by a structured interview. Because of the cognitive decline of the patients, the history was taken from the next of kin of the patient. To assure symmetry of data collection, we also interviewed a next of kin for the control subject. For 174 cases (88%) and 188 controls (95%) the informant was the spouse or an adult child. As etiologic factors were studied, questions referred to exposure of the patient before the age of onset of dementia. Onset age of Alzheimer's disease was estimated as the age at which memory loss or change in behavior was first noted by the next of kin or any other relative. For control subjects, a "reference age" was defined

based on the age of onset of Alzheimer's disease in the matched case and only exposures before this "reference age" were considered.

The interviews were done by three interviewers, who in general obtained the information in both the patient and his or her control. We aimed to conduct the interview shortly after the diagnosis was made. In practice, patients were interviewed three months to three years following the diagnosis of Alzheimer's disease. The interview included questions on (1) family history of dementia and related disorders, (2) parental age at birth, (3) medical history, including head trauma and psychiatric history, (4) environmental factors including smoking, alcohol and occupational history, and (5) life events. The disease history of patients and controls was evaluated by closed questions with additional open ended questions about medical treatment and admission to hospital.

Pedigree studies. In the case-control study, full pedigree information on dementia was obtained from the next of kin as part of the structured interview. All first degree relatives were listed and we asked specifically about the occurrence of dementia, Down's syndrome and Parkinson's disease in them. To increase the validity, all data on family history were verified by a sibling of the participant. Verification was obtained in the period from 1989 to 1990, as part of a follow-up study on mortality of the patients. Again, we asked about the occurrence of dementia and related disorders in all first degree relatives but in addition we questioned siblings extensively on the cause and the course of the dementia in the affected relatives. If the patient had been admitted to hospital, the diagnosis was checked in independent medical records.

The pedigree structure of 17 patients (9%) was consistent with autosomal dominant inheritance of Alzheimer's disease. The criteria for autosomal dominant inheritance were: (1) at least three patients with reported dementia in two generations; (2) at least two patients with detailed records with the clinical diagnosis of Alzheimer's disease. Within the 17 families in which the disease was apparently inherited as an autosomal dominant disorder, all relatives (first to third degree) were screened for dementia. Data on risk factors for Alzheimer's disease were collected in affected and in unaffected relatives along with a blood sample for DNA extraction. Again, informed consent was obtained from family members for affected relatives and directly from the non-demented relatives.

Population

Tables 2.1.1 and 2.1.2 give a brief overview of the study population. Table 2.1.1 shows

Table 2.1.1 Age at disease onset and at diagnosis of the 198 clinically diagnosed Alzheimer patients

	Men n=74	Women n=124
Age at onset:		
-54 years (number)	22	34
55-59 years (number)	28	48
60-69 years (number)	24	42
Mean (years and SD)	56.3 (6.0)	57.1 (4.8)
Age at diagnosis:		
-59 years (number)	21	38
60-69 years (number)	53	86
Mean (years and SD)	61.1 (3.0)	61.3 (3.9)

Table 2.1.2 Family history of dementia and education in 198 patients with clinically diagnosed Alzheimer's disease and 198 age- and gender-matched controls*

Variable	Yes	No	OR†	OR‡
Family history:				
Cases	96	102	4.5 [2.7-7.7]	4.9 [2.8-8.4]
Controls	37	161		
Less than 7 years education:				
Cases	112	86	1.4 [0.9-2.1]	1.7 [1.1-2.7]
Controls	88	110		

* In first degree relatives
† Odds ratio, with 95% confidence interval within parentheses
‡ Odds ratio adjusted for dementia in first degree relatives or education

the age at onset and age at diagnosis of the patients. Of the 198 patients, 74 were men and 124 were women. The mean age at onset was 56 years in men and 57 years in women. At the time that we interviewed the informants, 41 of the patients were living at home and 157 in nursing homes or other institutions. One hundred twenty-seven patients lived in the four northern provinces of The Netherlands, whereas 71 lived in the region of the city of Rotterdam. Table 2.1.2 shows that Alzheimer's disease was associated with family history of dementia and education. Adjusted for education, the odds ratio for those with at least one first degree relative with dementia was 4.9 (95% confidence interval 2.8-8.4). Familial aggregation of Alzheimer's disease is further discussed in chapter 3.2. The odds ratio for those with less than seven years of education was 1.7 (95% confidence interval 1.1-2.7), when adjusting for family history of dementia. Possible explanations for the relation between education and Alzheimer's disease are addressed in chapter 6.3. Family history of dementia and education were always considered as putative confounders when studying other risk factors for Alzheimer's disease.

Acknowledgements

This research was supported by the SOOM Foundation, the Netherlands Organisation for Scientific Research (NWO) and the Eurodem EC Concerted Action on the Epidemiology and Prevention of Dementia. We thank Drs Wim Schulte, Teun Tanja, Rob Haaxma, Arie Lameris and Rolf Saan for their contributions to this study and Helen de Bruijn, Micheline de Haes, Jeanette Kamman, Hanneke van Meurs, Verona Otten and Caroline Valkenburg for data collection and genealogy studies.

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The EURODEM collaborative re-analysis of case-control studies of Alzheimer's disease

A collaborative re-analysis was conducted based on raw data of 11 case-control studies of risk factors for Alzheimer's disease to evaluate the evidence for the association of Alzheimer's disease with various putative risk factors.[1,2] The aim of the analysis was to study risk factors for Alzheimer's disease, with sufficient power to detect associations with relatively rare exposures and with specific subgroups of Alzheimer's disease. In addition, individual studies were re-analyzed to see whether associations were consistently found across studies. Here we will give a brief overview of the case-control studies that contributed data to the re-analysis[3-13] and discuss the strategy that has been followed in the analysis.

Description of the individual studies

All case-control studies of Alzheimer's disease conducted before January 1, 1990, were traced through medline search, review papers, and personal contacts. Studies in which the patients did not meet the NINCDS-ADRDA or DSM III criteria for the clinical diagnosis of Alzheimer's disease were excluded.[14,15] Thus, eleven studies were identified as eligible for the re-analysis. Table 2.2.1 gives an overview of studies included in the re-analysis. The selection of cases and controls in each study is described in table 2.2.2. In the tables, the studies are ordered alphabetically by country and city of origin. In nine of the eleven studies data collection has been symmetrical for cases and controls. In most studies, data were collected by interviewing a next of kin in person. There were three

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This chapter is based on: Van Duijn CM, Stijnen T, Hofman A. Risk factors for Alzheimer's disease: Overview of the EURODEM collaborative re-analysis of case-control studies. Int J Epidemiol 1991;20 (suppl 2):S4-S12.

Table 2.2.1 Studies included in the EURODEM collaborative re-analysis of case-control studies

Country	Principal investigator	Number of (response rate) Cases	Controls	Origin Cases	Controls	Data collection
Australia[11]	Broe, Henderson, Jorm, Creasey	170 (-)	170 (-)	Hospital/GP	Population	Structured interview at home with next of kin
Finland[3]	Soininen	63 (61%)	91 (50%)	Population	Population Nursing home	Structured interview with next of kin for cases and with controls in person/ Parish records
Italy[6]	Amaducci, Fratiglioni	116 (79%)	213 (-)	Hospital	Hospital:116 Neighborhood/ friend:97	Structured interview at home with next of kin
Japan[13]	Kondo	34 (-)	68 (-)	Hospital	Neighborhood	Structured interview with spouse for cases and with controls in person
The Netherlands[10]	Hofman	198 (99%)	198 (61%)	Population	Population	Structured interview at home with next of kin
USA - Bedford[8]	Shalat	106 (77%)	214 (31%)	Hospital	Neighborhood (registered voters)	Mailed questionnaire to next of kin with additional phone calls
USA - Denver[7]	Chandra	64 (100%)	64 (100%)	Hospital	Hospital	Structured interview with next of kin
USA - Durham[4]	Heyman	46 (100%)	92 (100%)	Hospital	Population random digit dialing	Structured interview with next of kin
USA - Minneapolis[5]	Schuman, Mortimer	78 (98%)	124 (84%; 64%)	Hospital	Hospital:76 Neighborhood:48	Structured with informant who lived >5 years with case/control
USA - Rochester[9]	Kokmen	392 (-)	392 (-)	Register	Register	Medical records
USA - Seattle[12]	Graves	130 (69%)	130 (-)	Hospital	Friend/Non-blood relative of case	Structured interview with next of kin by telephone

studies that differed in this respect. In the study conducted in USA Bedford,[8] data were collected by mailed questionnaires, in the USA Rochester study[9] only medical records were used and in the USA Seattle study[12] the data were collected by telephone interview. Four studies (Australia,[11] Finland,[3] The Netherlands,[10] USA Rochester[9]) can be considered as population-based, i.e., they aimed at a full ascertainment of cases with Alzheimer's

Table 2.2.2 Selection of cases and controls in the 11 case-control studies

Study	Diagnosis: criteria*; period;onset (years)	Exclusion criteria cases†	Exclusion criteria controls†	Matching variables
Australia[11]	NINCDS-ADRDA possible/probable; 1986-1988	Not English speaking No suitable informant	As for cases MMSE ≤26	Gender, GP, age (± 3 years)
Finland[3]	NINCDS-ADRDA possible/probable; 1979; onset > 65	Oligophrenia, chromosomal abnormality, MID (HIS>7) secondary dementias	As for cases Dementia, meningitis encephalitis	Gender, age, duration hospitalisation
Italy[6]	NINCDS-ADRDA probable; 1982-1983 onset 40-80	No next of kin available Residence outside study region	As for cases Dementia (Blessed) Relative of case	Gender, age (± 3 years), residence, hospital
Japan[13]	DSM-III -	No suitable informant Cerebrovascular lesions	-	Gender, age (± 5 years), residence
The Netherlands[10]	NINCDS-ADRDA probable;1980-1987 onset <65	MID (HIS>7), Parkinson's disease, secondary dementias	Dementia (SPMSQ)	Gender, age (± 5 years), residence
USA - Bedford[8]	DSM-III, NINCDS-ADRDA; 1975-1982	Alcoholism, severe head trauma, residence outside Eastern Massachusetts, females	Household<2 Non-voters	Gender, age, residence
USA - Denver[7]	NINCDS-ADRDA probable;1975-1985 onset >70	No suitable informant	As for cases Dementia (SPMSQ)	Gender, age (± 3 years), residence, relationship
USA - Durham[4]	NINCDS-ADRDA; onset <67	Stroke, alcoholism Parkinson's disease	MMSE≤21	Gender, age (± 5 years)
USA - Minneapolis[5]	NINCDS-ADRDA probable;1979-1982	Secondary dementias Females	As for cases Dementia	Gender, age, (± 3 years), residence, race
USA - Rochester[9]	NINCDS-ADRDA; onset 1960-1974	Residence outside Rochester Other dementias	Symptoms dementia	Gender, age (± 3 years)
USA - Seattle[12]	DSM-III; NINCDS-ADRDA possible/probable; 1980-1985	MMSE>26, Stroke, Parkinson's disease, major affective disorder, hypothyroidism No suitable informant	As for cases Memory loss	Gender, age (± 10 years)

* NINCDS-ADRDA denotes the criteria for Alzheimer's disease from the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association;[15] DSM-III denotes the criteria for Alzheimer's disease from the Diagnostic and Statistical Manual for mental disorders, 3rd edition[16]

† MID denotes Multi-Infarct Dementia; HIS denotes Hachinski Ischemic Score;[17] MMSE denotes Mini-Mental State Examination[18]; Blessed denotes Blessed information, memory and concentration test;[19] SPMSQ denotes Short Portable Mental Status Questionnaire[20]

disease in a defined geographical area.[4,9,10,11] An important feature of all case-control studies is that each is based on prevalent cases, who may have been diagnosed with Alzheimer's disease for several years, as well as incident or newly diagnosed cases.

Australia

This study was conducted by Broe, Henderson, Creasey, Jorm and coworkers and comprised 170 cases and 170 population controls.[11] The study aimed at a complete ascertainment of Alzheimer cases in a series of general practices in Sydney. Ascertainment was carried out through dementia clinics at two hospitals and the general practitioners in the catchment area of these hospitals. For cases, there were no inclusion criteria for onset age, but the large majority was of late-onset. Cases were diagnosed between 1986 and 1988. Controls were matched for age, gender and general practice. Data collection was completely symmetrical for cases and controls, i.e., for both cases and controls data were obtained by a structured interview of a next of kin.

Finland

The study of Soininen and coworkers comprised 63 Alzheimer cases with an onset of disease of 65 years or older.[3] This study was conducted in 1979 and aimed to ascertain all patients with the diagnosis of dementia in a defined geographical area of eastern Finland. The response rate for cases was 61%. Controls (n=91) were randomly drawn from nursing homes and the general population. Cases and controls were matched for gender, age and institutionalisation and length of stay. Data collection was not symmetrical for cases and controls: control subjects were interviewed directly whereas the information of the cases was obtained by interviewing an informant.

Italy

Amaducci, Fratiglioni and coworkers conducted a hospital-based multi-centre study of 116 Alzheimer cases.[6] Cases were diagnosed in 1982 and 1983. Patients with a disease onset between 40 and 80 years were eligible. Most cases were of early-onset: the onset of 91% of the sample was before 70 years. The response rate for cases was 79%. There were two control populations in this study: 116 hospital controls and 97 neighborhood controls. Cases and controls were matched for gender, age, area of residence and hospital. Response rates for controls were unknown. For both cases and controls an informant was interviewed.

Japan

The study of Kondo et al comprised 34 cases and 68 neighborhood controls.[13] Case selection was hospital-based and there were no inclusion criteria for onset age. Information for the cases was obtained from the spouse. Cases and controls were matched for age and gender. Data collection was not symmetrical for cases and controls. For cases an informant was interviewed whereas controls were interviewed directly.

The Netherlands

The study of Hofman et al comprised 198 cases with early-onset Alzheimer's disease and 198 population controls and has been described extensively in chapter 2.1.[10] The study aimed at a complete ascertainment of Alzheimer patients in whom the diagnosis was made before the age of 70 years in four Northern provinces of the Netherlands and the area of metropolitan Rotterdam. Ascertainment was carried out through all neurologic, psychiatric and geriatric services in the study areas. All cases were diagnosed between 1980 and 1987 and the response rate for cases was 99%. Control subjects were drawn randomly from the same municipality as the cases. Cases and controls were matched for age, gender and area of residence. The response rate for control subjects was 61%. For both cases and controls an informant was interviewed.

USA, Bedford, Massachusetts

The study of Shalat et al comprised 106 Alzheimer patients diagnosed between 1975 and 1982.[8] Since the cases were derived from a Veterans hospital, all cases were male. Although there were no restrictions for onset age, the onset of disease of the majority (61%) of the patients was before the age of 65 years. Neighborhood controls (n=214) were drawn from lists of registered voters. The cases could be matched for gender and age to 162 controls. The response rate was 77% for cases and 31% for controls. Data were collected by self-administered questionnaires, mailed to a next of kin of cases and controls. Additional phone calls were made for verification and clarification of incomplete questionnaires. The study aimed to collect the data symmetrically for cases and controls in that informants of controls were asked not to consult the study subject for information.

USA, Denver, Colorado

Chandra and coworkers have studied 64 cases with late-onset Alzheimer's disease, i.e. onset age after 70 years.[7] Cases were diagnosed in the period 1975-1985. Cases and controls were derived from the same outpatient clinic. Cases were matched to the

controls for gender, age and race. In addition, cases and controls were matched for relationship to informant. Information was collected symmetrically for cases and controls. The response rate was 100% for cases as well as controls.

USA, Durham, North Carolina

The study of Heyman et al included 46 cases with early-onset Alzheimer's disease and 92 population controls.[4] Case selection was hospital-based. The response rate for patients was 100%. Controls were selected from the population by random-digit dialing. Cases and controls were matched for gender and age. The response rate was 100% for controls. For cases as well as controls, data were collected by a structured personal interview with a next of kin.

USA, Minneapolis, Minnesota

The study of Schuman, Mortimer and coworkers, was conducted in a Veterans hospital.[5] The study comprised 78 male cases, diagnosed between 1979 and 1982. There were two control populations in this study: 76 hospital controls and 48 neighborhood controls. Cases and controls were matched for gender, age, residence and race. For both cases and controls an informant was interviewed. The response rate for cases was 98%. For hospital controls and neighborhood controls, the response rates were 84% and 64% respectively.

USA, Rochester, Minnesota

The study of Kokmen et al was based on the Rochester register.[9] The study included all patients with Alzheimer's disease with an onset of disease between 1960 and 1974. The register is considered to give a nearly complete ascertainment of Alzheimer patients admitted to hospitals and outpatient facilities in the Rochester area. Control subjects were also drawn from the register. However, since the register has been shown to cover over 95% of the Rochester population, the control subjects may be considered population-based. This is the largest case-control study conducted to date. The study comprised 392 cases and 392 control subjects. Since the data collection was completely based on medical records, this study only yielded information on medical history.

USA, Seattle, Washington

The study of Graves et al was a hospital-based study of 130 cases diagnosed between 1980 and 1985.[12] For cases, there were no exclusion criteria for onset age. The response

rate was 69%. Cases were compared to 130 neighborhood controls, matched for gender and age. Furthermore, cases and controls were matched for relationship to informant. Data were collected by telephone interview. Information was collected symmetrically for cases and controls.

Strategy of analysis

The raw data of all eleven studies were centralized at the Department of Epidemiology and Biostatistics of the Erasmus University Medical School, Rotterdam, The Netherlands. The principal investigators of the studies were invited to a first workshop on the collaborative re-analysis and they all attended this meeting. During this workshop, the strategy of analysis was discussed with other invited epidemiologists and biostatisticians. In working groups, the analyses for the putative risk factors were prepared. On the basis of these discussions, the data were re-analyzed and the results of these analyses were discussed during a second workshop.

In the re-analysis, only studies in which the data were collected symmetrically for cases and controls have been included. Two studies (Finland and Japan) did not fulfil this criterion, in that control subjects were interviewed personally but the patient's history was taken indirectly from an informant.[3,13] A second restriction concerned the control subjects. Only three studies in which the data were collected symmetrically included a group of hospital controls,[5-7] and two of these studies also had a population control group.[5,6] To increase comparability with the other studies, the analysis was restricted to population controls for studies with two control groups. A separate analysis based on hospital controls gave generally similar findings and did not change any of the conclusions. Since we were interested in etiologic factors, only exposures more than one year before the disease onset were included in the analysis. In control subjects, only exposures before the age of onset of the matched case were considered.

The strength of the association between Alzheimer's disease and the putative risk factors was assessed by computing of the odds ratio as an estimate of the relative risk.[16] Odds ratios were estimated by maximum likelihood and the 95% confidence intervals were based on the asymptotic standard errors. Since all included studies were matched for age and gender, odds ratios were estimated using conditional logistic regression analyses.[16] Thus, possible confounding by age and sex was taken care of by the matched design and the matched analysis. Potential confounding by family history of dementia and

education was controlled by entering these variables into the logistic regression model. For family history of dementia, cases and controls with one or more first degree relatives affected with dementia were considered to have a positive family history. The number of years of education was available in ten studies[3-8,10-13] and was added as a potential confounder to the conditional logistic regression model. Education was also considered as a dichotomous variable (less than twelve years education versus twelve years education or more), to allow for a possible threshold effect. To test whether risks differed significantly across studies, covariables representing the interaction between the studies and the determinant were entered into the model. If the overall test for heterogeneity was significant, the study that differed was excluded and heterogeneity across the other studies was again tested. In case of heterogeneity, pooled odds ratios were estimated including and excluding the deviant studies. Stratified analyses were conducted based on gender, onset age and family history of dementia. For onset age, we did not break the matching of the patients and controls.

Two additional analyses were conducted to exclude some possible sources of bias. First, since the studies were partly based on prevalent cases, selection bias may result from differential survival. The observed risk factors may therefore relate to predictors of survival, rather than to the risk of Alzheimer's disease. To overcome this problem, subgroup-analyses were conducted in incident cases, i.e. in patients who participated in the study within one year after diagnosis. Another subgroup-analysis was related to information bias, which may occur in particular when cases and controls are not matched for relationship to informant. To investigate the effect of this type of possible bias, we conducted analyses including only matched pairs which were concordant for relationship to informant. These analyses gave results very similar to the overall analysis and did not alter any of the conclusions.

A great variety of risk factors has been studied in the eleven case-control studies (table 2.2.3). For this re-analysis, the risk factors were grouped into six categories: (1) family history of dementia and related disorders; (2) parental age at birth; (3) head trauma; (4) medical history; (5) psychiatric history; (6) environmental factors.For each risk factor, exposure definition and comparability of measurement across studies was evaluated before re-analyzing the data. This procedure and the results of the collaborative re-analysis are presented in seven separate papers published as a supplement to the June 1991 issue of the International Journal of Epidemiology.[21-27]

Table 2.2.3 Risk factors assessed in the EURODEM collaborative re-analysis of case-control studies

Risk factor n=number of studies	Studies that assessed the risk factor	Number of cases and controls
Family history of:		
Dementia n=9	Australia, Finland, Italy, Japan, The Netherlands, US-Bedford, US-Denver, US-Durham, US-Seattle	883 cases 1190 controls
Down's syndrome n=7	Australia, Finland, Italy, Japan, The Netherlands, US-Denver, US-Durham	678 cases 877 controls
Parkinson's disease n=2	Italy, The Netherlands	314 cases 411 controls
Patient history of:		
Parental age n=7	Australia, Italy, Japan, The Netherlands, US-Durham, US-Minneapolis, US-Seattle	653 cases 759 controls
Head trauma n=11	Australia, Finland, Italy, Japan, The Netherlands, US-Bedford, US-Denver, US-Durham, US-Minneapolis, US-Rochester, US-Seattle	1385 cases 1739 controls
Anesthesia n=8	Australia, Italy, Japan, The Netherlands, US-Durham, US-Minneapolis, US-Rochester, US-Seattle	1145 cases 1352 controls
Thyroid disease n=10	Australia, Finland, Italy, The Netherlands, US-Bedford, US-Denver, US-Durham, US-Minneapolis, US-Rochester, US-Seattle	1350 cases 1668 controls
Epilepsy n=8	Australia, Finland, Japan, The Netherlands, US-Bedford, US-Durham, US-Minneapolis, US-Rochester	1145 cases 1401 controls
Viral infections n=10	Australia, Finland, Italy, Japan, The Netherlands, US-Denver, US-Durham, US-Minneapolis, US-Rochester, US-Seattle	1165 cases 1321 controls
Bacterial infections n=7	Australia, Finland, Japan, The Netherlands, US-Durham, US-Minneapolis, US-Rochester	975 cases 1123 controls
Atopy n=5	Australia, Italy, The Netherlands, US-Minneapolis, US-Seattle	692 cases 835 controls
Osteoarthritis n=3	Australia, US-Durham, US-Minneapolis	288 cases 374 controls
Blood transfusion n=5	Australia, Italy, The Netherlands, US-Durham, US-Minneapolis	602 cases 785 controls
Severe headaches n=4	Australia, The Netherlands, US-Durham, US-Minneapolis	486 cases 572 controls
Psychiatric disorders n=8	Australia, Finland, Japan, The Netherlands, US-Bedford, US-Durham, US-Minneapolis, US-Rochester	1145 cases 1401 controls
Alcohol intake n=9	Australia, Finland, Italy, Japan, The Netherlands, US-Bedford, US-Durham, US-Minneapolis, US-Seattle	935 cases 1288 controls
Smoking habits n=10	Australia, Finland, Italy, Japan, The Netherlands, US-Bedford, US-Denver, US-Durham, US-Minneapolis, US-Seattle	991 cases 1300 controls
Occupational exposures n=5	Australia, Japan, US-Bedford, US-Durham, US-Minneapolis	428 cases 656 controls
Education n=10	Australia, Finland, Italy, Japan, The Netherlands, US-Bedford, US-Denver, US-Durham, US-Minneapolis, US-Seattle	996 cases 1349 controls

Acknowledgements

The collaborative re-analysis of case-control studies was supported by the Commission of the European Community for the EURODEM concerted action on the epidemiology of dementia, the Netherlands Organisation for Scientific Research (NWO) and was conducted in collaboration with the National Institute on Aging of the US National Institutes of Health and with the World Health Organization. For the acknowledgements of the individual studies this re-analysis is based upon please see appendix (page 33). We thank Marcel Eijgermans, Gerrit-Anne van Es and Eric Neeleman for their help in data analysis.

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Appendix

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Chapter 3

Genetic factors

Introduction

It is generally agreed upon that genetic factors are implicated in Alzheimer's disease.[1] Although there is evidence for autosomal dominant inheritance in a considerable number of families with multiple affected relatives,[2] the majority of cases appear to be sporadic[3] and there is still wide disagreement about the percentage of patients with Alzheimer's disease that may be explained by genetic factors.[1]

This chapter aims to delineate the role of genetic factors in Alzheimer's disease. Firstly, chapter 3.2 addresses familial aggregation of Alzheimer's disease, Down's syndrome and Parkinson's disease. Secondly, chapter 3.3 deals with the genetics of early-onset Alzheimer's disease. Risk of Alzheimer's disease and segregation of disease were studied in first degree relatives of 198 patients with early-onset Alzheimer's disease and 198 age- and sex-matched controls. Thirdly, chapter 3.4 gives the results of a study of 100 patients with early-onset of Alzheimer's disease, who were screened for a putative causal mutation in the amyloid precursor protein that has been reported by Goate et al.[4]

The findings presented in chapter 3.2 are based on the collaborative re-analysis of case-control studies, as described in chapter 2.2. The other chapters (3.3-3.4) are based on the case-control study of early-onset Alzheimer's disease, which has been described in chapter 2.1.

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Familial aggregation of Alzheimer's disease and related disorders

Although the cause of Alzheimer's disease is still unknown, genetic factors seem to play an important role in its etiology.[1,2,3-5] Together with age, a positive family history of dementia is one of the few established risk factors for Alzheimer's disease.[6] Alzheimer's disease has also been linked with a family history of Down's syndrome and of Parkinson's disease.[6,7] In this paper we present a re-analysis of case-control studies that examined familial aggregation of Alzheimer's disease and other disorders.[7-16] The aims of this analysis were to compare risk estimates from the individual studies, to analyze the pooled data in order to obtain stable risk estimates, and to study subgroups in the pooled data set, based on gender and onset age. First, we will briefly review the evidence for an association of Alzheimer's disease with family history of dementia, Down's syndrome and Parkinson's disease.

Familial aggregation of Alzheimer's disease has been long recognised.[17,18] In a number of families the disease is apparently inherited as an autosomal dominant disorder.[2] It has been suggested that all cases of Alzheimer's disease may be due to autosomal dominant inheritance.[1,19-22] Other studies have suggested a more complex mechanism, in which genetic as well as environmental factors may be implicated.[2,23-25] There is some evidence from genetic studies that the strength of familial aggregation of Alzheimer's disease may vary with age of onset. Heston has suggested that familial aggregation of Alzheimer's disease may be specific to early-onset patients.[26] In his study, familial aggregation was observed only in early-onset patients. The risk in relatives of autopsy-proven patients diagnosed after the age of 70 years was not statistically different from the risk in the general population. Thal and coworkers reported that nearly 50% of the patients with early-onset of disease (before 55 years) had a positive family history of dementia, as

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This chapter is based on: Van Duijn CM, Clayton D, Chandra V, Fratiglioni L, Graves AB, Heyman A, Jorm AF, Kokmen E, Kondo K, Mortimer JA, Rocca WA, Shalat SL, Soininen H, Hofman A, for the EURODEM Risk Factors Research Group. Familial aggregation of Alzheimer's disease and related disorders: a collaborative re-analysis of case-control studies. Int J Epidemiol 1991;20 (suppl 2):S13-S20.

compared to only 25% of those with a late-onset.[27] Although several studies have also supported this hypothesis,[12,28,29,30] these findings were not supported by others.[31-33]

Family history of dementia has been studied in a variety of case-control studies, which, in contrast to the genetic studies, did not adjust for the age of the relatives when assessing family history.[7-9,11-13,15,16,35-36] Nine studies reported a significantly higher risk of Alzheimer's disease for relatives of patients with dementia. The only study that failed to show familial aggregation of Alzheimer's disease was of late-onset patients.[12] In studies that comprised only early-onset cases, an increase in risk of Alzheimer's disease for subjects with a positive family history of dementia was consistently reported.[7,9,35]

Family history of Down's syndrome has been associated with Alzheimer's disease. There is much evidence for a link between Alzheimer's disease and Down's syndrome. The Alzheimer type neuropathologic changes have been shown in patients with Down's syndrome[37-39] and genetic linkage to chromosome 21 has been reported in a number of families in which Alzheimer's disease was apparently inherited as an autosomal dominant disorder.[3-5] These observations have led to the hypothesis of familial aggregation of Alzheimer's disease with Down's syndrome. A higher frequency of presenile Alzheimer's disease than expected has been observed in relatives of patients with Down's syndrome,[40] but this finding was not confirmed by a study of the family history of dementia of 188 patients with trisomy 21 and 185 controls.[41] It cannot be excluded, however, that Alzheimer's disease is associated with family history of Down's syndrome due to translocations on chromosome 21.[41] Studies of the family history of Down's syndrome of patients with Alzheimer's disease have also yielded equivocal results. Family history of Down's syndrome has been studied in 10 studies.[9,11,12,16,19,20,26,30,33,42] Although seven studies observed more patients with a positive family history of Down's syndrome as compared to controls,[9,11,12,16,30,33] a significant association was established in only three studies.[9,16,26] The study of Heston suggested that the risk was only increased for early-onset Alzheimer's disease.[26] In three studies that have examined family history of Down's syndrome, no patients or controls with a positive family history were found.[19,20,42] So far it has not been possible to discern whether the negative findings of these studies reflect the low rate of occurrence of Down's syndrome (1 in 700 in the general population) or a true lack of association. Another issue to resolve is whether familial aggregation of Alzheimer's disease with Down's syndrome is found more in familial cases specifically, as would be predicted by a genetic link between Alzheimer's disease and Down's syndrome. This issue is addressed in *chapter 5.4.*

Parkinson's disease is a neurologic disorder that has been associated with Alzheimer's

disease. Alzheimer's disease and Parkinson's disease share several neuropathologic characteristics[43] and it has been suggested that Alzheimer's disease and Parkinson's disease may have a common etiology.[44] Two case-control studies of Alzheimer's disease have investigated family history of Parkinson's disease.[7,11] In both studies there were more Alzheimer patients with a first degree relative with Parkinson's disease as compared to age- and sex-matched population controls. In the largest study, a significant increase in risk was observed, particularly in men with early-onset of Alzheimer's disease.[7]

Methods

Family history data have been assessed in seven case-control studies in this re-analysis[45] in which the data had been collected symmetrically for patients and controls (table 3.2.1).[7,9,11-13,15,16] Family history was obtained in five studies by a personal interview,[7,9,11,12,16] in one study by telephone interview[15] and in one study by a questionnaire mailed to the informant.[13] In the Dutch study, family history data were always verified by a second informant who was a first degree relative of the participant. The analysis was restricted to disorders in first degree relatives. To increase comparability, we restricted the case-control comparisons to control subjects derived from the population.

All seven studies collected data on family history of dementia. The analysis included all first degree relatives with a history of dementia, not Alzheimer's disease specifically, because anamnestic information on the cause of dementia is not likely to be reliable. In addition, affected relatives may have been diagnosed years before, when the diagnosis of Alzheimer's disease may have been less accurate. Family history of Down's syndrome has been assessed in five studies eligible for the re-analysis.[7,9,11,12,16] In two studies, no distinction was made between Down's syndrome and mental retardation.[9,12] Family history of Parkinson's disease has been studied in two investigations.[7,11] Both studies have excluded patients with a history of Parkinson's disease before the onset of Alzheimer's disease. In the Dutch study the diagnosis of Parkinson's disease was checked with independent medical records.[7]

Family history was considered positive for those who had at least one first degree relative with dementia. The number of first degree relatives, a putative confounder, was not available in one of the seven eligible studies (USA Bedford[13]). The strength of the association was assessed by computing the odds ratio (OR) as an estimate of the relative risk. Conditional logistic regression analysis was used to take the effects of education, the

Table 3.2.1 Family history of dementia in first degree relatives and the risk of Alzheimer's disease

Study	Cases	Controls	OR*	95% CI
Australia[16]	58/170	21/170	3.8	2.1-6.9
Italy[11]	29/116	12/97	2.6	1.0-7.5
The Netherlands[7]	96/198	37/198	4.8	2.8-8.1
USA, Bedford[13]	21/103	9/162	4.4	1.8-10.7
USA, Denver[12]	21/54	18/50	1.0	0.5-2.2
USA, Durham[9]	25/44	14/87	7.2	2.7-19.1
USA, Seattle[15]	55/129	29/130	2.5	1.4-4.4
Overall analysis	305/814	140/894	3.5	2.6-4.6
Excluding USA, Denver[12]	284/760	122/844	3.6	2.7-4.9

* Odds ratio adjusted for age, gender, number of siblings and education

number of siblings and the matching variables age and gender into account.[45] In this paper, we present the adjusted odds ratios with 95% confidence intervals (95% CI). Stratified analyses were conducted based on gender and onset age.

Results

Family history of dementia

Table 3.2.1 shows the odds ratios for family history of dementia in first degree relatives in the seven individual studies and in the pooled analysis. Overall, the odds ratio for those with at least one first degree relative with dementia was 3.5 (95% CI 2.6-4.6). The test for heterogeneity indicated no evidence for heterogeneity in odds ratios, except for the risk estimate observed in the Denver study.[12] The latter study of late-onset Alzheimer's disease (onset age 70 years or over) deviated significantly (p=0.01) from the other studies in the overall analysis as well as in a subgroup analysis of late-onset Alzheimer patients. In the present study, the odds ratios were similar for men (OR 3.9; 95% CI 2.5-6.5) and women (OR 3.3; 95% CI 2.3-4.6). Stratification according to age of onset of Alzheimer's disease showed that the odds ratio decreased with increasing onset age (table 3.2.2). Although there were still significantly more late-onset patients with a positive family history of dementia than controls, the odds ratio differed significantly from the odds ratio observed in early-onset patients (onset before 70 years). Table 3.2.3 shows

Table 3.2.2 Family history of dementia in first degree relatives and the risk of Alzheimer's disease by onset age

Onset age (years)	Cases	Controls	OR*	95% CI
-59	97/272	37/327	4.0	2.4-6.1
60-69	76/183	31/205	5.3	2.8-10.0
70-79	72/196	43/198	2.3	1.4-3.6
80+	41/122	20/123	2.6	1.3-5.2

*Odds ratio adjusted for age, gender, number of siblings and education

Table 3.2.3 Family history of dementia and the risk of Alzheimer's disease by relationship

Family history dementia	Cases	Controls	OR*	95% CI
Parents	190/814	95/894	2.3	1.8-3.1
Siblings	101/814	23/894	4.8	2.9-7.8
Onset case before 70 years:				
Parents	115/453	43/528	3.5	2.3-5.2
Siblings	40/453	9/528	4.4	2.1-9.0
Onset case at 70 years or over:				
Parents	63/308	47/307	1.4	0.9-2.1
Siblings	57/308	14/307	4.7	2.5-9.0

*Odds ratio adjusted for age, gender, number of siblings and education

that for early-onset Alzheimer's disease, similar risk estimates were found for parents and siblings. There was a lower risk for late-onset Alzheimer's disease for subjects with a demented parent observed. The percentage of patients of whom one of the parents was affected with dementia decreased with increasing onset age (figure 3.2.1). The prevalence of dementia in parents of control subjects did not show a trend across the age strata. Figure 3.2.2 shows that the percentage of patients with one or more affected siblings increased with increasing onset age. Table 3.2.4 gives the odds ratio according to the number of first degree relatives with a history of dementia. There were 49 patients with two or more first degree relatives as compared to 7 controls (OR 7.5; 95% CI 3.3-16.7). The risk of Alzheimer's disease increased with the number of affected relatives (p-value for trend=0.008). This trend was observed in early-onset patients (p=0.013) as well as late-onset patients (p=0.005).

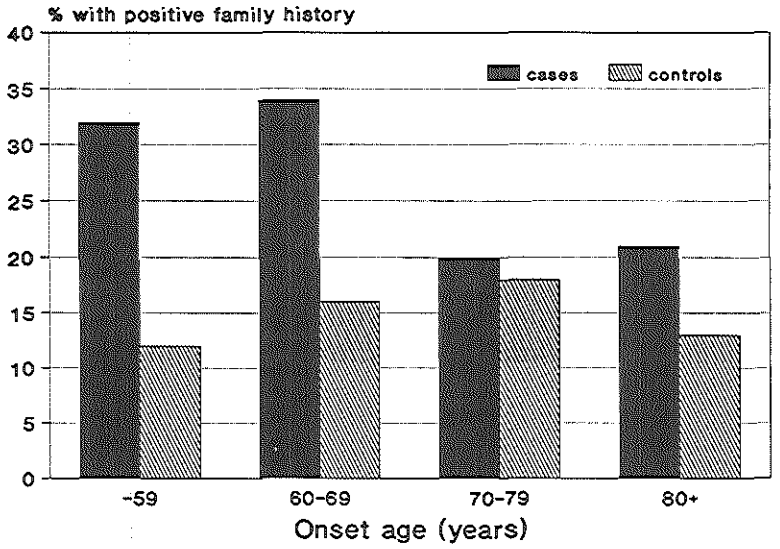

Figure 3.2.1 Percentage of Alzheimer cases and controls with a positive family history of dementia in the parents

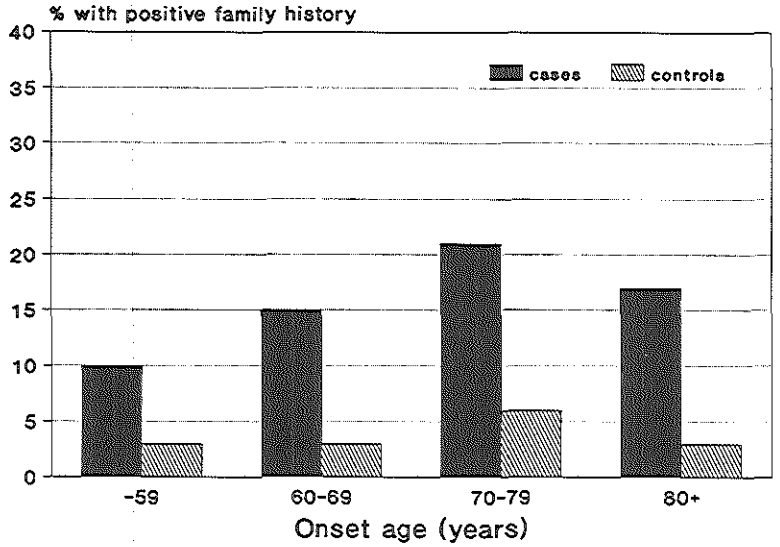

Figure 3.2.2 Percentage of Alzheimer cases and controls with a positive family history of dementia in the siblings

Table 3.2.4 Family history of dementia in first degree relatives and the risk of Alzheimer's disease by number of affected relatives

Number of relatives with dementia	Cases	Controls	OR*	95% CI
0	509	709	1	reference
1	206	103	2.6	2.0-3.5
2+	49	7	7.5	3.3-16.7

* Odds ratio adjusted for age, gender, number of siblings and education

Family history of Down's syndrome

In all studies included in the re-analysis there were more patients with a positive family history of Down's syndrome in first degree relatives as compared to controls (table 3.2.5). The test for heterogeneity indicated that there was no evidence for heterogeneity across studies. Overall, the odds ratio was 2.7 (95% CI 1.2-5.7) for family history of Down's syndrome. When excluding the studies which did not differentiate between Down's syndrome and mental retardation,[9,12] the risk estimate did not change materially (OR 3.3; 95% CI 0.9-12.1). Similar risk estimates were observed for men and women and for early-onset and late-onset Alzheimer's disease (Table 3.2.6).

Table 3.2.5 Number of subjects with a family history of Down's syndrome in first degree relatives in Alzheimer cases and controls

	Cases	Controls	OR*	95% CI
Australia[16]	5/165	0/165	-	-
Italy[11]	1/116	0/97	-	-
Netherlands[7]	5/198	3/198	1.7	0.3-13.0
USA, Denver[12]	2/64	0/64	-	-
USA, Durham[9]	7/45	4/91	3.5	1.2-5.7
Overall analysis	20/588	7/615	2.7	1.2-5.7
Excluding USA, Denver[12] and USA, Durham[9]	11/479	3/460	3.3	0.9-12.1

* Odds ratio adjusted for age, gender, number of siblings and education

Table 3.2.6 Down's syndrome in first degree relatives and the risk of Alzheimer's disease: stratification by gender and onset age

	Cases	Controls	OR*	95% CI
Stratification by gender:				
Women	15/382	5/398	3.0	1.2-7.3
Men	5/206	2/216	2.6	0.6-10.5
Stratification by onset age:				
Before 65 years	9/327	3/348	2.8	1.1-7.5
65 years or over	9/243	4/241	2.6	0.7-10.0

* Odds ratio adjusted for age, gender, number of siblings and education

Family history of Parkinson's disease

Family history of Parkinson's disease in first degree relatives was assessed in two studies (table 3.2.7). In both studies, there were more patients with a positive family history of Parkinson's disease as compared to controls. Pooling of the studies yielded an odds ratio of 2.4 (95% CI 1.0-5.8) for family history of Parkinson's disease in first degree relatives. No significant differences in odds ratios were observed comparing men versus women (table 3.2.8). However, odds ratios tended to be higher for men. Risk estimates were very similar for patients with early-onset versus patients with late-onset Alzheimer's disease (table 3.2.8).

Table 3.2.7 Number of subjects with a family history of Parkinson's disease in first degree relatives in Alzheimer cases and controls

	Cases	Controls	OR*	95% CI
Italy[11]	6/114	3/96	2.0	0.4-14.8
Netherlands[7]	14/198	5/198	2.8	1.0-10.8
Overall analysis	20/312	8/294	2.4	1.0-5.8

* Odds ratio adjusted for age, gender, number of siblings and education

Table 3.2.8 Parkinson's disease in first degree relatives and the risk of Alzheimer's disease: stratification by gender and onset age

	Cases	Controls	OR*	95% CI
Stratification by gender:				
Women	11/198	6/186	1.6	0.5-4.9
Men	9/114	2/108	4.4	0.9-20.9
Stratification by onset age:				
Before 65 years	16/272	8/258	2.4	1.0-5.8
65 years or over	4/29	0/25	-	-

* Odds ratio adjusted for age, gender, number of siblings and education

Discussion

This re-analysis of case-control studies of Alzheimer's disease showed familial aggregation of dementia in both early-onset and late-onset Alzheimer's disease. Significantly more Alzheimer patients than controls had a first degree relative with Down's syndrome. Furthermore the re-analysis supported a higher frequency of Parkinson's disease in first degree relatives of patients with Alzheimer's disease.

These findings must be interpreted in light of the various problems encountered in case-control studies of Alzheimer's disease. As the majority of studies were hospital-based for case selection, selection bias may result from differential referral of patients according to family history of dementia.[45] The pooled risk estimate, however, was very similar to the odds ratios observed in the population-based studies of Hofman et al[7] and Broe et al.[16] Another issue related to selection bias is that all studies included prevalent patients.[45] Observed associations may therefore relate to predictors of survival rather than to the risk of Alzheimer's disease. However, in a sub-analysis of incident patients, i.e. patients included in the study within 1 year following diagnosis, risk estimates remained virtually the same. As to information bias, non-differential misclassification may occur when assessing disease history in first degree relatives. A related issue is that we did not distinguish the type of dementia in relatives, which may not always have been of the Alzheimer type. This has most likely led to an underestimate of the strength of association between Alzheimer's disease and these disorders. Another problem in assessing family history of late-onset disorders such as Alzheimer's disease and

Parkinson's disease is that misclassification may also occur because relatives are still at risk of the disease after the study or may have died before the expression of the disease. Assuming that such a censoring mechanism has been similar for first degree relatives of patients and controls, this type of bias may dilute a true association rather than to create a spurious one. Recall bias may be another important source of bias in these studies. Spurious associations may occur if relatives of patients pay more attention to the occurrence of other diseases in their family than relatives of control subjects. This is more likely to occur in assessment of disease in relatives who are more distantly related. We have therefore restricted the re-analysis to first degree relatives.

In this analysis of 814 patients with clinically diagnosed Alzheimer's disease[46] and 894 age- and gender-matched control subjects we observed aggregation of dementia in the families of patients with early-onset as well as those with late-onset Alzheimer's disease. For early-onset Alzheimer's disease, the risk was about four times elevated, which was significantly higher than the 2.5 elevation in risk for Alzheimer's disease after the age of 70 years. The lower risk estimate resulted from a lower prevalence of dementia in parents of late-onset patients. No difference was observed in risks for early-onset and late-onset Alzheimer's disease for history of dementia in siblings. An explanation for these findings may be related to the clustering of onset age within families.[2,47] Relatives of late-onset patients are more likely to have a late-onset of the disease and may therefore have a higher chance of dying before the disease onset. Since the life expectancy has most likely been higher for siblings than for parents, the chance of expressing the disease may have been higher in siblings of late-onset patients. Alternatively, if there is a true difference in risk for late-onset Alzheimer's disease between those who have an affected sibling and those who have an affected parent, this would suggest that familial aggregation of late-onset Alzheimer's disease may be of non-genetic or multifactorial origin. This finding is even compatible with a recessive disorder. A second finding which may point to heterogeneity is that the risk of Alzheimer's disease was significantly different for those with two or more first degree relatives with dementia as compared to those with one.

There were significantly more patients with a first degree relative with Down's syndrome than control subjects. Although each of the individual studies showed a higher frequency of Down's syndrome in the family of patients, a significant increase in risk of Alzheimer's disease for subjects with a positive family history of Down's syndrome could only be shown after pooling of the data. This may be explained by the fact that Down's syndrome is a relatively rare disorder and large numbers of relatives are therefore

needed to establish an increase in risk. Our findings confirm earlier studies of family history of Down's syndrome.[9,16,26] The re-analysis of case-control studies did not confirm the hypothesis that the increase in risk was specific to early-onset patients.[26] The risk estimates were very similar when stratifying by onset age. In etiologic terms, this finding may be of interest because if there is a true association between Alzheimer's disease and Down's syndrome, our finding would predict a link with chromosome 21 for both early-onset as well as late-onset Alzheimer's disease. However, to date, genetic linkage of Alzheimer's disease to chromosome 21 has been shown in families with early-onset Alzheimer's disease[3-5] but not in families with late-onset of disease.[48,49] It is important to note that maternal age at birth of the patients with Down's syndrome was not known and may have been an important confounder. Therefore we cannot rule out the possibility that the familial aggregation of Alzheimer's disease and Down's syndrome may be explained by a factor associated with the family history of both disorders, e.g. social class.

In this analysis we observed familial aggregation of Alzheimer's disease with Parkinson's disease, although patients with Parkinson's disease before the onset of Alzheimer's disease were excluded in the case selection. The odds ratio tended to be higher in men as compared to women. In the interpretation of these findings it is important to note that the diagnosis of Alzheimer's disease was clinically assessed. Since the type of dementia was not confirmed pathologically, we cannot exclude the possibility that the higher frequency of Parkinson's disease has occurred specifically in the family of patients who suffered from dementia caused by parkinsonism or Lewy body disease.

In conclusion, this re-analysis confirmed earlier studies that reported familial aggregation of early-onset Alzheimer's disease. In contrast to earlier studies, the re-analysis also showed familial aggregation of late-onset Alzheimer's disease. The association between Alzheimer's disease and family history of dementia, however, was weaker in late-onset patients. Pooling of the data showed a significant increase in risk of Alzheimer's disease for subjects with a first degree relative with Down's syndrome. Individual studies may have lacked statistical power to assess an association. The re-analysis also supported the hypothesis of familial aggregation of Parkinson's disease with Alzheimer's disease. However, this finding should be confirmed in a study of autopsied patients.

Acknowledgements

The collaborative re-analysis of case-control studies was supported by the Commission of the European Community for the EURODEM concerted action on the epidemiology of dementia, the Netherlands Organisation for Scientific Research (NWO) and was conducted in collaboration with the National Institute on Aging of the US National Institutes of Health and with the World Health Organization. For the acknowledgements of the individual studies this re-analysis is based upon please see appendix to chapter 2.2 (page 33). We thank Marcel Eijgermans, Gerrit-Anne van Es and Eric Neeleman for their help in data analysis.

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Genetics in early-onset Alzheimer's disease: risk and segregation of disease in first degree relatives

Alzheimer's disease is a disorder with a complex genetic etiology.[1] Heterogeneity is evidenced by studies suggesting genetic linkage to chromosome 19 as well as to chromosome 21.[2-6] Despite the evidence for autosomal dominant inheritance in a considerable number of families,[7] the large majority of patients do not have first degree relatives affected with dementia and for those the extent of genetic involvement is less clear.[8] Studies of the risk of Alzheimer's disease for first degree relatives of cases have yielded risk estimates ranging from 5% to 50%.[9-16] Differences in methodology may underlie this wide range in risk estimates. However, it is also conceivable that these discrepancies may be due to heterogeneity.[1] It has been suggested that genetics may differ between early- and late-onset Alzheimer's disease and that the early-onset form may be explained as an autosomal dominant trait.[1,16] Previous studies have been too small to yield precise risk estimates for early-onset Alzheimer's disease. Another problem in the interpretation of earlier investigations is that case-series have been hospital-based, which may have introduced selection bias when studying the extent of genetic involvement in the disease. As to the pattern of genetic transmission, to date only two studies on the segregation of Alzheimer's disease have been published, which have yielded contradicting results.[17,18]

We have studied the risk of early-onset Alzheimer's disease and the genetic transmission of disease in first degree relatives of 198 patients and in 198 age- and sex-matched controls in a population-based study.

This chapter is based on:
Van Duijn CM, Farrer LA, Cupples LA, Hofman A. Risk of dementia in first degree relatives of patients with Alzheimer's disease. In: Iqbal K, McLachlan DRC, Winblad B, Wisniewski HM (eds); Alzheimer's disease: Basic mechanisms, diagnosis and therapeutic strategies. John Wiley & Sons, Chichester, 1991, 423-426.
Van Duijn CM, Farrer LA, Cupples LA, Hofman A. Genetic transmission for Alzheimer's disease among patients identified in a Dutch population-based epidemiological study. Submitted.

Methods

Subjects. Patients were derived from an epidemiologic study of risk factors for clinically diagnosed Alzheimer's disease. The study aimed at a complete ascertainment of cases in whom the diagnosis was made before 70 years and who were living in the four northern provinces of The Netherlands and the area of metropolitan Rotterdam. Ascertainment was carried out through all neurologic, psychiatric, geriatric services in the study areas. All cases were diagnosed in the period of January, 1980 to July, 1987. Control subjects were randomly drawn from the same municipality as the cases. Cases and controls were matched for age (within 5 years), sex and residence. An extensive description of the design of the study, case diagnosis and response rates has been given in chapter 2.1. The study comprised a total of 198 patients and 198 controls.

Data collection. Detailed data on family history were collected by interviewing a next of kin of the patient or control. All first degree relatives were listed and we asked specifically about the occurrence of dementia in them. To increase the validity of these data, the information was always verified by a sibling of the patient or control. Because subjects were born outside The Netherlands, we could not contact siblings in four cases and these subjects were excluded from the analysis. For patients, we have assessed the onset age of dementia as the age at which memory loss or change in behavior was first noted. For non-demented relatives, the censoring age was determined, i.e., the age at time of the study or the age at death.

We questioned informants extensively on the cause and the course of the dementia in affected relatives. If the patient had been admitted to hospital the diagnosis was checked in independent medical records. Based on the reports of the informants and the information derived from medical records, we have classified relatives with a history of neurologic, psychiatric or metabolic disorders that may also lead to dementia (e.g. stroke, Parkinson's disease, epilepsy, depression or alcoholism) as unaffected. Those subjects were considered at risk of Alzheimer's disease until the onset of the disorder that led to the censoring. As Alzheimer's disease may have been diagnosed years before when the diagnosis was less accurate, all other relatives with a type of dementia that was reported as being irreversible and progressive were classified as affected with possible Alzheimer's disease.

Data analysis. Risks of Alzheimer's disease and the age of onset distribution among first degree relatives were estimated using a maximum likelihood method.[19] The maximum likelihood estimates take into account the possibility that a proportion of relatives asymptomatic at the time of study may be susceptible and express the disease later in life and that some deceased relatives may have succumbed to causes unrelated to Alzheimer's disease although they may have developed symptoms had they survived. This method considers affected persons with known onset ages, unaffected persons with known censoring ages and persons for whom onset age or censoring age data are missing.[19] For these latter relatives, a censoring age distribution was estimated based on the censoring distribution of the unaffected relatives with known data. The risk estimates are presented with 95% confidence intervals. We tested differences in life time risks with the large sample z statistic, using the risk estimate and standard error of the oldest onset age common to both groups.

Segregation analysis was carried out using a maximum likelihood method implemented in the computer program POINTER.[20] The model parameters are: d, the degree of dominance, such that $d=0$ corresponds to a recessive gene, $d=1$ to a dominant gene, $d=0.5$ to a codominant gene and $0<d<1$ corresponds to some additivity; t, the displacement at the major locus; q, the frequency of the high risk allele A; H, the polygenic heritability in offspring; Z, the parent to child heritability ratio; and $\tau 1$, $\tau 2$ and $\tau 3$, the respective probabilities of genotypes AA, Aa, and aa transmitting the allele A. For Mendelian transmittance of a major locus, $\tau 1=1$, $\tau 2=0.5$ and $\tau 3=0$, whereas the t's are equal if there is no transmission of a major effect. No families were ascertained through two or more cases in the study. The ascertainment probability we have used for this population-based study is 0.01, corresponding to single ascertainment. Analyzing the data with an ascertainment probability of 0.98 resulted in minor changes in the model parameters and did not change the conclusions. Liability to developing dementia was assessed using the population risk of dementia, which was estimated as a cumulative incidence of 6.5 per 1000.[21] Based on the results of the survival analysis, we have adjusted the liability for age and sex (table 3.3.1). We have examined various models assuming: (1) Mendelian inheritance; (2) familial aggregation without an underlying major gene; (3) a mixed model postulating both of the above; (4) no familial clustering. To compare the models the likelihood ratio test was used.

Table 3.3.1 Liability classes as defined for the segregation analysis

Class	Age range (years) Men	Women	Cumulative Incidence
1	0-50	0-45	0.000132
2	51-65	46-62	0.000674
3	66-75	63-70	0.001300
4	76-80	71-78	0.002167
5	81+	79-80	0.002768
6	-	81-85	0.004285
7	-	86-89	0.004971
8	-	90+	0.006500

Results

The 195 patients had 1,308 first degree relatives compared with 1,219 in the 195 controls. The risk of Alzheimer's disease in first degree relatives of patients is given in figure 3.3.1. The risk in relatives of the cases increased rapidly after age 55. By the age of 90 years the risk was 0.39 (95% CI 0.27-0.51). Although this is lower than the risk expected when the disease is to be explained solely as an autosomal dominant disorder, the 95% confidence interval included the predicted value of 0.50. The risk of Alzheimer's disease was significantly lower in relatives of control subjects (0.14; 0.06-0.22). The relative risk for Alzheimer's disease for those with an affected relative was 2.8 (1.5-5.2).

When stratifying the data for sex of the proband, onset age of the proband, sex of the relatives, relationship of the relatives to the case or control, the risk was significantly higher among female relatives as compared to males when comparing risk estimates at the oldest common onset age (table 3.3.2). Further stratification revealed that this was due to the 0.70 risk among female relatives of early-onset cases (onset before the age of 58 years) by age 90. At the oldest common age (80 years), the risks in male relatives of early-onset cases (0.16), male relatives of late-onset cases (0.20), female relatives of early-onset cases (0.29) and female relatives of late-onset cases (0.21) did not differ significantly.

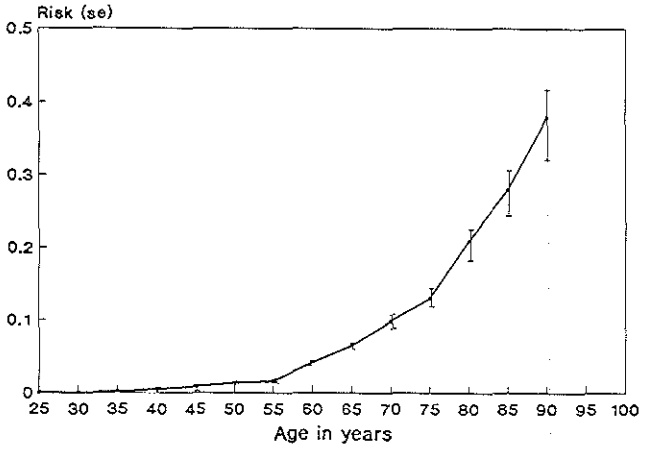

Figure 3.3.1 Risk of dementia in first degree relatives of patients with Alzheimer's disease up to age 90 years

Results of the segregation analysis are presented in table 3.3.3. The model assuming no familial aggregation of Alzheimer's disease (model 1) was rejected when we compared this model to the most general Mendelian model (model 8; χ_6^2 882.32; $p<10^{-6}$) or the models assuming multifactorial inheritance (model 2; χ_1^2 853.54;$p<10^{-6}$ and model 3; χ_2^2 853.57;$p<10^{-6}$). When compared to the unrestricted mixed model (model 13), models postulating no Mendelian inheritance (model 2; χ_6^2 39.26;$p<10^{-6}$), no multifactorial component (model 8; χ_1^2 10.48;p=0.0012) and no transmission of a genetic defect (model 16; χ_3^2 42.42;$p<10^{-6}$) could be excluded. As to the mode of inheritance among the mixed models (models 9-12), recessive inheritance was rejected because the degree of dominance (d) differed significantly from 0 (model 9 versus model 12; χ_1^2 29.37;$p=10^{-6}$). Since d was not significantly different from 1 (model 11 versus model 12; χ_1^2 0;p=1) or 0.5 (model 10 versus model 12; χ_1^2 0.49;p=0.484), our data are compatible with the existence of a dominantly transmitted gene with reduced penetrance. While the multifactorial component was accounted for, more offspring was affected than expected based on Mendelian inheritance, i.e., $\tau 2$ was significantly higher than 0.5 (model 12 versus model 13; χ_3^2 9.89;p=0.0195). The best fit to our data was achieved in model 15. This model suggests that the penetrance of the Alzheimer's disease susceptibility allele at the major locus is 96%,

Table 3.3.2 Risk for Alzheimer's disease and age of onset distribution in first degree relatives of cases with Alzheimer's disease and age- and sex-matched controls

Subgroup	Number Relatives Affected	Unaffected	Life-time Risk* (years)	Estimated Onset age* (years)	Oldest Onset age	Risk at Age Lowest Common Denominator*
All	127	1181	0.39 (0.27-0.51)	77.7 (74.6-80.8)	90	0.39 (0.27-0.51)
Controls	32	1187	0.14 (0.06-0.22)	81.4 (77.9-84.9)	90	0.14 (0.06-0.22)
Male probands	49	416	0.39 (0.21-0.57)	77.8 (72.7-82.9)	90	0.37 (0.19-0.55)
Female probands	78	765	0.38 (0.24-0.52)	77.2 (73.3-81.1)	89	0.38 (0.24-0.52)
Onset $\leq$ 58 years	68	566	0.48 (0.30-0.66)	78.5 (74.4-82.6)	90	0.48 (0.30-0.66)
Onset > 58 years	59	615	0.32 (0.18-0.46)	76.9 (72.2-81.6)	90	0.32 (0.18-0.46)
Males relatives	48	620	0.22 (0.14-0.30)	73.0 (69.3-80.6)	86	0.22 (0.14-0.30)
Females relatives	79	561	0.56 (0.36-0.76)	79.8 (76.3-83.3)	90	0.37 (0.17-0.57)
Parents	81	306	0.42 (0.30-0.68)	75.7 (72.2-79.2)	90	0.26 (0.14-0.38)
Siblings	46	875	0.18 (0.08-0.28)	71.4 (66.3-76.5)	81	0.18 (0.08-0.28)

* 95% confidence interval within parentheses

the frequency is 0.00097 and 35% of the transmission variance is accounted for by Mendelian inheritance. When assuming an ascertainment probability of 0.98, the estimate was for penetrance 100% and for gene frequency 0.0084. In this case only 9% of the transmission variance could be accounted for by the major locus.

Table 3.3.3 Segregation analysis of Alzheimer's disease in first degree relatives of cases with Alzheimer's disease using an ascertainment probability of 0.01 and a cumulative incidence of 0.0065

Model	d	t	q	H	Z	τ1	τ2	τ3	-2ln+c
1 Sporadic	-	-	(0)	(0)	(1.0)	-	-	-	672.91
Multifactorial									
2 No cohort effect	-	-	(0)	0.96	(1.0)	-	-	-	-180.63
3 Cohort effect	-	-	(0)	0.97	0.99	(1.0)	(0.5)	(0)	-180.66
Single locus									
4 Recessive	(0)	4.24	0.0680	(0)	(1.0)	(1.0)	(0.5)	(0)	-2.91
5 Co-dominant	(0.5)	5.68	0.00076	(0)	(1.0)	(1.0)	(0.5)	(0)	-202.70
6 Dominant	(1.0)	6.99	0.00390	(0)	(1.0)	(1.0)	(0.5)	(0)	-132.93
7 Unrestricted d	0.51	5.61	0.00076	(0)	(1.0)	(1.0)	(0.5)	(0)	-202.70
8 Unrestricted d+τ	0.53	5.94	0.00060	(0)	(1.0)	1.0	0.64	0	-209.41
Mixed model									
9 Recessive	(0)	5.07	0.06600	0.98	(1.0)	(1.0)	(0.5)	(0)	-180.63
10 Co-dominant	(0.5)	4.41	0.00180	0.67	(1.0)	(1.0)	(0.5)	(0)	-209.51
11 Dominant	(1.0)	2.16	0.00190	0.71	(1.0)	(1.0)	(0.5)	(0)	-210.00
12 Unrestricted d	1.0	2.16	0.00190	0.71	(1.0)	(1.0)	(0.5)	(0)	-210.00
13 Unrestricted d+τ	0.83	3.18	0.00089	0.79	(1.0)	1.0	0.69	0	-219.89
General transmission									
14 Mendelian major locus + cohort effects	1.0	2.20	0.00220	0.46	2.13	(1.0)	(0.5)	(0)	-224.33
15 Unrestricted	0.96	2.62	0.00097	0.65	1.52	1.0	0.64	0	-229.23
16 No cohort effect + no transmission major gene	0.14	2.26	0.0010	0.95	(1.0)	(1.0)	(1.0)	(1.0)	-177.47

Discussion

In this population-based study of early-onset Alzheimer's disease we observed that the risk of Alzheimer's disease was significantly higher for first degree relatives of patients with Alzheimer's disease as compared to relatives of controls. Among first degree relatives of patients the risk of Alzheimer's disease was lower than expected for an autosomal dominantly inherited disorder, although the risk estimate did not differ significantly from the expected risk (i.e., 0.50). Segregation analysis suggested that familial aggregation of disease is most likely explained by a dominant major gene effect that acts together with a multifactorial component.

A problem in the interpretation of our data may be that we have studied the occurrence of possible Alzheimer's disease in first degree relatives. The dementia may have been diagnosed years before when the diagnosis of Alzheimer's disease and other types of dementia was less accurate. To come to a diagnosis of possible Alzheimer's disease in relatives, we have questioned informants extensively on the cause and the course of dementia and have classified relatives with a history of neurologic, psychiatric or metabolic disorders that may lead to dementia other than Alzheimer's disease as unaffected. Furthermore, we have used multiple informants, which has been shown to increase the reliability of data of family history.[22] For all cases and controls, a sibling was interviewed who is likely to be the most knowledgable person about first degree relatives.

Previous studies of the risk of Alzheimer's disease for relatives of patients have yielded risk estimates ranging from 5% to 50%.[9-16] Differences in methodology, e.g. ascertainment of patients and definition of age of onset may account for the controversial findings of these studies.[1,14] In addition, the growing evidence that genetic heterogeneity may be related to age of onset of Alzheimer's disease may explain part of the apparently contradicting findings.[1] The present study aimed at a complete ascertainment of cases with early-onset Alzheimer's disease in the two study regions to anticipate the problem of heterogeneity and selection bias. Among first degree relatives of patients the risk of Alzheimer's disease (0.39) was lower than expected for an autosomal dominant inherited disorder (0.50), although the risk estimates did not differ significantly. It is important to note, however, that we may have overestimated the risk because of the inclusion of relatives that suffered from other types of dementia than Alzheimer's disease. The risk among female relatives of early-onset cases by age 90 years was higher than expected for an autosomal dominant disorder.

This finding may be explained by (1) difficulties in assessment of Alzheimer's disease at old age; (2) the existence of cases with Alzheimer's disease of non-genetic origin; (3) the low number of women who survive to such an old age leading to unstable risk estimates. When comparing the risk of relatives of cases and controls, this study confirms earlier findings of familial aggregation of Alzheimer's disease.[8]

The segregation analysis indicates that familial aggregation of disease is most likely explained by a dominant major gene effect that acts together with a multifactorial component. Our findings suggest that a major gene for Alzheimer's disease accounts for no more than 35% of the total variance in transmission of disease. Other genetic mechanisms or environmental factors may account for the familial aggregation of disease in a considerable number of cases. As to the possibility of bias, the inclusion of relatives with other types of dementia would most likely lead to an overestimation of the extend of genetic transmission.[17] Since no systematic bias in particular families is expected, this type of misclassification has probably not biased the data towards a specific model. Another methodological problem is related to censoring bias. Because the risk of Alzheimer's disease increases rapidly with age, genetic liability may have been underestimated. A final issue of concern relates to the statistical analysis. Although we were able to reject several models postulating no transmission, recessive inheritance, no Mendelian inheritance and no multifactorial inheritance, we could not distinguish between several mixed and general transmission models. Moreover, despite the large number of observations, several models could not be fitted easily because the likelihood surfaces were relatively flat or because of disturbances by local maxima.

The findings of the present segregation analysis are very similar to those reported by Farrer et al, who have studied first degree relatives of 232 patients affected with early- or late-onset Alzheimer's disease. Both studies showed: (1) Mendelian inheritance as well as a multifactorial component; (2) a higher number of offspring affected than expected based on autosomal dominant inheritance in the mixed model, which takes into account the multifactorial component. There are several explanations possible for the latter finding.[17] Firstly, this finding may be explained by the presence of non-genetic cases in the offspring, i.e., there may be subjects with disease but without the genotype (phenocopies). Secondly, there may be reduced ascertainment of carriers among parents. Thirdly, there may be more than one dominant locus involved in early-onset Alzheimer's disease. Thus, some families would be expected to have more than 50% chance of affected offspring if this is not a rare gene. In the only other study on segregation of Alzheimer's disease that has been reported to our

knowledge, two populations were studied.[18] No distinction could be made between the models postulating a single major locus, multifactorial inheritance and a mixed model using the data of one population.[23] In the other population, the multifactorial model was most likely. However, this population comprised a mixture of patients with Alzheimer's disease and Pick's disease.[24]

In conclusion, our findings are compatible with the view that multiple mechanisms may be involved in the transmission of early-onset Alzheimer's disease. Although our study confirms that a dominant gene may be implicated in early-onset Alzheimer's disease, the results suggest that other genetic factors as well as environmental factors may account for the disease a considerable number of patients.

Acknowledgements

This research was supported by the SOOM Foundation, the Netherlands Organisation for Scientific Research (NWO), the Eurodem EC concerted action on the epidemiology of dementia and the National Institutes of Health (NIH), Grant AG09029. We thank Drs Wim Schulte, Teun Tanja, Rob Haaxma, Arie Lameris and Rolf Saan for their contributions to this study and Helen de Bruijn, Micheline de Haes, Jeanette Kamman, Hanneke van Meurs, Verona Otten and Caroline Valkenburg for data collection and genealogy studies.

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Amyloid precursor protein gene mutation in early-onset Alzheimer's disease

A mutation within the amyloid precursor protein (APP) gene has recently been reported as the possible cause of Alzheimer's disease in two families in which the disease was apparently inherited as an autosomal dominant disorder.[1] This mutation in exon 17 of the APP gene has been shown to cause a valine-to-isoleucine substitution at amino acid 717 (transcript APP_{770}). Recombination events between Alzheimer's disease and the APP gene in other families with early-onset Alzheimer's disease,[2,3] however, imply that other genes must be involved and that the role of the 717 APP mutation as a cause of early-onset Alzheimer's disease in the general population remains to be established.[4] To assess the proportion of early-onset Alzheimer's disease that may be explained by the 717 APP mutation, we have screened 100 familial and sporadic patients.

Methods

Patients were derived from a population-based epidemiologic study of early-onset Alzheimer's disease.[5] The study aimed at a complete ascertainment of prevalent and incident Alzheimer patients in whom the diagnosis was made before the age of 70 years in four Northern provinces of the Netherlands and the area of metropolitan Rotterdam. For this study, the clinical diagnosis of Alzheimer's disease was independently confirmed using a standardised protocol according to the NINCDS-ADRDA criteria for Alzheimer's disease.[6] The response rate was 99% for patients. An extensive description of ascertainment of these cases been given in chapter 2.1. Blood samples for DNA extraction were collected for a sample of 100 out of the 198 Alzheimer patients

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This chapter is based on: Van Duijn CM, Hendriks L, Cruts M, Hardy JA, Hofman A, Van Broeckhoven C. Frequency of mutation in the amyloid precursor protein gene (Letter). Lancet 1991;337:978.

participating in the study, who were still alive during the follow-up in the period of 1989 to 1990. The mean age of onset of dementia for the 100 patients was 57 years (SD=5). Of these patients, 48 were sporadic, i.e., there were no first degree relatives known with Alzheimer's disease or dementia, and 52 patients had at least one first degree relative with dementia. Of these 52 familial patients, the pedigree structure of 14 was consistent with autosomal dominant inheritance of Alzheimer's disease. The criteria for autosomal dominant inheritance of Alzheimer's disease were: (1) at least three patients with reported dementia in two generations; (2) at least two patients with detailed medical records on the clinical diagnosis of Alzheimer's disease. Genealogy studies of second, third and fourth degree relatives of these 14 familial patients did not reveal evidence for a relationship between them.

For the 100 patients DNA was extracted from total blood using the standard phenol-chloroform extraction procedure. The DNA samples were screened for the 717 APP mutation. The mutation creates a BclI restriction site which allows detection of the corresponding polymorphism within the polymerase chain reaction (PCR) product. PCR was carried out using the intronic primers GTTGGGCAGAGAATATACTGA and GCCTAATTCTCTCATAGTCT, generating a DNA fragment of 355 base pairs. The PCR reactions were performed in a total volume of 50 μl containing 0.1 to 0.2 μg of genomic DNA, 1.5 Mm $MgCl_2$, 0.05 Mm KCl, 10 Mm Tris Ph 8.3, 0.001% (w/v) gelatine, 0.2 Mm nucleotide triphosphates, 50 pmol of each primer and 2 U of Taq DNA polymerase (BRL, Bethesda, USA). The samples were covered with 50 μl mineral oil and 30 three step cycles (1.30 min. 94°C; 1.30 min. 60°C, 2 min 72°C) were performed in a Cetus Thermocycler apparatus. The PCR products were digested with BclI (BRL) for 4 hours at 50 °C. The digested PCR products were analyzed on a 3% agarose gel (2.5% Nusieve and 0.5% Seakem (FMC, Maine, USA)) at 2.5 V/cm during 5 hours. A sample of family F23 which showed the 717 APP mutation,[1] was used in each screening as a positive control. The proportion of screen positives were estimated for the total group of patients and for the subgroups of familial and sporadic patients, separately. Exact 95% confidence intervals were calculated.

Results and Discussion

Screening of the 100 early-onset patients failed to show the mutation (table 3.4.1.). At a confidence level of 95%, this finding suggests that the APP mutation accounts for less

Table 3.4.1 Mutation in the amyloid precursor protein gene in 100 patients with early-onset alzheimer's disease

Number of first degree relatives with dementia	Number patients tested	Mean onset age in years (SD)	Number screen positives (%)	95% confidence interval
All	100	57 (5)	0	0-3.6
0	48	56 (5)	0	0-7.4
1	38	57 (5)	0	0-9.3
$\geq 2^{*}$	14	59 (5)	0	0-23.2

* Consistent with autosomal dominant inheritance of Alzheimer's disease

than 3.6% of all cases with early-onset Alzheimer's disease. Including only the 52 patients with a family history of dementia in first degree relatives in the analysis, our study indicates that it is unlikely that more than 6.8% of the familial cases may be explained by this mutation. Restricting the analysis to the 14 patients derived from families in which the disease was apparently inherited as an autosomal dominant disorder, our negative findings yield a 95% confidence interval ranging from 0 to 23.2%.

This study suggests that the 717 APP mutation is not a common cause of early-onset Alzheimer's disease in the general Dutch population. To date, screening of patients with late-onset Alzheimer's disease has also failed to show the 717 APP mutation.[1] The presence of this mutation in late-onset patients is less likely because families with late-onset Alzheimer's disease do not show linkage to chromosome 21.[7] Despite the significance the 717 mutation in the APP gene may have for the understanding of the pathogenesis of Alzheimer's disease, we conclude that for case diagnosis the mutation is at present of limited value.

Acknowledgements

This research was funded by the Flemish Biotechnology Program, the National Fund for Scientific Research (CVB) Belgium, the Eurodem EC Concerted Action of dementia, the SOOM Foundation and the Netherlands Organisation for Scientific Research (NWO). We thank Hubert Backhovens and Dirk Goyvaerts for DNA extraction, Drs Wim Schulte, Teun Tanja, Rob Haaxma, Arie Lameris, Rolf Saan for case diagnosis and Helen de Bruijn, Micheline de Haes, Jeanette Kamman, Hanneke van Meurs, Verona Otten, Caroline Valkenburg for genealogy studies.

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Chapter 3.5

Decreased DNA repair capacity in inherited Alzheimer's disease

Alzheimer's disease is a common neurodegenerative disorder characterized by the premature death of neurons.[1] It has been reported that fibroblast and lymphoblastoid cell lines of patients with Alzheimer's disease have an increased sensitivity to the cell killing effects of alkylating agents and X-rays.[2-4] This phenomenon has been attributed to a defect in the capacity to remove DNA damages.[2,3,5] Comparison of DNA-damage levels in brain tissue of patients with Alzheimer's disease and controls indicated an at least twofold higher level of DNA breaks in patients with Alzheimer's disease as compared to controls.[6] It is conceivable that *in vivo* such a defect will be pathogenic specifically in neuronal cells of Alzheimer patients, but that after exposure to certain DNA-damaging agents *in vitro* the defect may be detected in cells other than brain cells.[2]

Decreased levels of DNA repair have been found in fibroblast and lymphoid cell lines of patients with Alzheimer's disease after treatment with methyl methanesulfonate (MMS) or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG),[5,7-9] although some of these results have been challenged.[10-11] Thus far, DNA repair capacity has not been determined in freshly isolated cells of patients with Alzheimer's disease. Direct testing for general DNA repair defects will circumvent artifacts that may be introduced when using cell lines and may be used for the diagnosis of Alzheimer's disease.

In the present study a highly sensitive alkaline filter elution assay was used to determine the induction and disappearance of single-strand breaks in peripheral blood lymphocytes after exposure to N-ethyl-N-nitrosourea (ENU). We found that the mean percentage single-strand breaks disappearance was similar in patients with no (42.5%) or one first degree relative with dementia (43.0%) and controls (41.4%). Single-strand breaks disappearance was significantly lower in patients with two or more affected relatives with dementia (23.6%) when compared to controls or the other patients.

This chapter is based on: Boerrigter METI, Van Duijn CM, Mullaart E, Eikelenboom P, Van der Togt CMA, Knook DL, Hofman A, Vijg J. Decreased DNA repair capacity in familial, but not in sporadic Alzheimer's diseases. Neurobiol Aging 1991;12:367-370.

Methods

Patients were derived from an epidemiologic study of risk factors for clinically diagnosed Alzheimer's disease. The study aimed at a complete ascertainment of cases in whom the diagnosis was made before 70 years and who were living in the four northern provinces of the Netherlands or the area of metropolitan Rotterdam. Ascertainment was carried out through all neurologic, psychiatric, geriatric services in the study areas. All cases were diagnosed in the period of January 1980 to July 1987. Control subjects were randomly drawn from the same municipality as the cases. Cases and controls were matched for age (within 5 years), sex, and residence. An extensive description of the design of the study, case diagnosis and response rates has been given in chapter 2.1. The study comprised a total of 198 patients and 198 controls.

In the period 1989 to 1990, all patients and controls who were still alive were revisited. During 91 consecutive visits of 43 cases and 48 controls, blood was drawn for the present study of DNA repair capacity. Lymphocytes were isolated from 10 ml of coded blood samples, using Ficoll-Paque (Pharmacia) gradients[12] and washed twice in RPMI 1640 medium (Flow laboratories) plus 2% fetal calf serum. All steps were performed at 4°C. Viability of recovered blood lymphocytes was always higher than 95% as measured by trypan blue dye exclusion. ENU (Sigma) was dissolved in dimethylsulfoxide (DMSO) immediately before use. A constant number of cells ($3x10^6$/ml) was used for each treatment in order to exclude variations in the amount of damage initially induced. Lymphocyte suspensions were exposed to either 0.5 mM ENU (20 minutes) in RPMI 1640 medium plus 20 mM Hepes, 2mM glutamine and 5% fetal calf serum at 37 °C. The DMSO concentration during ENU exposure was never higher than 1%. Control cells were treated with RPMI containing the same DMSO concentration. At the end of the exposure period, cells were centrifuged and resuspended in RPMI 1640 supplemented with 10% fetal calf serum and glutamine and incubated for repair at 37 °C. For treatment with 4Gy of ^{60}Co-gamma-rays, lymphocytes were suspended in RPMI 1640 medium plus 20 mM Hepes, 2mM glutamine and 5% fetal calf serum and were irradiated on ice in a Gamma-cell 100 (Atomic Energy of Canada Ltd.) at a dose rate of 6 Gy/minute. After irradiation the cells were centrifuged and resuspended in RPMI 1640, 10 FCS, 2mM glutamine for repair incubation.

The technique of alkaline filter elution, modified for analyzing nonradioactively labelled cells,[13] was used to measure ENU-induced DNA lesions, detected as single-strand breaks.[14] In brief, cells were collected, centrifuged and resuspended in ice-cold PBS (8.1

mM Na_2HPO_4, 15 mM KH_2PO_4, 0.14 M NaCl and 2.6 mM KCl) at a final concentration of $1.5x10^6$ cells/ml; $0.8x10^6$ cells were applied per filter. Loading and lysing of the cells, as well as the elution of the DNA, were performed under subdued lighting in order to minimize artificial induction of single-strand breaks. Elution was carried out at a flow rate of 0.03 ml/min. Six fractions were collected at 2.5 hour intervals. After the addition of Hoechst 33258, DNA in each fraction was quantified spectrofluorometrically, as described.[6] All determinations and subsequent calculations were performed in a blinded manner. The elution results were plotted as the log percent of DNA remaining on the filter as a function of elution time. Linear regression between the data points obtained at t=2½ and t=12½ hour of elution time was used to determine the slope of the elutions. Mean slopes of elution curves were used to calculate the percentage single-strand break disappearance. In all experiments, mean slopes were based on at least triplicate determinations and standardized with reference to mean slopes of untreated control cells assayed in the same experiment. Variation between the triplicate determinations of a single sample was typically less than 2%.

Results

Table 3.5.1 shows the general characteristics of both the patients with Alzheimer's disease and controls. There were no significant differences in the mean age between patients and controls. Out of 43 patients, twelve (28%) had one first-degree relative with dementia, as compared to 13 out of 48 controls (27%). Fifteen of the 43 patients (35%) had two or more first-degree relatives with dementia. The pedigree structure of these patients was consistent with autosomal dominant inheritance of Alzheimer's disease. These patients were not related to each other in first, second, or third degree. None of the controls had two or more first-degree relatives with dementia.

In a previous study on DNA repair in lymphocytes from normal human individuals we used the alkylating agent ENU at a dose of 0.5 mM, which induces approximately 4000 single-strand breaks per cell and has no significant effect on cell survival.[15] In that previous study we observed that lymphocytes of 10% of normal young subjects suffer from a low capacity to remove ENU-induced single-strand breaks.[15] The low level of repair appeared to correlate with a low level of survival of these cells at ENU doses of 1,2 and 5 mM.[15] Figure 3.5.1 shows representative elution curves for lymphocytes from a normal control subject, a patient with sporadic Alzheimer's disease, and a patient with

Table 3.5.1 Percentage disappearance of single-strand breaks in peripheral blood lymphocytes from patients with Alzheimer's disease and age-matched controls after in vitro exposure to 0.5 mM ENU (20 min, 37 °C) followed by an 1 hour repair incubation in fresh medium

Subjects	Family history*	Number subjects	age (years)†	Female (%)	Single-strand breaks induction‡ t=0	t=1	ENU repair‡ (%)
Cases	0	16	67.1 (1.47)	88	0.164 (0.011)	0.084 (0.009)	42.5 (8.2)
	1	12	69.3 (0.82)	83	0.180 (0.011)	0.101 (0.009)	43.0 (4.4)
	2	15	68.8 (1.74)	75	0.181 (0.017)	0.131 (0.011)	23.6§ (5.8)
	All	43	68.3 (0.84)	81	0.176 (0.008)	0.105 (0.006)	36.1 (4.0)
Controls	0	35	68.2 (1.15)	72	0.181 (0.011)	0.096 (0.007)	40.8 (3.4)
	1	13	70.0 (1.55)	70	0.173 (0.013)	0.100 (0.012)	44.3 (5.5)
	All	48	68.9 (0.93)	71	0.179 (0.0009)	0.097 (0.006)	41.4 (2.9)

* Number of affected relatives
† Mean (S.E.M.)
‡ Number of single-strand breaks represented by the slope of the elution curve (mean and S.E.M.) at indicated time (hours) after 20 minute ENU treatment
§ Significantly different from the 48 control subjects (p=0.01) and significantly different from the 28 other patients (p=0.02)

two or more affected relatives, directly after exposure to 0.5 mM ENU and after a 1 hour repair incubation. The initial amount of single-strand breaks in lymphocytes from these three selected subjects did not differ significantly. However, following a 1 hour repair incubation the amount of single-strand breaks was significantly decreased in lymphocytes from the control subjects and the sporadic patient, where as the lymphocytes from the selected patient with two or more affected relatives appeared incapable of removing the ENU-induced single-strand breaks (figure 3.5.1, panels A-C). For lymphocytes from these same individuals gamma-ray-induced single-strand breaks were removed efficiently; repair was virtually complete within 1 hour of repair incubation (figure 3.5.1, panels D-F). The number of single-strand breaks induced by ENU over the 20-minute treatment period was

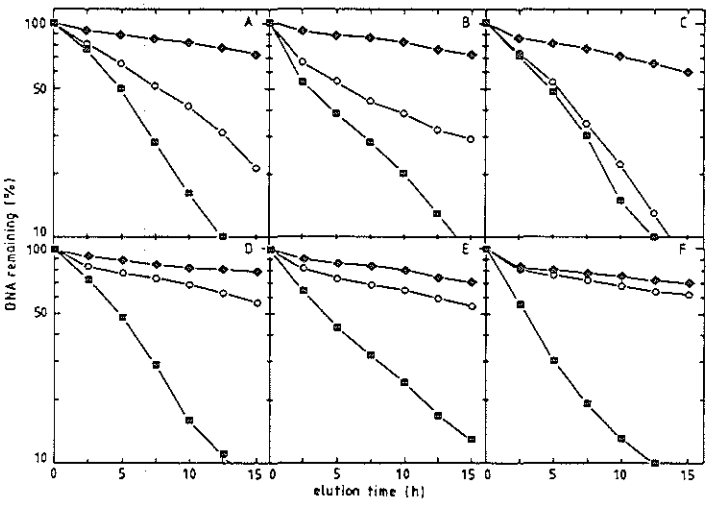

Figure 3.5.1 Representative elution curves of lymphocytes from a normal control subject (A and D), a sporadic Alzheimer patient (B and E) and a patient with inherited Alzheimer's disease (C and F) exposed in vitro to 0.5 mM ENU for 20 min at 37 °C (A,B, and C) or irradiated with 4Gy of gamma-rays at 4 °C (D, E, and F). Untreated control cells (◆); treated cells, no repair incubation (■); treated cells followed by an 1 hour repair incubation (○)

not significantly different between patients and controls (table 3.5.1). In the 48 controls the percentage single-strand breaks disappearance was 41.4% (SE 2.9%), which was not significantly different from the 36.1% (SE 4.0%) found in the 43 patients (p=0.75). Patients who had no or one first degree relative with dementia had a mean single-strand breaks disappearance of 42.5% (SE 8.2) and 43.0% (SE 4.4), respectively, which was very similar to the repair in the controls (41.4%). Patients with two or more affected first-degree relatives had a significantly lower percentage single-strand breaks disappearance (23.6%; SE 5.8%) than controls (p=0.01) or the other patients (p=0.02). In lymphocytes from six patients with two or more affected relatives that were checked at 2½ hour posttreatment, the percentage of ENU-induced single-strand breaks was 34.9% (SE 6.7), indicating that the rate rather than the total amount of repair was lower in these patients (results not shown). A similar decrease in rate of single-strand breaks disappearance in lymphocytes from some normal young subjects exposed in vitro to ENU was reported in our previous study.[15]

There was no statistically significant correlation between age and the percentage single-strand breaks disappearance in patients with Alzheimer's disease, controls or all groups combined. No differences were observed between men and women among control subjects. The amount of single-strand breaks disappearance was not associated with onset-age, severity or duration of disease in the cases.

Discussion

Our data suggest a delayed DNA repair in patients from families in which Alzheimer's disease is apparently inherited as an autosomal dominant disorder. No evidence for a decrease in DNA repair capacity was found in the other patients. This study confirms earlier observations of a significantly lower survival of fibroblast and lymphoid cell lines from patients with Alzheimer's disease[2] and of DNA repair deficiencies in Alzheimer's disease cell lines with regard to exposure to MMS or MNNG.[5,7-9] However, other studies reported conflicting evidence.[10,11] There may be several explanations for these contradicting findings. The number of patient-derived cell lines used in earlier studies has been limited (n=7 to n=9) resulting in a low statistical power to show a decrease in DNA repair. Also, the application of long-term cell culture instead of using freshly isolated cells may have introduced bias. Moreover, it cannot be excluded that (genetic) heterogeneity explains part of the contradicting results. To overcome these problems, we have studied DNA repair capacity in freshly isolated cells of 43 patients with early-onset Alzheimer's and conducted stratified analysis by family history of dementia, age and sex.

In a previous study, we have used the excision repair inhibitor 1-ß-arabino-furanosylcytosine to provide evidence that the most likely type of defect responsible for the low ENU-induced single-strand breaks repair observed in some individuals is a lesion-specific step in excision repair, e.g. glycosylation.[15] In this regard it should be noted that different alkylating agents induce a different spectrum of DNA lesions, the removal of which may require different repair pathways and possibly different glycosylases. Therefore, the previously reported DNA repair defect in cells from sporadic Alzheimer patients, detected after treatment with MMS,[5] is not necessarily in conflict with our present results but could be the consequence of the different alkylating agents used.

A deficiency in the ability of cells to repair alkylating agent-induced single-strand breaks may imply a hypersensitivity to DNA lesions induced by those agents. There may be several explanations for a diminished DNA repair in patients from families in which

the disease is apparently inherited as an autosomal dominant disorder but not in the other patients. Firstly, a decrease in DNA repair may be related to medication. As treatment was very similar for all patients involved in this study, this explanation is not very likely. Secondly, our findings may be explained by misdiagnosis of Alzheimer's disease as within this group of patients the diagnosis has not been confirmed by pathological examination. Although for this study the clinical diagnosis of Alzheimer's disease was verified in all patients (chapter 2.1), it may be argued that the diagnosis may be more certain in patients with two or more other affected relatives. As misdiagnosis among the other patients will tend to attenuate relationships, a pattern suggesting genetic heterogeneity as observed in the present study may be expected. Thirdly, the difference among subgroups of patients may be a consequence of a different pathogenesis. At present, it is not clear how a defective DNA repair may be related to the etiology and/or pathophysiology of familial Alzheimer's disease. It may be speculated that a decrease in DNA repair may aggregate within families. It remains to be resolved whether this may lead to an increased susceptibility for Alzheimer's disease for subjects from such families or whether this has led to a mutation causing Alzheimer's disease in a progenitor, which subsequently has been transmitted as an autosomal dominant trait within these families. In patients with no or one affected relatives, DNA repair may not be implicated in the etiology or there may be other DNA repair processes involved than we have examined in the present study.

In conclusion, our findings suggest a delayed DNA repair in lymphocytes of patients from families in which Alzheimer's disease is apparently inherited as an autosomal dominant disorder. However, as this is the first study to show an association to ENU-induced single-strand breaks specifically in a rather small subgroup of patients, our findings remain to be confirmed by other studies.

Acknowledgements

This research was supported by the SOOM Foundation, the Netherlands Organisation for Scientific Research (NWO), the Eurodem EC concerted action on the epidemiology of dementia, Senetek p.l.c., the Dutch Ministry of Welfare and Health, and the Sandoz Foundation for Gerontological Research. We thank Drs Wim Schulte, Teun Tanja, Rob Haaxma, Arie Lameris and Rolf Saan for their contributions to this study and Helen de Bruijn, Micheline de Haes, Jeanette Kamman, Hanneke van Meurs and Caroline Valkenburg for data collection and genealogy studies.

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Chapter 4

Environmental factors

Introduction

In this chapter, the evidence for environmental (non-genetic) risk factors for Alzheimer's disease is presented. Case-control studies have yielded few leads about the etiology of Alzheimer's disease.[1] Although advanced age and a positive family history of dementia have been consistently associated with Alzheimer's disease, studies on the role of other putative risk factors have yielded contradicting results. A major concern in the interpretation of these findings is the low statistical power of the individual case-control studies. Although the largest study comprised 392 cases and 392 age- and sex-matched controls, the size of the other studies varied from 34 to 198 cases.[2] Given an exposure frequency of 0.10 in controls, a significance level of 0.05 (two-sided) and a power of 0.90, the largest detectable relative risk is 3.4 in a study of 100 cases and 100 controls and 2.5 in a study of 200 cases and 200 controls.[3] The relatively small sample size of the individual studies may therefore explain some of the apparently conflicting results.

In the collaborative re-analysis based on raw data of case-control studies (see chapter 2.2), we evaluated the evidence for the association of Alzheimer's disease with parental age, head trauma, medical and psychiatric history, and environmental factors. An overview of the findings is given in chapter 4.2. Chapter 4.3 deals with further evidence for a relationship between head trauma and Alzheimer's disease and chapter 4.4 presents the findings of a study of serum levels of interleukin-6 in patients with Alzheimer's disease. The latter two chapters are based on the on the case-control study of early-onset Alzheimer's disease, which has been described in chapter 2.1.

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Risk factors for Alzheimer's disease: The EURODEM collaborative re-analysis of case-control studies

Case-control studies have yielded few leads about the etiology of Alzheimer's disease.[1,2] Although advanced age and a positive family history of dementia have been consistently associated with Alzheimer's disease, studies of other putative risk factors have yielded contradicting results. These contradicting findings may in part be explained by the relatively small sample size of the individual studies. Although the largest study comprised 392 cases and 392 controls, the size of the other studies varied from 34 to 198 patients.[3-14] The low statistical power of such studies to detect a significant association with rare exposures may explain some of the apparently conflicting results.

In this paper, we present a re-analysis of case-control studies of Alzheimer's disease. The aim of this analysis was (1) to re-analyze the data in order to compare risk estimates from the individual studies, (2) to estimate risks of Alzheimer's disease using the pooled data-set, and (3) to study subgroups of Alzheimer's disease, based on onset age and gender. The risk factors considered in this collaborative analysis were maternal age at index birth, head trauma, medical and psychiatric history and environmental factors.

Methods

For this analysis, the original data of eleven case-control studies were sent to the Department of Epidemiology and Biostatistics of the Erasmus University Medical School Rotterdam, The Netherlands. An extensive description of the individual studies and the re-analysis is given in chapter 2.2. In all studies, the diagnosis of the patients met the NINCDS-ADRDA or DSM-III criteria.[15,16]

This chapter is based on: Van Duijn CM, Hofman A, for the EURODEM Risk Factors Research Group. Risk factors for Alzheimer's disease: The EURODEM collaborative re-analysis of case-control studies. Neuroepidemiology. In press.

In the re-analysis, we excluded two studies in which the data were not collected symmetrically for patients and controls. Three studies can be considered population-based, i.e., they aimed to detect all patients in a defined geographical area.[9,10,12] All studies comprised prevalent as well as incident patients.

The strength of association between Alzheimer's disease and the putative risk factors was assessed by computing of the odds ratio (OR) as an estimate of the relative risk. Since all included studies were matched for age and gender, odds ratios were estimated using conditional logistic regression analysis.[17] Confounding by family history of dementia, number of siblings and education was evaluated by entering those variables into the logistic regression model. For family history of dementia, a dichotomous variable was created, categorizing individuals by having at least one first degree relative with dementia. In this paper, the adjusted odds ratios are presented with 95% confidence intervals (95% CI). Stratified analyses were conducted based on gender and onset age. Age of onset was categorized in two groups: onset before 70 years and at age 70 or older. For controls, a "reference age" was defined based on the onset age of the matched case.

Results

Parental age

Of the nine studies that were included in this re-analysis, seven studies assessed parental age as a putative risk factor.[4-6,8,10-12] Of those studies, three (USA Denver,[8] USA Durham[4] and USA Minneapolis[5]) were excluded because data were available of less than 60% of either the cases or the controls. Thus, only four studies contributed data to the analysis of parental age.[6,10-12] As there is evidence for an increase in risk of Alzheimer's disease for young as well as late maternal age, subjects whose mother was aged 25 to 29 years at birth were taken as the reference category in the pooled analysis.[18]

An increased risk of Alzheimer's disease was observed for those whose mothers age was 40 years or older at the time of the subjects birth (table 4.2.1). The increase in risk was mainly determined by the Italian study[6] (OR 4.8; 95% CI 1.2-19.3), while the other studies showed a non-significant small increase in the odds ratio (Australian study[12] OR 1.4; 95% CI 0.5-3.7; Dutch study[10] OR 1.4; 95% CI 0.6-3.4; USA, Seattle[11] OR 1.2; 95% CI 0.3-4.5). Although the association was strongest in women, odds ratios were not statistically significant different when comparing men and women and early- and late-onset Alzheimer's disease.

Table 4.2.1 Maternal age and the risk of Alzheimer's disease*

Maternal age (years)	Number of: Cases	Controls	OR†	95% CI
15-19	25	17	1.5	0.8-3.0
20-24	87	97	0.9	0.6-1.4
25-29	130	135	1	reference
30-34	104	114	0.9	0.6-1.3
35-39	53	55	1.0	0.6-1.6
40+	47	28	1.7	1.0-2.9

* Studies included in the analyses are Australia,[12] Italy,[6] The Netherlands[10] and USA, Seattle[11]
† Odds ratio adjusted for age, gender, family history of dementia, number of siblings and education

The risk was also increased for very young maternal age in three studies,[6,10,11] although the association did not reach statistical significance in the overall analysis (table 4.2.1). In the Dutch Study[10] of early-onset Alzheimer's disease, the odds ratio was significantly increased for those whose mother was 19 years or younger at birth (OR 6.5; 95% CI 1.4-30.3). However, in the Seattle study[11] an inverse association was found (OR 0.3; 95% CI 0.1-1.3). When stratifying for onset age, an association with young maternal age was found only with early-onset Alzheimer's disease (OR 4.2; 95% CI 1.4-13.0), but this finding was mainly determined by the Dutch study.

There was no evidence for an association between early- or late-onset Alzheimer's disease and paternal age at birth, when maternal age was adjusted for.

Head trauma

Only studies which collected information on head trauma with loss of consciousness that occurred more than one year before the onset of dementia or the reference age in controls were considered in the re-analysis (table 4.2.2).[19] In all these studies, the odds ratio exceeded one (table 4.2.2). The pooled odds ratio estimated from these studies was 1.8 (95% CI 1.3-2.7). Subgroup analysis showed a significant (p=0.005) difference between men and women. The odds ratio was for women 0.9 (95% CI 0.4-1.7) and for men 2.7 (95% CI 1.6-4.4). No significant difference was observed when stratifying for onset age. Since patients with Alzheimer's disease may have a higher risk of head trauma due to the dementia, odds ratios were estimated for head trauma occurring more and less than ten years prior to the disease onset. The highest odds ratio was observed for head

Table 4.2.2 Head trauma and the risk of Alzheimer's disease

Study	Exposure frequency Cases	Controls	OR*	95% CI
Australia[12]	7/152	6/152	1.2	0.3-4.2
Italy[6]	6/95	3/95	2.0	0.4-12.4
The Netherlands[10]	22/197	17/197	1.3	0.7-2.8
USA, Denver[8]	6/48	1/48	6.0	0.7-276.0
USA, Minneapolis[5]	16/45	7/45	2.8	1.0-9.9
USA, Rochester[9]	11/392	8/392	1.4	0.5-4.4
USA, Seattle[11]	19/130	8/130	2.4	1.0-6.3
Overall analysis	87/1059	50/1059	1.8	1.3-2.7

* Odds ratio adjusted for age, gender, family history of dementia, number of siblings and education

trauma that occurred within ten years before onset (OR 5.5; 95% CI 1.6-18.3). A significant elevation in odds ratio (OR 1.6; 95% CI 1.0-2.57) was also observed for head trauma that occurred more than ten years before disease onset, which are not likely due to the dementia.

Medical history

Several disorders have been assessed as putative risk factors in the case-control studies (table 4.2.3).[20] In the pooled analysis, only disorders that occurred more than one year before the onset of Alzheimer's disease or the reference age in controls were considered. No association was found between Alzheimer's disease and thyroid disease, when the type of thyroid disorder was unspecified. A significant association with hypothyroidism was observed (OR 2.3; 95% CI 1.0-5.4). Although numbers were too small to stratify for lag-time between diagnosis of hypothyroidism and onset age of dementia, in most cases hypothyroidism was diagnosed long before the onset of dementia (median time 20 years). An increased risk was found for those with and without a family history of dementia.

No association of Alzheimer's disease was found with neurotropic viruses. Since in most studies data were collected by interview, no distinction could be made between encephalitis or meningitis. There was no association of Alzheimer's disease with exposure to either of these disorders. The pooled analysis of epilepsy yielded a small, non-significant increase in the odds ratio. The increase in risk was highest for epilepsy which first occurred within ten years before the onset of Alzheimer's disease (OR 2.5; 95% CI 0.4-13.9), suggesting the seizures may have been an early symptom of the disease.

Table 4.2.3 Medical history and the risk of Alzheimer's disease

Risk factor*	Exposure frequency Cases	Controls	OR†	95% CI
All thyroid disease[4-6,9,10,12]	110/994	115/991	1.0	0.8-1.3
Hyperthyroidism[6,11,12]	14/408	12/390	1.2	0.5-2.6
Goitre[6,11,12]	12/408	18/390	0.6	0.3-1.4
Hypothyroidism[7,9,12]	17/655	8/732	2.3	1.0-5.4
Neurotropic viruses:				
Herpes zoster[4-6,9-12]	100/1103	105/1110	0.9	0.6-1.2
Herpes simplex I[6,10]	94/314	78/295	1.2	0.8-1.8
Polio myelitis[4,5,10,11]	5/451	7/467	0.8	0.3-2.7
Encephalitis/meningitis[4-7,9,10,12]‡	7/1081	5/1167	1.6	0.5-5.3
Epilepsy[7,9,10,12]	11/459	6/538	1.6	0.7-3.5
Atopy[5,7,10-12]	172/670	190/684	0.9	0.6-1.2
Hay fever[5,7,10,11]	31/445	38/454	0.8	0.5-1.2
Arthrosis/arthritis[4,5,12]‡	131/277	168/302	0.7	0.5-1.0
Migraine/severe headaches[4,5,10,12]‡	68/463	101/490	0.7	0.5-1.0
Blood transfusions[4-6,10,12]	71/523	112/562	0.6	0.4-0.9
General anaesthesia[4,5,9-12]	827/1098	838/1114	1.0	0.8-1.3

* Reference to studies included in the analysis
† Odds ratio adjusted for age, gender, family history of dementia, number of siblings and education
‡ Based on the anamnestic data, no distinction could be made between these disorders

There was no association of Alzheimer's disease with allergies nor with general anaesthesia. Inverse associations were observed with several chronic disorders (arthrosis/arthritis, migraine/severe headaches) and with blood transfusions. For migraine and severe headaches, subgroup analysis showed a significant (p=0.05) difference between men and women. The odds ratio for men was 1.1 (95% CI 0.6-2.0) and for women was 0.6 (95% CI 0.4-0.9).

History of depression

Four studies have assessed episodes of depression disorder occurring more than one year before the onset of Alzheimer's disease or the reference age in controls (table 4.2.4).[21] In three out of the four studies there were more patients with a history of depression as compared to controls. It is important to note, that the association was observed in the Rochester study[9], which is based on medical records. A significant association was observed for episodes of depression more than ten years before disease onset (OR 1.9; 95% CI 1.1-3.3), suggesting that the association may be a true risk factor rather than merely be the result of the dementia. There was no difference in risks

Table 4.2.4 History of depression and the risk of Alzheimer's disease

Study	Exposure frequency Cases	Controls	OR*	95% CI
Australia[12]	11/170	4/170	2.8	0.9-8.6
USA, Bedford[8]	4/103	6/162	0.8	0.3-3.1
USA, Minneapolis[5]	4/78	1/48	3.0	0.3-28.6
USA, Rochester[9]	36/392	21/392	1.8	1.0-3.3
Overall analysis	55/743	32/772	1.8	1.2-2.9

* Odds ratio adjusted for age, gender, family history of dementia, number of siblings and education

observed after stratifying for gender. An increase in risk of Alzheimer's disease for history of depression was only observed in late-onset patients (OR 2.4; 95% CI 1.4-4.4). The odds ratio in early-onset patients was 1.0 (95% CI 0.5-2.3). Data on anti-depressant treatment (unspecified) were available only in two studies.[5,12] No association with Alzheimer's disease could be established.

Data of life events have been assessed in six studies.[4-6,8,10,12] A pooled analysis of these studies did not show a significant association of Alzheimer's disease with death of a spouse, death of a child or divorce.

Environmental factors

Table 4.2.5 gives the odds ratios for smoking (ever smoked yes/no).[22] With the exception of two studies,[4,7] there was a consistent trend towards an inverse relationship. The pooled analysis of the studies showed a borderline significant decrease in the odds ratio (OR 0.8; 95% CI 0.6-1.0). When smokers were classified according to the number of pack-years (number of cigarettes smoked a day (packs) x smoking duration (years)), the odds ratio decreased with increasing number of pack-years (p=0.0003). The odds ratio was 0.7 (95% CI 0.5-1.1) for smoking less than 15.5 pack-years, 0.6 (95% CI 0.4-1.0) for smoking 15.5-37.0 pack-years and 0.5 (95% CI 0.3-0.8) for smoking more than 37 pack-years. There was no evidence for effect modification by onset age or gender.

Data on alcohol consumption were available from five studies.[5-8,10] Odds ratios were estimated for low (0.6-3.2 ounces pure alcohol per week), moderate (3.2-6.0 ounces/week) or high intake (more than 6.0 ounces/week).[22] The odds ratios were for low intake 1.1 (95% CI 0.7-1.6), for moderate intake 1.0 (95% CI 0.6-1.7) and high intake 0.8 (95% CI 0.5-1.3).

Table 4.2.5 Smoking and the risk of Alzheimer's disease

Study	Exposure frequency Cases	Controls	OR*	95% CI
Australia[12]	80/168	89/169	0.8	0.5-1.3
Italy[6]	51/116	52/97	0.6	0.3-1.4
The Netherlands[10]	89/193	102/195	0.7	0.5-1.2
USA, Bedford[7]	85/102	127/161	1.5	0.7-3.2
USA, Denver[8]	17/63	23/63	0.6	0.2-1.4
USA, Durham[4]	25/46	50/91	1.0	0.5-2.1
USA, Minneapolis[5]	54/78	37/48	0.5	0.2-1.7
USA, Seattle[11]	66/129	75/129	0.7	0.4-1.2
Overall analysis	467/895	555/953	0.8	0.6-1.0

* Odds ratio adjusted for age, gender, family history of dementia, number of siblings and education

Occupational exposures to solvents and lead have been studied in four investigations included in this re-analysis.[4,5,7,8] The pooled risk estimate was for occupational exposure to solvents 0.8 (95% CI 0.5-1.2) and for exposure to lead 0.7 (95% CI 0.4-1.4).[23]

Discussion

This collaborative analysis of case-control studies of patients with clinically diagnosed Alzheimer's disease suggests that the risk for Alzheimer's disease is increased for late maternal age, head trauma, hypothyroidism and history of depression. There were inverse associations with smoking, arthrosis/arthritis, migraine/severe headaches and blood transfusions. No evidence was observed for a role in Alzheimer's disease of alcohol, occupational exposures to solvents and lead, general anaesthesia, neurotropic viruses, encephalitis or meningitis, atopy or hay fever.

There are several ways in which the findings of the re-analysis of case-controls studies of Alzheimer's disease may be biased.[24,25] Selection bias in the case-series may have occurred, since the studies were generally based on prevalent patients. The observed risk factors may therefore relate to predictors of survival, rather than to the risk of Alzheimer's disease. Another issue related to selection bias is that spurious associations may occur in studies which are not population-based. Patients with certain conditions, e.g. head trauma, hypothyroidism and depression may have a higher chance to be included

in the study. As to the possibility of information bias, case-control studies relying on reports of informants are prone to misclassification and recall bias. In chapter 6.2 the possibilities of bias will be discussed in depth and in chapter 6.3 the results of the re-analysis will be reviewed in light of the findings of previous studies. Here, we will discuss the evidence for the association between Alzheimer's disease with parental age, head trauma, medical and psychiatric history, and environmental factors based on the findings of the re-analysis of case-control studies. Case-control studies of Alzheimer's disease conducted to date have been hampered by small sample size. Pooling of data into a single risk estimate may be an efficient way to overcome this problem. Yet, it is well recognized that case-control studies are prone to bias.[24] Consistency of findings across studies that have been conducted at different locations with different methodology, however, may strengthen the evidence for a causal relationship. Therefore, an important aim of the re-analysis has been to test for heterogeneity across studies (chapter 2.2).

Parental age

The association to maternal age derives limited support from this re-analysis. The majority of the individual studies showed a weak non-significant association of Alzheimer's disease with late maternal age (40 years and over), although all studies showed a consistent increase in risk. The increase in risk for young maternal age (age 19 years or younger) was less consistent since one study showed an inverse association. No association could be shown with paternal age. Weak associations as observed here may be explained by bias or confounding. On the other hand one may argue that true weak associations may be blurred by error in exposure measurement. Therefore, studies in which maternal age is verified in birth records are needed to discern whether maternal age is indeed a risk factor for Alzheimer's disease.

Head trauma

The association between Alzheimer's disease and head trauma was consistently shown across studies. In this analysis we were able to show a significant elevation in risk of Alzheimer's disease for head trauma that occurred more than ten years before disease onset. Such events are not likely due to the dementia. However, we cannot exclude the possibility of recall bias that may have operated in a similar way in all studies as informants for patients may be more eager to recollect head trauma than informants for control subjects. The interaction with gender was unexpected and it is difficult to find a biologic explanation for the absence of an association in women. Although we only

included head trauma with loss of consciousness, the explanation may be related to the head trauma generally being more severe in men. However, the possibility of recall bias and the unexpected effect modification by gender indicate that the association between head trauma and Alzheimer's disease should be treated with caution. In chapter 4.3, the role of head trauma will be discussed further.

Medical history

It is difficult to interpret the findings on medical history. Methodological pitfalls such as selection bias, information bias, confounding, multiple testing and data dredging apply particularly to these analyses.[24] A negative indication when interpreting the findings may therefore be an association contradicting the *a priori* hypothesis. Although the association with hypothyroidism is of interest because of a possible role of the thyroid hormone on neuritic outgrowth,[20] *a priori* this association was not expected and since hypothyroidism can be a cause of secondary dementia, caution is warranted in the interpretation of this finding. From a statistical point of view skepticism on a relationship to a specific kind of thyroid disease results from the lack of an association to all thyroid disease combined. Also the inverse associations with several chronic disorders (arthrosis/arthritis, migraine/severe headaches) and with blood transfusions were unexpected. One may argue that these inverse associations may be artifacts due to bias in the control population. Control subjects, who suffer from chronic diseases may have been more willing to participate in epidemiologic studies. An issue to consider when interpreting the findings on viral infections is, that one may argue that measurement of exposure has been too crude to pick up an association to a specific virus.

Psychiatric history

Perhaps one of the most interesting findings is the association with history of depression as: (1) the association was observed in the Rochester study[9], which is based on medical records; (2) a significant association was observed for episodes of depression more than ten years before disease onset, suggesting that the association may be a true risk factor rather than merely be the result of the dementia. However, in a slowly progressive disorder as Alzheimer's disease, the underlying disease process may already be in progress although no clinical symptoms can be detected. Therefore, we cannot fully exclude the possibility that the depression may be a prodrome of Alzheimer's disease and it may be concluded that the association between Alzheimer's disease and history of depression requires further research. In particular, the role of prior medication with

anticholinergic effects needs to be addressed. The data on medication in the present analysis did not allow such an analysis.

Environmental factors

The inverse association to smoking was an unexpected finding. With the exception of two studies, the association was consistently found across studies and there was evidence for a dose-response relationship. In the interpretation of these findings it is important to note that prevalent cases were included in the studies. Patients that survived longer, i.e. non-smokers, may have been overrepresented in the study. The association to smoking will be discussed more extensively in the chapters 5.3 and 5.4. The absence of an association with alcohol intake should be treated with caution as cases with high intake may have been excluded in each study when applying the criteria for possible or probable Alzheimer's disease.[15,16] This may have led to an underestimation in risk. This re-analysis does not support an association with occupational exposure to lead or solvents. However, frequency of exposure was low and exposure definition may have been too crude to detect an association with Alzheimer's disease.

To date, a great variety of risk factors have been associated with Alzheimer's disease. Many of these associations appeared in one or two studies and were not replicated by others.[2] In the present analysis we tried to review the evidence for an association of Alzheimer's disease with maternal age at birth, head trauma, medical and psychiatric history and environmental exposures by re-analyzing the data of nine case-control studies. Although the study has supported an association of Alzheimer's disease with a number of these risk factors, prospective studies are needed to discern whether the observed associations are of true etiologic relevance. Case-control studies nested within prospective follow-up studies of Alzheimer's disease, are probably the most efficient way to overcome the problems encountered in retrospective case-control studies.

Acknowledgements

The re-analysis of case-control studies was supported by the Commission of the European Community for the Eurodem concerted action on the epidemiology of dementia, the Netherlands Organisation for Scientific Research (NWO) and was conducted in collaboration with the National Institute on Aging of the US National Institutes of Health and with the World Health Organization. For the acknowledgements of the studies see appendix to chapter 2.2 (page 33). We thank M. Eijgermans, Dr G.A. van Es and E. Neeleman for their help in data analysis and Dr T Stijnen for statistical advice.

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Head trauma and the risk of early-onset Alzheimer's disease

Repeated head trauma in boxers has been linked to dementia pugilistica (punch drunk syndrome).[1] Although no classical senile plaques were observed in patients with this syndrome,[1] neurofibrillary tangles indistinguishable from those seen in Alzheimer's disease are found.[2] These findings have led to the hypothesis that head trauma may be implicated in Alzheimer's disease. In four case-control studies a significant increase in risk of Alzheimer's disease was observed for those with a history of head trauma.[3-6] With the exception of two small studies,[7,8] each of the previous studies reported an excess of head trauma in patients with Alzheimer's disease, although no significant association could be established.[9-14] Little is known of the mechanism through which head trauma may be implicated in Alzheimer's disease. Graves et al reported that the risk of Alzheimer's disease increased when the period between the head trauma and the onset of disease decreased.[5] As other studies have been too small to consider the timing of the head trauma relative to the onset of disease, this inverse trend remains to be confirmed. Another issue to resolve is the interaction of head trauma with other risk factors for Alzheimer's disease, in particular genetic factors.

The emphasis of this study of the relationship between head trauma and Alzheimer's disease was on the lag-time between the head trauma and disease onset and on the interaction with other risk factors, i.e. family history of dementia, gender and education.

Methods

Patients were derived from an epidemiologic study of risk factors for clinically diagnosed Alzheimer's disease. The study aimed at a complete ascertainment of cases in

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This chapter is based on: Van Duijn CM, Tanja TA, Haaxma R, Schulte W, Saan RJ, Lameris AJ, Antonides-Hendriks G, Hofman A. Head Trauma and the risk of Alzheimer's disease. Am J Epidemiol. In press.

whom the diagnosis was made before 70 years and who were living in the four northern provinces of The Netherlands and the area of metropolitan Rotterdam. Ascertainment was carried out through all neurologic, psychiatric, geriatric services in the study areas. All cases were diagnosed in the period of January 1980 to July 1987. Control subjects were randomly drawn from the same municipality as the cases. Cases and controls were matched for age (within 5 years), sex, and residence. An extensive description of the design of the study, case diagnosis and response rates has been given in chapter 2.1. The study comprised a total of 198 patients and 198 controls.

Data collection. We assessed data on head trauma and other putative risk factors for Alzheimer's disease by a structured interview. Because of the cognitive decline of the patients, the history was taken from the next of kin of the patient. To assure symmetry in data collection, we also interviewed a next of kin for the control subject. For 174 cases (88%) and 188 controls (95%) the informant was the spouse or an adult child. As etiologic factors were studied, questions referred to exposure of the patient before the age of onset of dementia. Age of onset of Alzheimer's disease was estimated as the age at which memory loss or change in behavior was first noted by the next of kin or any other relative. For control subjects, a "reference age" was defined based on the age of onset of Alzheimer's disease in the matched case and only events occurring before the "reference age" were considered. Head trauma was assessed in closed questions with additional open-ended questions about: 1) loss of consciousness and duration of unconsciousness; 2) age at the time of the head trauma; 3) circumstances surrounding the event; 4) medical treatment.

Full pedigree information on dementia was obtained as part of the structured interview.[15] To increase the validity of these data, we verified the information about the family history through an additional interview of a first degree relative. If the patient had been admitted to hospital, the diagnosis was checked in independent medical records. Patients were considered to have a positive family history of dementia when there was at least one first degree relative with dementia.

Data analysis. We have restricted the analysis to head injuries with loss of consciousness. The duration of the unconsciousness varied from one minute to two hours. According to the informant, all patients had regained normal intellectual cognitive function after the head trauma. We assessed the strength of the association between head trauma and Alzheimer's disease by the odds ratio (OR) as an estimate of the relative risk. Conditional logistic regression analysis was used to take the matched design into account.[20] The odds ratio was estimated by maximum likelihood and the 95% confidence

interval (CI) was based on the asymptotic standard error. Possible confounding by family history of dementia and education was adjusted for by adding these variables to the conditional logistic regression model. To control for bias which may result from the fact that cases and controls were not matched for informant, we performed a separate analysis based on the 111 matched case-control pairs that were concordant for informant. The results of this analysis were very similar to those of the overall analysis and did not change any of the conclusions. Modification of the relationship between head trauma and Alzheimer's disease by gender and education was studied by stratified analysis. For family history of dementia, interaction was tested as described by Ottman.[21] Those without a head trauma and without a family history of dementia were taken as a reference category. Risks were then estimated simultaneously for those with a head trauma but without a family history of dementia, for those with a positive family history but without a head trauma and for those with a head trauma as well as a positive family history.

Results

There was a non-significant increase in risk (OR 1.3; 95% CI 0.7-2.6) for those with a history of head trauma with loss of consciousness (table 4.3.1). When we restricted the analysis to case-control pairs that were concordant for informant, the risk estimate virtually did not change (OR 1.4; 95% CI 0.5-4.1). In the overall analysis, the OR for head trauma adjusted for family history of dementia and education was 1.6 (95% CI 0.8-3.4). When stratifying for gender, an increase in risk was observed only in men (p-value for interaction 0.17). There was no evidence for effect-modification by education (p-value for interaction 0.56).

The mean period of time between the occurrence of the head trauma and the onset of dementia or the reference age was 16.8 years (SD=16.3) for patients and 29.9 years (SD=12.9) for controls. Stratification for the time-lag until the onset of Alzheimer's disease showed that those with a head trauma within ten years before the occurrence of Alzheimer's disease had eight times the chance of having Alzheimer's disease (table 4.3.2). The odds ratio was 3.0 (95% CI 0.3-28.8) in women. In men, five cases and no controls had a head trauma within ten years before disease onset (p=0.03). There was no significant increase in odds ratio for head trauma that occurred more than ten years before the disease onset or the reference age in men (OR 1.2; 95% CI 0.3-4.7) or in women (OR 0.4; 95% CI 0.1-1.7).

Table 4.3.1 Head trauma with loss of consciousness in 198 patients with clinically diagnosed Alzheimer's disease and 198 age- and gender-matched controls: The Netherlands, 1980-1987

	Head trauma Yes	No	OR*	OR†	OR†
All (n=198):					
Cases	22	176	1.3 [0.7-2.6]	1.8 [0.8-3.8]	1.6 [0.8-3.4]
Controls	17	181			
Men (n=74):					
Cases	16	58	2.0 [0.8-5.0]	2.7 [1.0-7.4]	2.5 [0.9-7.0]
Controls	9	65			
Women (n=124):					
Cases	6	118	0.8 [0.3-2.2]	1.0 [0.3-3.2]	0.9 [0.3-2.8]
Controls	8	116			

* Odds ratio, with 95% confidence interval within parentheses.
† Odds ratio adjusted for dementia in first degree relatives
‡ Odds ratio adjusted for dementia in first degree relatives and education

Table 4.3.2 Timing of head trauma with loss of consciousness relative to onset of dementia in 198 patients with clinically diagnosed Alzheimer's disease and 198 age- and gender-matched controls: The Netherlands, 1980-1987

Timing of head trauma relative to onset*	Number of: Cases	Controls	OR†	OR‡	OR§
No head trauma	176	181	1.0 reference	1.0 reference	1.0 reference
Head trauma ≤ 10 years	8	1	8.0 [1.0-64.0]	8.8 [1.0-76.9]	10.0 [1.0-96:8]
Head trauma > 10 years	12	15	0.8 [0.3-1.8]	1.0 [0.4-2.6]	0.9 [0.4-2.2]

* Data are missing for 2 cases and 1 control
† Odds ratio, with 95% confidence interval within parentheses
‡ Odds ratio adjusted for dementia in first degree relatives
§ Odds ratio adjusted for dementia in first degree relatives and education

Table 4.3.3 shows the interaction with family history of dementia. For those with a head trauma and a first degree relative with dementia the risk of Alzheimer's disease was nine times elevated. On a multiplicative scale, there was no evidence for effect-modification by family history (p=0.90). The odds ratio for head trauma was 1.7 (95% CI 0.8-4.0) for those without a first degree relative with dementia and 2.0 (95% CI 0.4-10.0) for those with a positive family history. The risk estimates did not change materially when adjusting for education.

Table 4.3.3 Head trauma with loss of consciousness and Alzheimer's disease, effect modification by family history of dementia in first degree relatives: The Netherlands, 1980-1987

Component	Number of: Cases	Controls	OR*	OR†
Head trauma - Family history -	89	147	1.0 reference	1.0 reference
Head trauma - Family history +	87	34	4.7 [2.7-8.1]	4.9 [2.8-8.6]
Head trauma + Family history -	13	14	1.7 [0.8-4.0]	1.5 [0.6-3.6]
Head trauma + Family history +	9	3	9.2 [1.8-48.3]	9.5 [1.8-48.8]

* Odds ratio, with 95% confidence interval within parentheses
† Odds ratio adjusted for education

Discussion

In this study the association between Alzheimer's disease and head trauma with loss of consciousness was confined to head trauma that occurred within a period of ten years before the onset of dementia. Although overall no relationship could be established in women, there was an excess of head trauma among female patients within this ten year period as compared to control subjects. The association was not modified by family history of dementia, as measured on a multiplicative scale.

Ten previous studies consistently showed a higher frequency of head trauma in patients with Alzheimer's disease than in controls.[3-6,9-14] Four studies reported the risk of Alzheimer's disease to be significantly elevated.[3-6] Pooling of the data from all formal

case-control studies of head trauma with loss of consciousness showed a significant association (OR 1.8, 95% CI 1.3-2.7).[4,5,9,10,12,13,15,22] It is important to note that in the only prospective follow-up study based on data obtained from medical records of the Rochester register[12] a slight elevation in risk of 1.3 (p=0.70) was reported, similar to the risk unadjusted for family history of dementia observed in our study. Family history of dementia, however, was not assessed as a putative confounder in the Rochester study and the data did not allow stratification by timing of the head trauma relative to the onset of disease. In the re-analysis of case-control studies the association between Alzheimer's disease and head trauma could only be established in men.[22] Although the association was strongest for head trauma that occurred within ten years before disease onset, a significant elevation in risk (OR 1.6; 95% CI 1.0-2.6) was also observed for head trauma that occurred more than ten years before the onset of disease.[22] Such an inverse trend has been shown earlier by Graves et al,[5] who reported an association between Alzheimer's disease and head trauma (with or without loss of consciousness) that occurred one to nine years (OR 9.7; 95% CI 1.1-83.3) and ten to 29 years (OR 5.0; 95% CI 1.3-9.8) before the onset of disease. In contrast, our study of head trauma with loss of consciousness suggests that the risk of Alzheimer's disease was increased only within the ten year period before the disease onset. The finding of this association in men and women argues against effect-modification by gender. As to the interaction with family history of dementia, it has been suggested that head trauma may play a role only in those who are genetically susceptible,[23] while others have reported that such environmental risk factors may be associated with sporadic Alzheimer's disease rather than with the familial form of the disease.[22,24] Using family history of dementia as an indicator for genetic susceptibility,[21] a similar odds ratio for Alzheimer's disease was observed in those with and without an increased genetic risk. This suggests that head trauma may influence the risk of Alzheimer's disease in those who are not genetically susceptible and that there is no evidence for synergy or antagonism in a multiplicative model. One may argue, however, that the power to show interaction in our study has been low.

The type of study reported here may suffer from bias. A first problem to be discussed is recall bias. Since relatives of patients may be eager to find an explanation for the disease of the patient, they may overreport the exposure to factors such as head trauma which are thought to be associated with the disease. We have therefore restricted the analyses to severe head trauma with loss of consciousness. These events are less vulnerable to recall bias since it is unlikely that relatives of patients report serious events which did not occur and that relatives of controls have forgotten such a severe head

trauma. A second methodologic problem to be discussed concerns the diagnosis of Alzheimer's disease, which was in most cases based on clinical data. Although we firmly applied the criteria for the clinical diagnosis of Alzheimer's disease and the diagnosis was confirmed in all ten cases who had an autopsy,[15] misclassification may have occurred in disease status in some cases. This type of misclassification tends to diminish a true association. Misclassification may also bias the comparison of sporadic and familial Alzheimer's disease. In the absence of a genetic marker for population studies,[25] we have classified the patients on the basis of their family history of dementia assuming that those with a positive history are most likely of primarily genetic origin and those with no first degree relatives with dementia are most likely of primarily environmental origin. Indeed, relatives of patients without a family history may carry the gene and express the disease later in life, whereas familial aggregation of Alzheimer's disease may be due to clustering of non-genetic disease. This has perhaps led to non-differential misclassification,[21] which may have reduced the power to show effect-modification. As to the possibility of selection bias, case selection aimed to be population-based in the two study areas. Although selection may have occurred in the control series because of the higher non-response as compared to the cases, it is unlikely that this was associated with a history of head trauma. Finally, confounding bias may have occurred because of the fact that putative risk factors may not have been measured or may have been measured with so much error that effective control for confounding was impossible.

Two interpretations of a relationship between recent head trauma and Alzheimer's disease are possible: 1) the head trauma may be a consequence of an early stage of the dementia; and 2) head trauma may be implicated in the pathogenesis of Alzheimer's disease. As to the first explanation, there is evidence for an increase in risk of head trauma and falls for Alzheimer patients as compared to non-demented subjects.[26-28] It is difficult in a case-control study to distinguish events preceding or following the disease onset, in particular when studying a disorder like Alzheimer's disease in which the onset age is not easily assessed. However, we questioned relatives extensively about symptoms of the disease prior to the occurrence of the head trauma and excluded those patients with changes in behavior or memory loss before the head trauma occurred.

The second explanation, that head trauma may lead to Alzheimer's disease, is supported by the finding of the Alzheimer type neurofibrillary degeneration in patients with dementia pugilistica.[2,29] This type of pathology has been associated with repeated head trauma in boxers.[1,30] Although initially no classical senile plaques were observed,[1] recent studies of patients with dementia pugilistica have shown diffuse plaques.[31-33]

Because of the similarity in pathology, a common pathogenesis of Alzheimer's disease and dementia pugilistica has been suggested.[1,2] The mechanism through which head trauma may be implicated in dementia is still unclear. Head trauma may induce changes in the blood brain barrier, which may lead to Alzheimer's disease.[1,3] On the other hand, it is conceivable that head trauma may cause rupture of brain vessels, which are already weakened by amyloid deposits.[1] Thus, the trauma may trigger a subclinical pathological process and provoke early-onset of dementia. This hypothesis is supported by the finding of an earlier onset of disease in patients with a history of head trauma[34,35] and by the short lag-time between the head trauma and disease onset observed in the present study.

Although the findings of our study are compatible with a role of head trauma in early-onset Alzheimer's disease, we interpret our findings with caution. Despite the apparently consistent findings of case-control studies,[22] we cannot exclude the possibility that all studies may have been biased in a similar way. The validity of case-control studies of Alzheimer's disease conducted to date is limited by the possibility of confounding by recall bias and selection bias. To determine the etiologic significance of the relation between head trauma and Alzheimer's disease, the association remains to be confirmed in a prospective follow-up study.

Acknowledgements

This research was supported by the SOOM Foundation, the Netherlands Organisation for Scientific Research (NWO) and the Eurodem EC concerted action on the epidemiology of Dementia. We thank Drs Hans Valkenburg and Walter Rocca for their contributions to this study and Helen de Bruijn, Micheline de Haes, Jeanette Kamman, Hanneke van Meurs, Verona Otten and Caroline Valkenburg for data collection and genealogy studies.

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Serum levels of interleukin-6 in patients with early-onset Alzheimer's disease

In patients with Alzheimer's disease, extracellular amyloid fibrils accumulate in the cores of the senile plaques and in cerebral and meningeal microvessels.[1] Two lines of evidence suggest that the formation of amyloid deposits in Alzheimer's disease may be accompanied by an acute-phase response, the reaction of the body to tissue damage and inflammatory processes. First, the amyloid in the senile plaques has been demonstrated to be tightly associated with the acute-phase protein α1-antichymotrypsin.[2,3] Second, increased serum levels of the acute-phase proteins have been reported in patients with Alzheimer's disease.[4,5]

Recently, it has been demonstrated that the production of acute-phase proteins is at least in part mediated by two lymphokines, interleukin-1 (IL-1) and interleukin-6 (IL-6).[5-11] These lymphokines are produced by a variety of cells and have several functions inside and outside the immune system.[10] The increased serum levels of IL-6 that have been demonstrated in patients rejecting their kidney transplantant,[12] patients with serious skin injury,[6] and in patients with rheumatoid arthritis[13,14] indicate that inflammatory processes can be sensitively monitored by an IL-6 bio-assay. Therefore, we have used this assay to study serum levels of IL-6, as a serologic marker of disease and severity of dementia in 97 patients with clinically diagnosed Alzheimer's disease, and in 79 population control subjects.

Methods

Patients were derived from an epidemiologic study of risk factors for clinically diagnosed Alzheimer's disease. For this study all early-onset patients (diagnosis $\leq$ 70 years) living in Rotterdam or the four northern provinces of the Netherlands in whom

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This chapter is based on: Van Duijn CM, Hofman A, Nagelkerken L. Serum levels of interleukin-6 are not elevated in patients with Alzheimer's disease. Neuroscience Letters 1990;108:350-354.

the diagnoses was made in the period of January 1980 to July 1987 were ascertained. Ascertainment was carried out through all neurologic, psychiatric, geriatric services in the study areas. Control subjects were randomly drawn from the same municipality as the cases. Cases and controls were matched for age (within 5 years), sex, and residence. An extensive description of the design of the study, case diagnosis and response rates has been given in chapter 2.1. The study comprised a total of 198 patients and 198 controls, of whom 127 patients and 127 controls were derived from the four Northern provinces. The latter patients participated in this study of IL-6. Serum samples were available for 97 patients (76%) and 79 controls (62%).

Serum levels of IL-6 were determined in stored serum samples (-80 °C) with the use of a bio-assay.[6] IL-6 dependent B9 hybridoma cells as well as human recombinant IL-6 were kindly provided by Dr. L. Aarden, Central Laboratory of the Blood Transfusion Service, Amsterdam. The cells were cultured in Iscoves' modified Dulbecco's medium (Seromed, Berlin, FRG), supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 2 Mm L-glutamin, $5x10^{-5}$ M 2-mercapto-ethanol and 5 % (v/v) foetal calf serum (Seralab, Crawley Down, UK). Sera from patients with Alzheimer's disease and from control subjects were serially diluted in duplicate in the same medium in flat-bottom microtiter plates (Greiner, Nürtingen, FRG). Five thousand B9 cells were added to each well and the final volume was adjusted to 200 μl. After 66 hours of culture, the cells were pulsed with 0.25 μCi ^{3}H-methyl-thymidine (specific activity 2 Ci/mMol; Radiochemical Center, Amersham, UK). Six hours later, the cultures were terminated by harvesting the DNA on nitrocellulose filters (Skatron, Tranby, Norway). Filters were counted in toluene, containing 4 g/l PPO and 0.05 g/l POPOP. One unit IL-6/ml was defined as the concentration at which half-maximal proliferation was found; recombinant IL-6 served as a standard.

As appears from figure 4.4.1, the B9 assay is highly sensitive to IL-6. The B9 cells proliferated in a dose-dependent manner in response to rIL-6. The assay detected levels of RIL-6 as low as 0.1 U/ml. Maximal proliferation of the cells occurred in the presence of approximately 5 U/ml. Parallel dose response curves were obtained when positive sera were diluted. These three individual sera numbered 1052, 2063 and 2082 were found to contain 25, 96 and 650 U IL-6/ml, respectively.

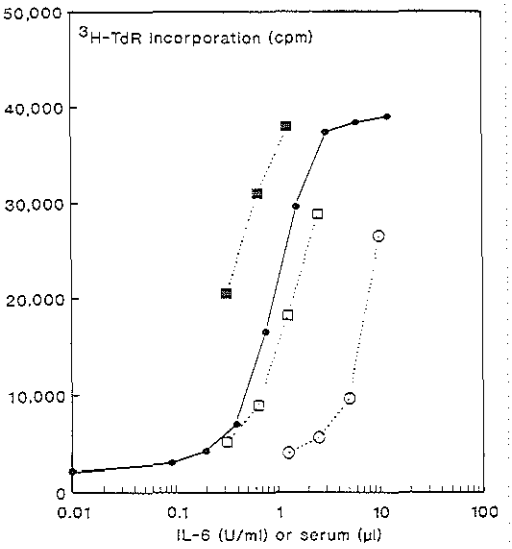

Figure 4.4.1 Measurement of IL-6 in serum. Several amounts of human recombinant IL-6 (●), serum from one patient with Alzheimer's disease (○), and serum from two controls (□ and ■) were compared with regard to their ability to induce proliferation by B9 cells

Results and Discussion

Median concentrations of serum IL-6 did not differ significantly (p=0.40) between Alzheimer patients (8.6 U/ml, range:4.6-34.0) and controls (8.4 U/ml, range:5.8-650.0). Five patients with Alzheimer's disease as well as five controls had a history of rheumatoid disease. In two subjects with rheumatoid arthritis increased IL-6 levels (94.0 U/ml and 650 U/ml) may have been due to the fact that these patients suffered from rheumatoid arthritis. Upon exclusion of all patients with rheumatoid arthritis median levels of serum IL-6 were 8.6 U/ml (range:4.6-34.0) for patients with Alzheimer's disease and 8.2 U/ml (range:5.8-30.0) for the controls (figure 4.4.2). In 5% (4/74) of the controls the concentration of serum IL-6 was above 13.0 U/ml; serum levels over 13.0 U/ml were found in twice as many Alzheimer patients (11%=10/93; p=0.17). Serum levels of IL-6 were similar for the 43 sporadic patients (those with no family history of dementia) and the 50 familial patients (those with one

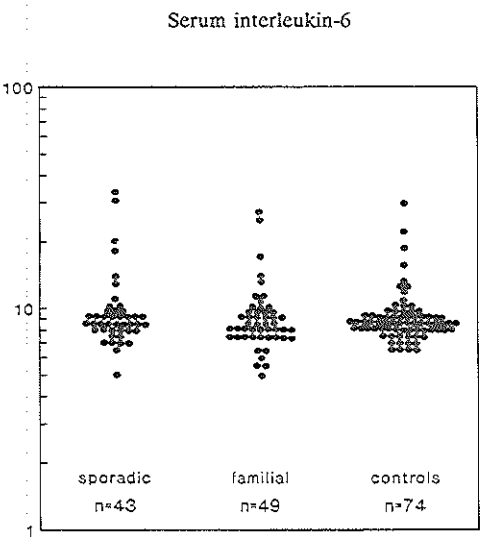

Figure 4.4.2 IL-6 in serum of patients with Alzheimer's disease and age- and sex-matched controls

or more first degree relatives with dementia). Median levels of serum IL-6 were 8.6 U/ml (range:4.6-34.0) for sporadic patients and 8.2 U/ml (range:4.8-28.0) for familial patients (p=0.42). High concentrations of IL-6 were not associated with the severity of the dementia as measured by the Clinical Dementia Rating-scale or with the duration of the disease since the first symptoms.

Earlier studies have reported increased serum levels of acute-phase proteins in patients with Alzheimer's disease.[4,5] In the present study of well-defined patients with clinically diagnosed Alzheimer's disease we do not obtain evidence for elevated serum levels of IL-6, one of the mediators of the acute-phase response, as compared to age- and sex-matched population controls. In some patients and controls elevated levels of IL-6 were found in serum. Since no obvious symptoms accounted for these findings, it is likely that subclinical inflammatory processes were responsible. Our findings do not exclude of course the possibility that the concentration of IL-6 is locally elevated within the central nervous system. Recently an increased number of IL-1 positive cells have been reported in patients with Alzheimer's disease and Down's Syndrome.[15] Support for a local synthesis of IL-6 is provided by observations in mice.[16] It has been suggested that IL-6 may be involved in repair mechanisms by increasing the secretion of nerve growth factor by astrocytes.[16] Therefore it is conceivable that IL-6 production, like IL-1, may be altered in Alzheimer's disease and further studies of levels of IL-6

within autopsy material will certainly be of interest.

Acknowledgements

This research was supported by the SOOM Foundation, the Netherlands Organisation for Scientific Research (NWO) and the Eurodem EC concerted action on the epidemiology of Dementia. We thank Drs Wim Schulte, Teun Tanja, Rob Haaxma, Arie Lameris and Rolf Saan for their contributions to this study and Helen de Bruijn, Micheline de Haes, Jeanette Kamman, Hanneke van Meurs, Verona Otten and Caroline Valkenburg for data collection.

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Chapter 5

Gene-environment interaction

Introduction

Alzheimer's disease is a disorder with a complex genetic etiology.[1] There is some evidence that genetic and environmental mechanisms may be implicated in Alzheimer's disease.[2-4] Little is known of how these genetic and environmental factors may interact. Risk factors may merely modify the expression of a disorder of primarily genetic origin. It is also conceivable that non-genetic risk factors may increase the risk of Alzheimer's disease independently.

This chapter deals with gene-environment interaction. Chapter 5.2 addresses the question which factors determine the onset-age in familial early-onset Alzheimer's disease. For this study, data of families in which the disease was apparently inherited as an autosomal dominant disorder were used. In chapter 5.3 a study of the relationship between smoking and family history of Alzheimer's disease and Parkinson's disease is presented. This chapter is based on the case-control study of early-onset Alzheimer's disease (see chapter 2.1) and on a study of risk factors in families in which the disease was apparently inherited as an autosomal dominant disorder. In chapter 5.4, interaction between genetic and environmental risk factors is studied using data from the collaborative re-analysis of all case-control studies (see chapter 2.2).

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Onset age in familial early-onset Alzheimer's disease

There are over 100 reported families in which Alzheimer's disease segregates as an autosomal dominant disorder.[1-4] Three studies have shown genetic linkage between early-onset Alzheimer's disease and polymorphic DNA markers on the proximal long arm of chromosome 21.[5-7] Although age of onset of Alzheimer's disease may differ considerably between families, similar ages of onset of dementia have been reported previously within several large families in which Alzheimer's disease is apparently inherited as an autosomal dominant disorder.[1,8,9] The origin of the family resemblance in onset age of dementia is not yet clear. The age of onset within a family may be determined by one or more genetic loci. But on the other hand, given a common genetic predisposition environmental factors shared by relatives may be involved in the onset of Alzheimer's disease. In addition, selection bias may account for the family specific onset age since relatives with similar ages of onset are more likely to be detected for genetic studies.

To investigate which factors determine the age of onset of familial Alzheimer's disease, we have examined 30 families multiply affected by Alzheimer's disease. These families were selected for molecular linkage studies or were ascertained for an epidemiologic study with complete ascertainment of patients with early-onset Alzheimer's disease. Thus the analysis was performed in two groups of pedigrees of: (1) families with known linkage to chromosome 21, and (2) a population-based unbiased sample of families for which no linkage data were available. Although early- and late-onset Alzheimer's disease are considered as one neuropathological entity, we have excluded late-onset patients because inaccuracies in diagnosis of Alzheimer's disease in this group may seriously bias the analysis. Moreover, there is some evidence that genetics may differ between early- and late-onset Alzheimer's disease.[2,10]

This chapter is based on: Van Duijn CM, Van Broeckhoven C, Hardy JA, Goate AM, Rossor MN, Vandenberghe A, Martin J-J, Hofman A, Mullan MJ. Evidence for allelic heterogeneity in familial early-onset Alzheimer's disease. Br J Psychiat 1991;158:471-474.

Methods

Patients. We have studied age of onset of Alzheimer's disease within and between 30 families derived from Belgium,[7] Great Britain[6] and The Netherlands[11] (table 5.2.1). The families from these three studies contained totals of 62, 72 and 80 affected individuals respectively. All families had a pedigree structure consistent with autosomal dominant inheritance of Alzheimer's disease. The criteria for including a family in the study were: (1) at least three individuals with clinically diagnosed Alzheimer's disease in two or more generations;[12] (2) detailed medical records available on the clinical diagnosis of Alzheimer's disease of at least two affected relatives; (3) mean age of onset below 60 years in the Belgian and British families and (4) age of onset of the proband before the age of 60 years in the Dutch study. The cutoff point of 60 years to select families with early-onset Alzheimer's disease was based on the findings of a study of risk for familial Alzheimer disease using age of onset data of 70 families.[2] In the Belgian and British studies, families were ascertained for genetic linkage analysis. Therefore the mean age of onset of all affected relatives was used to select early-onset families. Different inclusion criteria were used for the Dutch epidemiologic study because individual patients, not families, had been ascertained. Moreover, using the age of onset of probands to select families from this population-based study did not put any restrictions on the onset age of the relatives. The Dutch study comprised all patients diagnosed with early-onset Alzheimer's disease during the period 1980-1987 in two areas of the Netherlands;[11] in the families of 17 patients there were at least three affected individuals known in two generations. All molecular genetic analyses conducted with the Belgian and British pedigrees were consistent with linkage to chromosome 21.[6,7] Although in one British family, a cross-over between the disease locus and one of the markers (D21S1/S11) was inferred, there was no significant evidence for heterogeneity. Data of onset age of Alzheimer's disease were determined through a personal interview of the next of kin of each patient, so that in each family there were multiple informants. Age of onset was estimated as the age at which memory loss or change in behavior was first noted. Information of onset age of dementia was available for 139 (67%) of the 214 affected individuals.

Data analysis. Analysis of variance was used to compare the age of onset among and within families.[13] To see whether the onset age changed over generations, the difference in age of onset was calculated for all possible combinations of sibships in a

Table 5.2.1 Clinical characteristics of families multiply affected with Alzheimer's disease.

Family number	Country	Onset age (years) Mean	range	Number of: Pathological confirmations	Cases
ADA*	Belgium	35	26-45	38	11
ADB*	Belgium	35	30-39	24	6
14	Great Britain	60	55-65	3	-
15*	Great Britain	52	45-70	3	-
23*	Great Britain	55	51-62	14	-
32*	Great Britain	54	47-59	6	-
34	Great Britain	44	37-52	3	1
53	Great Britain	52	43-60	5	-
74*	Great Britain	43	39-50	6	2
75	Great Britain	51	44-58	10	-
105	Great Britain	38	36-39	7	-
121	Great Britain	37	35-39	4	-
126	Great Britain	54	48-63	4	-
127	Great Britain	46	41-48	7	-
1005	Netherlands	60	50-78	9	-
1025	Netherlands	56	52-60	3	-
1034	Netherlands	63	59-70	4	-
1049	Netherlands	55	50-61	4	1
1066	Netherlands	41	38-49	9	2
1068	Netherlands	55	50-67	4	-
1070	Netherlands	59	52-67	4	-
1072	Netherlands	60	55-75	6	1
1085	Netherlands	57	50-63	4	-
1097	Netherlands	56	48-60	3	-
1100	Netherlands	39	35-42	7	-
1104	Netherlands	53	47-58	6	-
1125	Netherlands	53	49-58	5	-
1230	Netherlands	55	49-60	6	-
1264	Netherlands	61	56-66	4	-
1270	Netherlands	58	47-65	4	-

* Families with evidence of linkage to chromosome 21[6,7]

family. In the case of first degree relatives (genetic distance of 1/2), the difference in mean onset age of a sibship and the onset age of the affected parent is given. The comparison of second degree relatives (genetic distance of 1/4) comprised the difference between the mean onset age of a sibship and the mean onset age of all second degree relatives of this sibship (i.e., uncles, aunts and grandparents). In the same way, the differences were calculated between third (genetic distance of 1/8), fourth (genetic distance of 1/16), fifth (genetic distance of 1/32), sixth (genetic distance of 1/64), seventh (genetic distance of 1/128) and eighth (genetic distance of 1/256) degree relatives.

Results

The age of onset of Alzheimer's disease was more similar within than between families. 77% of variance in age of onset of dementia was due to differences between families (table 5.2.2). Restriction of the analysis to the pedigrees which we have previously reported linkage data on gave essentially similar results: 81% of variance was accounted for by between family differences. In the Dutch data, based on complete ascertainment of patients, 67% of the variance was due to differences between families (table 5.2.2). Unaffected siblings of these patients, however, are still at risk of Alzheimer's disease. Since ages of siblings tend to be similar, bias may occur

Table 5.2.2 Analysis of variance between and within families multiply affected with early-onset Alzheimer's disease

Families	Source of variation	Degrees freedom	Mean sum of squares	p-value	Intra-class correlation
All families	Between	29	407.20	0.0005	0.77
	Within	109	24.46		
Families with evidence of linkage to chromosome 21	Between	5	663.04	0.0005	0.81
	Within	39	22.20		
Population-based families	Between	15	184.91	0.0005	0.67
	Within	42	25.11		

towards a limited variation in age of onset of dementia. Upon exclusion of siblings of the probands, the intra-class correlation did not change materially (intra-class correlation 0.63; $p<0.005$).

Table 5.2.3 shows that the onset age has remained constant over generations and is independent of the genetic distance. The differences in onset age between sibships are given stratified for degree of relationship. The two extensive Belgian pedigrees with affected persons in 6 generations show that the age of onset remained constant in seventh and eighth degree relatives who may have no more than 1/128 and 1/256 of their genes in common. Essentially similar results were obtained if the mean age of death of affected individuals was used. Although data on environmental toxins were

*Table 5.2.3 Difference (±SD) in age of onset between sibships by degree of relationship**

Relationship (genetic distance)	Belgian Families	Dutch Families	British Families	All Families	Families linked to chromosome 21
First (1/2)	6.0±4.4 n=3	4.8±3.3 n=19	5.9±5.8 n=14	5.3±4.5 n=36	5.4±3.2 n=7
Second (1/4)	4.0±3.2 n=7	7.6±3.0 n=10	3.8±3.6 n=18	4.9±3.7 n=35	4.3±3.5 n=12
Third (1/8)	5.5±2.8 n=10	5.0 --- n=1	3.0±2.3 n=14	4.1±2.7 n=25	5.1±2.7 n=12
Fourth (1/16)	4.2±2.7 n=8	-	1.7±2.9 n=3	3.5±2.9 n=11	4.2±2.7 n=8
Fifth (1/32)	3.3±2.3 n=8	-	-	3.3±2.3 n=8	3.3±2.3 n=8
Sixth (1/64)	4.7±3.8 n=12	-	-	4.7±3.8 n=12	4.7±3.8 n=12
Seventh (1/128)	4.7±3.2 n=10	-	-	4.7±3.2 n=10	4.7±3.2 n=10
Eighth (1/256)	3.3±2.7 n=8	-	-	3.3±2.7 n=8	3.3±2.7 n=8

* The difference in age of onset between sibships of two different generations was calculated for all possible combinations of sibships within a family. The average of the differences (absolute values) are given by genetic distance. In the case of a genetic distance of 1/2, the difference in mean onset age of a sibship and the onset age of the affected parent is given. The genetic distance of 1/4 comprises the difference between the mean onset age of a sibship and the mean onset age of all second degree relatives (i.e., uncles, aunts and grandparents). Essentially similar results were obtained if the mean age of death of affected individuals was used.

not available, we compared onset ages of relatives raised apart to see whether there was evidence for a modifying role of environmental factors. The average difference in onset age between sibships of a family born more than 50 km apart (3.7 years; SD=3.0; n=13) was similar to the difference found for sibships born less than 50 km apart (4.4 years; SD=3.0; n=54).

Discussion

These data show that age of onset of Alzheimer's disease is more similar within families multiply affected with Alzheimer's disease than between families. The analysis of the population-based data indicates that this finding is not due to ascertainment bias. Indeed, determination of onset ages may still be subject to error and collecting data of age of onset through interviews of relatives may have led to artifactual clustering of onset ages within families. However, in our study onset age was always obtained through taking of a family history of the next of kin of each patient, i.e., there were multiple informants within a family. Since in the large pedigrees, onset ages were obtained from informants who did not know of the relationship to other patients in the pedigree, this information can be considered as independent. The finding that the age of onset remained constant even in eighth degree relatives strongly supports the existence of a family specific onset age within decades. Moreover, an analysis of age of death, dates which were always checked in independent medical records, demonstrated the same effect.

Our findings do not support a predominant role of non-genetic factors. Similarity in exposure to environmental factors and lifestyle are likely to decrease if members of a family are more distantly related. Onset age, however, did not differ significantly with distance in relationship or birth place in our analysis. In accordance with our findings, a constant age of onset has been reported within a large Italian pedigree although relatives have lived as far apart as France, Italy and the USA.[14] These findings suggest that genetic rather than environmental factors determine the family specific onset age within decades. However, within families in which the disease appears to be inherited as well as within sets of twins,[15] differences in onset age may vary from 3 to 30 years. Therefore, it is conceivable that non-genetic factors may be implicated in that they may alter the genetically determined onset age or that there may be phenocopies in

such families, i.e., patients who do not have the genotype, but in whom the disease may be primarily of non-genetic origin.

The data of families with linkage to chromosome 21 support the view that the characteristic age of onset of a family may have a genetic origin. There are two possible explanations for this: (1) other genes, which are coinherited with the locus on chromosome 21, may modify the onset age; (2) different loci on chromosome 21 or different alleles at the same genetic locus (allelic-heterogeneity) predispose to different ages of onset. The first explanation would predict that within a family recombination between the Alzheimer's disease locus and the putative onset determining locus may occur. Thus would the variation in age of onset increase when family members are more distantly related. Since age of onset appears to be constant within a family through different generations this explanation is less likely. The family specific onset age of Alzheimer's disease observed in the analysis of families with evidence of linkage to the predisposing locus on chromosome 21 suggests that there may be different mutations on chromosome 21, either at various loci or at the same locus (allelic heterogeneity), which determine the onset age.

These findings may have implications for scientific research as well as clinical practice. It may be possible to detect those individuals who fall outside of the expected age of onset for a particular pedigree. Studies of these patients may reveal non-genetic risk factors for Alzheimer's disease because it is conceivable that such factors have modified the genetically determined onset age in these cases or that these patients may represent non-genetic forms of Alzheimer's disease. In linkage studies, it may be of great value to detect the latter group of patients, as they will reduce the statistical power of the study. As to genetic counselling, these findings imply that family specific age of onset curves should be used when assessing the risk to an individual to have inherited the disease.

Acknowledgements

This research was funded by Eurodem EC concerted action of dementia, the SOOM Foundation, The Netherlands, The Netherlands Organisation for Scientific Research (NWO), Innogenetics, Belgium, the Belgian National incentive-program on fundamental research in Life Sciences, the MRC, Great Britain, the Mental Health Foundation, Great Britain, and the Foundation for Age Research, Great Britain. We thank Drs Wim Schulte, Teun Tanja, Rob Haaxma, Arie Lameris, Rolf Saan, Jan Gheuens and Marc Bruyland for assisting with case diagnosis and Helen de Bruijn, Micheline de Haes, Jeanette Kamman, Hanneke van Meurs, Verona Otten, Caroline Valkenburg and Goedele De Winter for genealogy studies.

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Relation between nicotine intake, Alzheimer's disease and Parkinson's disease

Alzheimer's disease and Parkinson's disease have been suggested to have a common etiology.[1-3] These neurodegenerative disorders share several pathological and neurochemical characteristics. Lewy bodies, one of the hallmarks of Parkinson's disease, are often observed in Alzheimer's disease.[1,2] The Alzheimer type pathology is found more often in patients with advanced idiopathic Parkinson's disease than in the general population. In the most studies of Parkinson's disease, patients have been observed to smoke less than control subjects.[4,5] There is some evidence that nicotine may improve information processing and attention in Alzheimer patients,[6,7] which suggests that nicotine may have a protective role in Alzheimer's disease. To test this idea, we studied smoking habits before the onset of dementia in Alzheimer patients and population controls, as part of a genetic-epidemiologic study of Alzheimer's disease carried out in the Netherlands.

Methods

Risk factors for Alzheimer's disease were studied in 198 early-onset Alzheimer patients, 198 age- and gender-matched population controls, and in 17 families with a pedigree structure consistent with autosomal dominant inheritance of Alzheimer's disease. The smoking history and the family history of dementia and of Parkinson's disease were assessed by a structured interview of the next of kin of the patient or control subject. Patients were derived from an epidemiologic study of risk factors for clinically diagnosed Alzheimer's disease.[8,9] The study aimed at a complete ascertainment of cases in whom the

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This chapter is based on: Van Duijn CM and Hofman A. Relation between nicotine intake and Alzheimer's disease? Brit Med J 1991;302:1491-4.

diagnosis was made before 70 years and who were living in the four northern provinces of The Netherlands and the area of metropolitan Rotterdam. Ascertainment was carried out through all neurologic, psychiatric, geriatric services in the study areas. All cases were diagnosed in the period of January 1980 to July 1987. Control subjects were randomly drawn from the same municipality as the cases. Cases and controls were matched for age (within 5 years), gender, and residence. An extensive description of the design of the study, case diagnosis and response rates has been given in chapter 2.1. The study comprised a total of 198 patients and 198 controls.

Based on the information about the family history of dementia, patients with Alzheimer's disease were classified as sporadic (those with no first degree relatives with dementia) or familial (those with one or more first degree relatives with dementia). 96 out of the 198 patients with Alzheimer's disease (48%) had one or more first degree relatives with dementia. The pedigree structure of 17 patients (9%) was consistent with autosomal dominant inheritance of Alzheimer's disease. The criteria for autosomal dominant inheritance were: (1) at least three patients with reported dementia in two generations; (2) at least two patients with detailed records with the clinical diagnosis of Alzheimer's disease.[9] Within the 17 families in which the disease was apparently inherited as an autosomal dominant disorder, all relatives were screened for dementia.[10,11] Data on smoking habits and risk factors for Alzheimer's disease were collected in affected and in unaffected relatives.

Data collection. Information on putative risk factors for Alzheimer's disease was obtained by a structured interview of the next of kin of the patient. To assure symmetry in data collection, information on the control subject was also obtained from the next of kin. Since etiologic factors were studied, questions addressed exposures of the patient before the onset of dementia. Age of onset was defined as the age at which memory failure or changes in behavior were first noted. For controls a "reference age" was defined on the basis of the age of onset of Alzheimer's disease in the matched case. Patients were classified as non-smokers if they had never smoked before the disease onset and as smokers if they had ever smoked. Controls were classified similarly based on their smoking habits before the reference age. For smokers the number of cigarettes smoked daily was asked for each period they had smoked. As a measure of the life-time cigarette exposure, the number of pack-years was calculated by multiplying the number of cigarettes smoked a day (in packs) and the duration of cigarette use (in years). Data on the smoking history were not available of five patients and three control subjects.

The disease history of patients and controls was evaluated in close-ended questions

with additional open-ended questions about medical treatment and hospitalization. Full pedigree information on dementia and Parkinson's disease was obtained. All first degree relatives were listed and we asked specifically about the occurrence of dementia and Parkinson's disease in them. To increase the validity of these data, the information of family history was verified by a second first degree relative. If the patient had been hospitalized, the diagnosis was checked in independent medical records.[8]

Data analysis. The strength of association between Alzheimer's disease, smoking and other putative risk factors was assessed by the odds ratio (OR) as an estimate of the relative risk.[13] Odds ratios are presented with 95% confidence intervals (95% CI). Smoking was significantly correlated with alcohol intake ($r=0.34$; $p=0.04$) in controls. Conditional logistic regression analysis was used to take the alcohol consumption and the level of education as well as the matching variables gender and age into account. Modification of the relationship between smoking and Alzheimer's disease by the established risk factors age, gender and family history of dementia was tested. Since there was a significant interaction between smoking history and family history of dementia of the patient ($p=0.02$), all analyses were stratified for family history of dementia. Trends in risk of Alzheimer's disease by categories of smoking were tested by the Mantel test for trend.[13]

In the diagnosis of Alzheimer's disease, the Hachinski score was used to exclude patients with multi-infarct or vascular dementia.[12] This scale effectively excludes patients with evidence of atherosclerotic cardiovascular disease. Because smoking is associated with cardiovascular disease, this may have resulted in lower smoking rates in the non-vascular dementia patients, i.e. Alzheimer patients. To control for this possible bias, we performed a separate analysis in which all patients and all controls with a history of atherosclerotic cardiovascular disease, including coronary heart disease, stroke and hypertension, were excluded.

Results

Table 5.3.1 shows the family history of dementia and Parkinson's disease of the Alzheimer patients and their controls. Of the patients with Alzheimer's disease, 48% had at least one first degree relative with dementia, as compared to 19% of the controls (OR 4.32; 95% CI 2.71-6.82). Significantly more Alzheimer patients than controls had a first-degree relative with Parkinson's disease (OR 2.90; 95% CI 1.11-8.49).

Table 5.3.1 Family history of dementia and Parkinson's disease of 198 Alzheimer patients and 198 age- and gender-matched controls§*

	Cases n=198	Controls n=198	Odds ratio [95% CI] Crude	Adjusted†
Dementia:				
family history +	96	37	4.12	4.32
family history –	102	161	[2.61-6.43]	[2.71-6.82]
Parkinson's disease:				
family history +	14	5	2.90	2.90
family history –	184	193	[1.11-8.29]	[1.11-8.49]

* In first degree relatives
† Adjusted for age, gender, area of residence, number of first degree relatives and education by conditional regression analysis

The proportion of smokers was lower in patients with Alzheimer's disease than in control subjects (table 5.3.2). A significant inverse association was found in patients with familial Alzheimer's disease (those with one or more first degree relatives with dementia), whereas there was no evidence for an association of smoking with sporadic Alzheimer's disease. The inverse association between familial Alzheimer's disease and smoking remained after restricting the analysis to patients and controls without a history of atherosclerotic cardiovascular disease (table 5.3.3: OR 0.16; 95% CI 0.06-0.45).

A negative trend for smoking was observed when comparing Alzheimer patients and controls: the risk of Alzheimer's disease decreased as the number of cigarettes smoked daily increased (table 5.3.4). After exclusion of patients and controls with a history of atherosclerotic cardiovascular disease, the odds ratio for smoking 1-10, 11-20 and more than 20 cigarettes daily was 0.81 (95% CI 0.44-1.49), 0.67 (95% CI 0.34-1.32) and 0.26 (95% CI 0.11-0.61), respectively. A similar trend was observed when comparing patients and controls for the number of pack-years they had smoked. The odds ratio was 0.38 (95% CI 0.13-1.10) for those who had smoked 1-10 pack-years, 0.29 (95% CI 0.08-0.98) for 11-30 pack-years and 0.22 (95% CI 0.06-0.77) for more than 30 pack-years.

In the 17 families with a pedigree structure consistent with autosomal dominant inheritance of Alzheimer's disease, 33% of the 56 affected siblings had ever smoked, as compared to 67% of the 109 unaffected siblings (OR 0.27; 95% CI 0.11-0.67). To test whether smoking history modified the onset of disease, we used the data of six families

Table 5.3.2 History of smoking before the onset of dementia of 193 Alzheimer patients and 195 controls, stratified for family history of dementia in first degree relatives

	Cases n=193*	Controls n=195*	Odds ratio [95% CI] Crude†	Adjusted†
Family history dementia+				
Smoking: yes	40	55	0.54	0.35
no	55	41	[0.31-0.96]	[0.16-0.78]
Family history dementia –				
Smoking: yes	49	47	1.11	1.19
no	49	52	[0.63-1.93]	[0.63-2.25]
All patients				
Smoking: yes	89	102	0.78	0.70
no	104	93	[0.52-1.16]	[0.43-1.15]

* Data on smoking history were missing for five patients and three control subjects
‡ Based on unmatched analysis
† Adjusted for age, gender, residence, education and intake of alcohol by conditional logistic regression analysis

Table 5.3.3 History of smoking before the onset of dementia of 193 Alzheimer patients and 195 controls, stratified for family history of dementia, without patients and controls with atherosclerotic disease†*

	Cases n=146	Controls n=122	Odds ratio [95% CI] Crude‡	Adjusted§
Family history dementia+				
Smoking: yes	27	41	0.28	0.16
no	44	19	[0.14-0.59]	[0.06-0.45]
Family history dementia–				
Smoking: yes	42	32	1.19	1.66
no	33	30	[0.61-2.34]	[0.67-4.13]
All patients				
Smoking: yes	69	73	0.60	0.61
no	77	49	[0.37-0.98]	[0.31-1.19]

* In first degree relatives
† All patients and controls with a history of coronary heart disease, stroke or hypertension excluded
‡ Based on unmatched analysis
§ Adjusted for age, gender, residence, education and intake of alcohol by conditional logistic regression analysis

Table 5.3.4 Number of cigarettes smoked daily of 193 Alzheimer patients and 195 controls, stratified for family history of dementia in first degree relatives

	All				Family history dementia* +			
Number of cigarettes	Cases n=193	Controls n=195	Odds ratio [95% CI] Crude*	Adjusted†	Cases n=95	Controls n=96	Odds ratio [95% CI] Crude*	Adjusted†
0	104	93	1	1	55	41	1	1
1-10	47	46	0.91 [0.56-1.50]	0.79 [0.46-1.34]	20	25	0.60 [0.29-1.22]	0.46 [0.20-1.06]
11-20	27	30	0.80 [0.45-1.45]	0.59 [0.29-1.21]	13	16	0.61 [0.26-1.40]	0.34 [0.11-1.05]
21+	15	26	0.52 [0.26-1.03]	0.39 [0.17-0.90]	7	14	0.37 [0.14-1.00]	0.18 [0.05-0.69]
Chi-square for trend			5.25 p=0.022				7.33 p=0.007	

* Based on unmatched analysis
† Adjusted for age, gender, residence, education and intake of alcohol by conditional logistic regression analysis

Table 5.3.5 Mean age of onset and history of smoking in 6 families with autosomal dominant inheritance of Alzheimer's disease

	Non-smokers			Smokers		
Family	Number of cases	Onset (years) Mean	Range	Number of cases	Onset (years) Mean	Range
1005	3	55	55-60	2	62	61-62
1011	1	62	—	1	62	—
1066*	3	39	38-49	2	45	41-49
1072*	1	58	—	1	63	—
1083	4	57	55-63	1	57	—
1125	1	51	—	1	58	—
All	13	54	38-63	8	59	41-63

* The clinical diagnosis of Alzheimer's disease was confirmed pathologically in two patients of family 1066 and 1 patient of family 1072.

in which there were both Alzheimer patients who had ever smoked and patients who had never smoked (table 5.3.5). There was a significantly later onset of 4.17 years in smoking as compared to non-smoking patients from the same family (standard error of the difference 1.35; p = 0.03).

Discussion

In this study we observed a strong inverse relationship between smoking and Alzheimer's disease. The association was restricted to Alzheimer patients with a positive family history of dementia and was independent of cardiovascular history and potential confounding variables such as age, gender and alcohol consumption. The risk of Alzheimer's disease decreased as the number of cigarettes smoked daily increased. Within families in which Alzheimer's disease was apparently inherited as an autosomal dominant disorder, we observed that the onset of Alzheimer's disease was later in smoking patients than in non-smoking patients. This study also showed familial aggregation of Parkinson's disease with Alzheimer's disease, even though Alzheimer patients with a history of Parkinson's disease were excluded.[8]

Before interpreting these findings, we would like to raise some methodologic issues. At present, it is not possible to distinguish between patients who are primarily of genetic origin and those who are primarily of environmental origin. Therefore, we have classified patients on the basis of their family history of dementia, assuming that patients with a positive family history are more likely to be of genetic origin. This has probably introduced misclassification, because relatives of sporadic patients may carry the gene and express the disease later in life, whereas familial aggregation of Alzheimer's disease may be due to clustering of non-genetic patients. The most likely effect of this misclassification is, that it has made familial and sporadic patients more similar. The difference in risk between familial and sporadic Alzheimer's disease may therefore be larger than reported in the present study. Another methodologic issue concerns the data collection. Although we have measured smoking habits in a symmetrical way in patients and controls,[8] there is a possibility of bias: (1) the non-response within the control group may have been associated with smoking; and (2) relatives of patients with Alzheimer's disease may have underreported smoking habits. We consider it less likely, however, that these types of bias have occurred only in patients with a positive family history of dementia or their matched controls. Since no association of smoking with sporadic Alzheimer's disease was observed, it is unlikely that the association with familial Alzheimer's disease can be fully explained by these sources of bias. Moreover, the comparison of age of onset between smoking and non-smoking patients largely overcomes the bias to which comparisons of patients with control subjects are prone. Since we have included both incident and prevalent patients in our study, another possible source of bias is that the survival rate may have been higher in non-smoking Alzheimer patients than in smoking patients. However, the

association between smoking and familial Alzheimer's disease remained significant after the exclusion of all patients and all controls with a history of atherosclerotic cardiovascular disease, the most common competing cause of death. Also, the finding of a later disease onset in smoking patients as compared to non-smoking patients argues against a clear effect of lower survival among smoking patients. A final point concerns the uncertainty in the clinical diagnosis of Alzheimer's and Parkinson's disease. The validity of the diagnosis of dementia and Parkinson's disease in relatives of patients has been discussed elsewhere.[8] For all patients and controls, the data on family history were confirmed by a second first degree relative. The diagnosis of Parkinson's disease could be confirmed by medical records for 11 patients (79%) and 4 controls (80%).

Previous studies of the association between Alzheimer's disease and smoking have yielded equivocal results.[14-26] In two studies, a significant positive association between smoking and Alzheimer's disease was reported,[19,24] but in three studies a significant inverse relationship was suggested.[14,22,23] Pooling of the data of all formal case-control studies, however, resulted in a significant inverse association (OR=0.78; 95% CI 0.62-0.98).[8,15-19,25,26,27] The advantage of the present study is that it comprises twice as many patients as the earlier investigations and therefore allows more careful analyses of subgroups and possible sources of bias.

The majority of studies of Parkinson's disease have reported a protective effect of smoking as well.[4] The mechanism underlying the association of smoking with Alzheimer's disease and Parkinson's disease is at present unclear. In both Alzheimer's disease and Parkinson's disease nicotinic receptors are reduced.[28,29] Decreased nicotinic receptor binding has been linked to pathological changes characteristic for Alzheimer's disease.[30,31] Nicotine has been reported to increase the density of nicotinic receptors in the brain.[32] It may be speculated that nicotine from cigarette smoke may compensate the loss of nicotinic receptors in Alzheimer's disease and Parkinson's disease and may thus postpone the onset of disease. However, it is also possible that preclinical changes in nicotinic receptors in Parkinson's disease and familial Alzheimer's disease may have resulted in a loss of desire to smoke in these patients.[21] The lack of association in patients with sporadic Alzheimer's disease suggests that smoking may be involved in a mechanism of primarily genetic origin.

There are two implications of our findings. Firstly, they suggest heterogeneity between familial and sporadic Alzheimer's disease. Secondly, they suggest a link between Parkinson's disease and familial Alzheimer's disease. Although the association of smoking with familial Alzheimer's disease and Parkinson's disease may be explained by shared

pathological characteristics of different etiology, the familial aggregation of Alzheimer's disease and Parkinson's disease supports the view of a joint pathogenesis. Both findings point to a genetic link between the disorders. For Parkinson's disease as well as familial Alzheimer's disease, it has been suggested that genetic and environmental factors may be implicated.[33,34]

We present our findings of an inverse association between smoking and Alzheimer's disease with caution, as they are not yet confirmed by prospective studies. Although the association we observed is compatible with a protective effect of smoking for familial Alzheimer's disease, it has no relevance for prevention of Alzheimer's disease because of the adverse health effects of smoking. These findings may lead to interesting speculations, however, as to why both familial Alzheimer's and Parkinson's disease may be inversely related to smoking. It may further suggest a joint etiology of these disorders.

Acknowledgements

This research was supported by the SOOM Foundation, the Netherlands Organisation for Scientific Research (NWO) and the Eurodem EC concerted action on the epidemiology of Dementia. We thank Drs Wim Schulte, Teun Tanja, Rob Haaxma, Arie Lameris and Rolf Saan for their contributions to this study and Helen de Bruijn, Micheline de Haes, Jeanette Kamman, Hanneke van Meurs, Verona Otten and Caroline Valkenburg for data collection and genealogy studies.

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Interaction between genetic and environmental risk factors for Alzheimer's disease

Genetic factors appear to play an important role in Alzheimer's disease. In a considerable number of families the disease is apparently inherited as an autosomal dominant disorder[1] and epidemiologic studies have shown familial aggregation of the disease.[2,3] It has been suggested, however, that Alzheimer's disease may be heterogenous and that there may be patients with sporadic Alzheimer's disease, in whom the disease may be attributed primarily to environmental causes.[4-6] Other factors that have been implicated in Alzheimer's disease include family history of Down's syndrome, family history of Parkinson's disease, late maternal age at birth, head trauma, depression, hyperthyroidism, smoking, aluminum and education.[2,3] Little is known of the interaction between genetic and other putative risk factors for Alzheimer's disease. The genotype and the risk factor may increase the risk of Alzheimer's disease independently. It is also conceivable that the genotype may exacerbate the effect of the risk factor (or vice versa) or that the presence of both the genotype and the risk factor may be required to increase the risk of disease.[7]

We have re-analyzed the original data of seven case-control studies of Alzheimer's disease to study the interaction between genetic and environmental factors. The data we present here are based on 814 patients with Alzheimer's disease and 894 control subjects.

Methods

For this study, all case-control studies of Alzheimer's disease conducted before January 1, 1990 were traced through medline search, review papers and personal contacts.[8,9]

Van Duijn CM, Clayton D, Chandra V, Fratiglioni L, Graves AB, Heyman A, Jorm AF, Kokmen E, Kondo K, Mortimer JA, Rocca WA, Shalat SL, Soininen H, Hofman A. Interaction between genetic and environmental risk factors for Alzheimer's disease: a re-analysis of case-control studies. Submitted.

We only included studies in which the patients met the criteria for the clinical diagnosis of Alzheimer's disease of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)[10] or the criteria of the Diagnostic and Statistical Manual of Mental Disorders for primary degenerative dementia (DSM-III).[11] Thus, we identified eleven studies. [12-22] Four studies were not included in the present analysis: two studies because no data on family history of dementia were collected[14,18] and two other studies because the data had not been collected symmetrically for cases and controls, i.e., control subjects were interviewed personally, but the patient's history was obtained from an informant.[12, 22] The seven studies on which this analysis is based have been described in chapter 2.2.

Selection of risk factors. A great variety of risk factors have been studied in the seven case-control studies. In this analysis of interaction among genetic and environmental factors we included only those factors which showed a significant association with Alzheimer's disease in the overall analysis.[9] A second criterion to include a risk factor in the present study was that the number of exposed cases and controls should allow stratification by family history of dementia in order to assess effect modification. Based on these criteria, we examined the interaction of family history of dementia with the following factors: family history of Down's syndrome and Parkinson's disease, late maternal age, history of head trauma, history of depression and history of smoking. For medical history a multitude of disorders have been studied and considerations of bias and multiple testing apply particularly to the analysis of these data. We therefore limited the analysis to disorders that *a priori* were expected to be associated, i.e., history of head trauma and depression.

For each risk factor, we evaluated the comparability of measurement across studies as described earlier.[8,9] As to exposure definition, we restricted the analysis of family history to disorders in first degree relatives. For late maternal age, cases and controls in whom the mother was aged 40 years or over at birth were considered exposed. We included in the analysis of patient history only exposures occurring at least one year before the disease onset. For control subjects we defined the age of onset of the matched case as reference and we considered only exposure before this reference age. To reduce the possibility of recall bias, we restricted the analysis of history of head trauma to that involving loss of consciousness and of episodes of depression to that medically treated. Smoking history was analyzed as a dichotomous variable; we classified patients and controls as non-smokers if they had never smoked before disease onset or the reference age, and as smokers if they had ever smoked.

Data analysis. The raw data of all the case-control studies were available for analysis. The results we present here are based on a comparison of cases with population or neighborhood controls. The association between Alzheimer's disease and the putative risk factors was assessed by the odds ratio (OR) as an estimate of the relative risk. Odds ratios are presented with 95% confidence intervals (95% CI). All studies were matched for age and gender and we used conditional logistic regression to take the matching variables into account.[23]

The present analysis is based on a strategy Ottman has outlined to study gene-environment interaction. This is a general genetic-epidemiologic approach, using family history of disease as a surrogate measure of genetic susceptibility in the absence of a genetic marker.[7] We defined those not exposed to the risk factor and without a history of dementia in first degree relatives as the reference category. Odds ratios were estimated (1) for those with a positive family history but not exposed to the risk factor; (2) for those exposed to the risk factor but without a family history of dementia; and (3) for those exposed to the risk factor having also a positive family history of dementia. If the genotype and the risk factor increase the risk of Alzheimer's disease independently, the model predicts that genetic susceptibility and risk factor will each increase the risk of Alzheimer's disease in the absence of the other. If the risk factor modifies the risk of a disorder primarily of genetic origin, one would expect the risk for those exposed to the risk factor to be increased only in genetically susceptible individuals. Conversely, if the genetic factor modifies the risk of a disorder primarily of environmental origin, one would expect an increased genetic relative risk only among those exposed to the risk factor, while the risk associated with the risk factor is expected to be higher among subjects that are genetically susceptible. It is also possible that the gene is causally related to the risk factor and that the risk factor increases the risk for the disease. In this case, one would also expect an increased genetic relative risk only among those exposed to the risk factor, while the risk associated with the risk factor is expected to be similar among subjects that are genetically susceptible and those who are not. Finally, if both the risk factor and genotype are needed to increase the risk of disease, an increased risk would be found only for those exposed to both factors. Since family history is an imperfect measure of genetic susceptibility, we would not expect to observe such extreme results. Nevertheless, gene-environment interaction should imply *statistical* interaction between the effects of family history and risk factor, although large sample sizes may be necessary to reach statistical significance.[24] Here we have interpreted statistical interaction in the multiplicative sense.

Results

Of the 814 patients, 399 (49%) were men and 415 (51%) were women. The mean age at onset was 66 years (SD=11 years). Three hundred and five (38%) patients had one or more first degree relatives with dementia as compared to 140 (16%) controls (OR 3.5; 95% CI 2.6-4.6). Table 5.4.1 shows exposure frequencies and the odds ratios for all factors studied in this analysis. Smoking history was inversely associated with Alzheimer's disease, whereas the other factors increased the risk of disease.

Table 5.4.1 Overview of risk factors included in the EURODEM collaborative re-analysis of interaction between genetic and environmental risk factors for Alzheimer's disease

Risk factor*	Exposure frequency		Odds ratio 95%confidence interval†
	Cases	Controls	
Family history:			
Dementia[13,15-17,19-21]	305/814	140/894	3.5 [2.6-4.6]
Down's syndrome[13,15,16,19,21]	20/588	7/615	2.7 [1.2-5.7]
Parkinson's disease[15,19]	20/312	8/294	2.4 [1.0-5.8]
Patient history:			
Maternal age 40+ years[15,19-21]	47/446	28/446	1.7 [1.0-2.9]
Head trauma[15,16,19-21]	60/622	35/622	1.8 [1.1-2.7]
Depression[17,21]	15/273	12/378	1.7‡ [0.8-3.9]
Smoking[13,15-17,19-21]	423/821	526/907	0.8 [0.6-1.0]

* References of studies with data available on this risk factor
† Risk estimates may differ slightly from estimates published earlier,[9] since this analysis is performed within a subset of studies that have collected data on family history of dementia
‡ Depression was significantly associated with Alzheimer's disease in the overall analysis (odds ratio 1.8; 1.2-2.9), when studies without family history data were included[31]

Odds ratios for family history of Down's syndrome and family history of Parkinson's disease, stratified by family history of dementia are given in table 5.4.2. In the absence of a family history of dementia, the odds ratios for those with a first degree relative with Down's syndrome (OR 2.6; 95% CI 0.8-8.5) and for those with a first degree relative with Parkinson's disease (OR 2.4; 95% CI 0.8-7.0) were increased although not statistically significant. For both risk factors the odds ratio tended to be higher for those with a positive family history of dementia. The effect was strongest for family history of Down's syndrome; the odds ratio of 4.2 (95% CI 0.9-20.0) was 1.6 times higher for those with a first degree relative with dementia as compared to those with no family history of dementia. The interaction was not statistically significant.

Table 5.4.2 Family history of Down's syndrome and Parkinson's disease and the risk for Alzheimer's disease, effect modification by family history of dementia: The EURODEM collaborative re-analysis of case-control studies

Family history of		Family history dementia		Odds ratio family history dementia*
		–	+	
Down's syndrome:	–	1 reference	3.3 [2.4-4.4]	3.3 [2.4-4.4]
	+	2.6 [0.8-8.5]	13.8 [3.0-63.8]	5.3 [0.8-36.9]
Odds ratio†		2.6 [0.8-8.5]	4.2 [0.9-20.0]	
Parkinson's disease:	–	1 reference	3.6 [2.4-5.6]	3.6 [2.4-5.6]
	+	2.4 [0.8-7.0]	12.0 [1.4-101.4]	5.0 [0.5-54.7]
Odds ratio†		2.4 [0.8-7.0]	3.3 [0.4-28.2]	

* Odds ratio [95% confidence interval] for those with a positive family history of dementia, computed within stratum of risk factor

† Odds ratio [95% confidence interval] for those exposed to risk factor, computed within stratum of family history of dementia

Table 5.4.3 Maternal age, head trauma and depression and the risk for Alzheimer's disease, effect modification by family history of dementia: The EURODEM collaborative re-analysis of case-control studies

Risk factor		Family history dementia		Odds ratio family history dementia*
		–	+	
Maternal age 40+:	–	1 reference	3.6 [2.5-5.1]	3.6 [2.5-5.1]
	+	2.0 [1.1-3.5]	6.0 [2.1-16.9]	3.0 [1.0-10.0]
Odds ratio†		2.0 [1.1-3.5]	1.7 [0.6-4.8]	
Head trauma:	–	1 reference	2.9 [2.2-3.9]	2.9 [2.2-3.8]
	+	1.9 [1.1-3.2]	5.0 [2.1-12.1]	2.6 [1.0-7.4]
Odds ratio†		1.9 [1.1-3.2]	1.7 [0.7-4.2]	
Depression:	–	1 reference	3.9 [2.4-6.5]	3.9 [2.4-6.5]
	+	2.1 [0.8-1.7]	7.9 [0.8-78.7]	3.8 [0.3-44.9]
Odds ratio†		2.1 [0.8-1.7]	2.0 [0.2-19.8]	

* Odds ratio [95% confidence interval] for those with a positive family history of dementia, computed within stratum of risk factor

† Odds ratio [95% confidence interval] for those exposed to risk factor, computed within stratum of family history of dementia

Table 5.4.3 gives the odds ratios for late maternal age, history of head trauma and history of depression, stratified by family history of dementia. Family history of dementia remained significantly associated with Alzheimer's disease in the absence of the other risk factors. For late maternal age, history of head trauma and history of depression the odds ratio virtually did not change when stratifying by family history of dementia, indicating that the risk for family history of dementia and these risk factors are multiplicative.

The inverse association between history of cigarette smoking and Alzheimer's disease tended to be stronger for those with a positive family history of dementia (table 5.4.4). For those with no family history of dementia there was no evidence for an association between smoking history and Alzheimer's disease (OR 0.9; 95% CI 0.7-1.2). For those with a positive family history an inverse relationship was observed (OR 0.6; 95% CI 0.4-1.0). The odds ratio for family history of dementia tended to be lower in smokers (OR 2.8; 95% CI 2.1-4.0) as compared to non-smokers (OR 3.9; 95% CI 2.6-5.7), although the two odds ratios did not differ significantly.

The high exposure frequency made it possible to perform an analysis of smoking history stratified further by the number of affected first degree relatives (table 5.4.5). The effect of smoking was most pronounced for those with two or more affected relatives.

Table 5.4.4 Smoking and the risk for Alzheimer's disease, effect modification by family history of dementia: The EURODEM collaborative re-analysis of case-control studies

Risk factor		Family history dementia		Odds ratio family history dementia*
		–	+	
History of smoking:				
	–	1 reference	3.9 [2.6-5.7]	3.9 [2.6-5.7]
	+	0.9 [0.7-1.2]	2.5 [1.8-3.6]	2.8 [2.1-4.0]
Odds ratio†		0.9 [0.7-1.2]	0.6 [0.4-1.0]	

* Odds ratio [95% confidence interval] for those with a positive family history of dementia, computed within stratum of risk factor

† Odds ratio [95% confidence interval] for those exposed to risk factor, computed within stratum of family history of dementia

Table 5.4.5 Smoking and the risk for Alzheimer's disease, effect modification by family history of dementia: The EURODEM collaborative re-analysis of case-control studies

History of smoking	Number of affected relatives*			Odds ratio† 1 affected relative	Odds ratio† 2+ affected relatives
	0	1	2		
–	1 reference	3.0 [2.0-4.7]	10.9 [2.6-46.9]	3.0 [2.0-4.7]	10.9 [2.6-46.9]
+	0.8 [0.6-1.1]	2.0 [1.3-3.0]	4.8 [1.8-13.2]	2.5 [1.7-3.5]	6.0 [2.2-16.1]
Odds ratio‡	0.8 [0.6-1.1]	0.7 [0.4-1.1]	0.4 [0.1-2.6]		

* Data on the number of affected relatives were not collected in two studies[13,17]
† Odds ratio [95% confidence interval] for those with a positive family history of dementia, computed within stratum of risk factor
‡ Odds ratio [95% confidence interval] for those exposed to risk factor, computed within stratum of family history of dementia

The odds ratio for those with two or more affected relatives was 1.9 times higher among non-smokers (OR 10.9; 95% CI 2.6-46.9) as compared to smokers (OR 6.0; 95% CI 2.2-16.1). However, the difference between the odds ratios was not statistically significant.

Discussion

This study shows that family history of Down's syndrome, family history of Parkinson's disease, late maternal age, head trauma and depression are associated with an increase in the risk for Alzheimer's disease in the absence of a family history of dementia, while family history of dementia remains strongly associated with Alzheimer's disease regardless of the presence or absence of these factors. As to the interaction between smoking and family history of dementia, there was no evidence for an association between history of smoking and Alzheimer's disease for those with no first degree relatives with dementia. The risk for family history of dementia tended to be lower in smokers as compared to non-smokers, although the difference was not statistically significant. This effect was most pronounced for those with two or more affected first degree relatives.

The results of this re-analysis of case-control studies must be interpreted bearing the crude measurement of genetic susceptibility in mind. The use of family history of dementia as an indicator in the absence of a genetic marker suitable for epidemiologic studies certainly incurs misclassification. Unaffected relatives may simply not have expressed the disease at the time of the study and patients classified as having "sporadic" Alzheimer's disease may in truth be gene carriers. Therefore the finding of an effect of a risk factor for those without a family history of dementia does not exclude the possibility that the risk factor operates only in those genetically susceptible. Yet, if the association in patients with "sporadic" Alzheimer's disease is to be explained by misclassification of genetically susceptible individuals, odds ratios must be higher for those with a positive family history. On the other hand, familial aggregation of Alzheimer's disease may result from clustering of patients with non-genetic disease and such misclassification may have reduced the statistical power to show interaction between genotype and other factors. Another issue in familial Alzheimer's disease is related to the growing evidence that more than one gene may be implicated in Alzheimer's disease.[25, 26] If a risk factor interacts with only one specific genotype, the use of family history as an indicator for genetic susceptibility may result in little statistical power to show such a mechanism. Moreover, due to genetic heterogeneity it is unlikely that we are able to assess a mechanism in which a gene exacerbates the effect of an environmental factor without increasing the risk for Alzheimer's disease by itself. The predicted lack of association with Alzheimer's disease for those with this gene but without the risk factor is likely to be blurred by the increase in risk for Alzheimer's disease for those with a positive family history due to other genes having an independent effect. A final point to be discussed when studying interaction with risk factors conferring only small or more moderate relative risks is that very large studies are necessary to achieve sufficient power to detect interaction.[24] Despite the pooling of all case-control studies conducted to date, one may argue that the sample size of this analysis has been too small.

The findings should also be interpreted in light of the validity of the case-control studies on which the re-analysis is based.[27, 28] Selection bias may have occurred in the first place because in all studies the control series had a higher non-response than the case series. Bias may have also resulted from the fact that the case series comprised a mixture of prevalent and incident cases. Although we performed a separate analysis for incident cases that did not suggest major differences in the risk estimates, we cannot fully exclude the possibility that the findings are biased by differential survival.[27,28] Despite the symmetric data-collection in cases and controls, recall bias may have occurred. This

applies particularly to data on family history, history of head trauma and history of depression. As biased recall is more likely to occur when assessing disorders in relatives more distantly related, we have restricted the analysis of family history to disorders in first degree relatives.[29] For history of head trauma, we restricted the analysis to that involving loss of consciousness in order to reduce the possibility of recall bias.[30] The association between depression and Alzheimer's disease was also shown within the cohort study based on medical records of the Rochester register, which suggests that the association can not be ascribed fully to recall bias.[18,31]

For family history of Down's syndrome, family history of Parkinson's disease, late maternal age, history of head trauma and history of depression a consistent increase in risk for Alzheimer's disease was observed across the individual case-control studies, although not always statistically significant.[29-32] The mechanism through which these risk factors may be implicated in Alzheimer's disease has been discussed earlier.[29-32] The present analysis showed that each factor increased the risk for Alzheimer's disease in the absence of a positive family history of dementia. These findings argue against the models in which (1) the risk factor merely exacerbates the genotype; and (2) the risk factor and the genotype are both required to increase the risk for Alzheimer's disease. As family history of dementia remained strongly associated with Alzheimer's disease in the absence of other risk factors, the results are most consistent with a model in which the genetic factor and these risk factors may increase the risk for Alzheimer's disease regardless of each others presence. For late maternal age, history of head trauma and history of depression, the similarity in relative risks for those with and without a family history of dementia suggests that there is no interaction with the genotype. The risk for Alzheimer's disease for those with a family history of Down's syndrome as well as for those with a family history of Parkinson's disease tended to be higher when having a positive family history of dementia in addition. These findings are compatible with a genetic link between the disorders. However, for both disorders the interaction was not statistically significant, but this may be due to the small number of exposed patients and controls.

Studies of the association between Alzheimer's disease and history of smoking have yielded equivocal results.[17,33] Pooling of the data of all formal case-control studies resulted in a significant inverse association.[34] This finding is supported by the lower incidence of late-onset Alzheimer's disease among smokers as compared to non-smokers in a prospective follow-up study.[35] The possibility that older smokers who develop Alzheimer's disease may be screened out of the case series due to conditions secondary to smoking should be considered as an explanation.[33,34] Yet, the finding of a trend (albeit non-

significant) towards an increased effect of smoking with increasing number of affected relatives argues against this explanation, as it is unlikely that this type of bias has occurred specifically in patients with familial Alzheimer's disease. The observation that pathological changes observed in Alzheimer's disease are associated with a decrease in nicotinic receptor density[36,37] makes the inverse association with smoking biologically plausible (see also chapter 5.3). Smoking increases the density of these receptors[38] and may thus delay the onset of dementia, resulting in a lower risk for Alzheimer's disease for smokers when comparing cases and age- and gender-matched controls. In the present analysis, the effect of cigarette smoking tended be stronger with increasing number of affected first degree relatives, suggesting that smoking interacts with a genetically determined process. This finding agrees with the delay in onset age observed in smoking patients as compared to non-smoking patients from families in which the disease is apparently inherited as an autosomal dominant disorder (chapter 5.3). The small non-significant decrease in risk for patients with no family history suggests heterogeneity in the etiology or pathology of familial and sporadic Alzheimer's disease.

Although the findings of our study are compatible with a role of several risk factors for Alzheimer's disease independent of genetic susceptibility, we interpret our findings with caution. Despite the large number of patients in the analysis, the validity of case-control studies of Alzheimer's disease conducted to date is limited by the possibility of recall bias and selection bias. Furthermore, we recognize the possibility of misclassification in genetic susceptibility and the relatively small sample size for studying interaction. These problems may be sufficient to account for our failure to find statistical significant evidence for interaction. Prospective follow-up studies incorporating biologic markers of genetic susceptibility would have a greater chance of success, but even then, large studies would be required to demonstrate gene-environment interaction.

Acknowledgements

The collaborative re-analysis of case-control studies was supported by the Commission of the European Community for the Eurodem concerted action on the epidemiology of dementia, the Netherlands Organisation for Scientific Research (NWO) and was conducted in collaboration with the National Institute on Aging of the US National Institutes of Health and with the World Health Organization. For the acknowledgements of the individual studies this re-analysis is based upon please see appendix to chapter 2.2 (page 33). We thank Theo Stijnen, Marcel Eijgermans, Gerrit-Anne van Es and Eric Neeleman for their help in data analysis.

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Chapter 6

General discussion

Introduction

A genetic-epidemiologic study aims at answering the following questions:[1-3] (1) Does the disease cluster in families? (2) If yes, can the familial aggregation be explained by genetic or shared environmental factors? (3) If there is evidence for a genetic component, can we identify the genetic mechanism(s)? (4) If genes are implicated in the genetic mechanism, on which chromosomes are these genes located? (5) Do environmental factors interact with genes to modify the expression of disease?

Research on the role of heredity in familial aggregation and the mode of inheritance requires pedigree research, whereas the study of the role of genetic and environmental factors in the occurrence of disease requires research of unselected populations. This thesis comprises several population studies as well as pedigree studies. In chapter 6.2 the limitations of the design of the studies will be reviewed in light of the interpretations of the findings and recommendations for further research. Chapter 6.3 discusses the inference that can be drawn from our findings with regard to the knowledge of risk factors for Alzheimer's disease along with recomdations for further research.

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Methodologic issues

Diagnosis

At present, there is no qualitative biologic marker for Alzheimer's disease. According to the criteria for the clinical diagnosis, Alzheimer's disease is a diagnosis by exclusion of other specific causes of dementia.[1,2] Perhaps the most difficult distinction to be made is with vascular dementia, as there is still debate on the diagnosis of this type of dementia.[3-7] The definite diagnosis of Alzheimer's disease can only be established by pathologic confirmation.[1] Autopsy studies have shown that the accuracy of the clinical diagnosis of Alzheimer's disease based on the NINCDS-ADRDA criteria varies from 85% to 100%.[8-12] Neuropathologic changes characteristic for Alzheimer's disease comprise neuritic plaques and neurofibrillary tangles. Extracellular amyloid fibrils composed of the amyloid protein (A4 protein) make up the core of the neuritic plaques.[13,14] Neurofibrillary tangles consist of intra-neuronal paired helical filaments, which are in part composed of altered forms of the microtubule-associated protein tau.[15,16] Both these pathologic changes have also been observed in normal aging, albeit to a lesser extent.[17-19]

As to the clinical diagnosis of Alzheimer's disease, in an early stage there is considerable chance for misclassification because of the insidious onset.[1,2] In particular for late-onset Alzheimer's disease, the cut-off points to separate dementia from normal aging and Alzheimer's disease from other types of dementia may be considered arbitrary.[20-22] Many of the methodologic problems that will be discussed in this chapter are related to the diagnosis of the disease. Another problem in studies of Alzheimer's disease is related to the measurement of genetic susceptibility as a determinant, confounder or effect modifier. Since the disease onset has been shown to be similar within families (chapter 5.2), particularly gene-carriers of the late-onset form have a high chance of dying before the expression of disease. This problem is referred to as censoring. As concern about the diagnosis and censoring apply to a lesser extent to early-onset Alzheimer's disease than to late-onset Alzheimer's disease, there is a strong point to be made to focus etiologic research on the early-onset form. Following this reasoning, we have conducted a

population-based study of risk factors for early-onset Alzheimer's disease, which is presented in this thesis.

A final issue to be considered related to the diagnosis of Alzheimer's disease is that although no clinical symptoms can be detected, the underlying disease process may already be in progress. Thus, although exposures are considered before the first symptoms of disease, risk factors may still relate to the progression of an ongoing process rather than be a cause of the disease. It is also conceivable that the risk factor merely is an early expression of the disease. Therefore, a risk factor should be viewed of as a measure of association, which may reflect a causal or non-causal relationship.

Bias

This thesis is based upon observational research. In epidemiologic terms, there are two principal types of observational studies: case-control studies and cohort studies. In a case-control study, present or previous exposure to a putative risk factor is measured after the onset of disease in patients and is compared to the level of exposure of subjects without the disease. Cohort studies follow people known to be exposed to the possible risk factor and compare the occurrence of disease to those who were not exposed. A pedigree study is conceptually a hybrid between a case-control and a cohort study. The risk of disease is compared within two cohorts, i.e., relatives of cases and controls, the exposure being the disease status of the proband. However, selection of study subjects and data-collection generally follow the case-control design. Therefore, the same problems of bias may apply to the case-control studies and the pedigree studies presented in this thesis. There are various ways in which the validity of these studies of Alzheimer's disease may be compromised:[23,24]

- *Firstly*, the studies presented in this thesis comprised a mixture of prevalent and incident cases. It is well known, that if prevalent cases are studied, i.e., when cases are not already included in the study at first occurrence of Alzheimer's disease, selection bias may result from mortality and migration related to the disease.[25] This would not be the case when subjects were incident or newly diagnosed. Although a separate analysis on incident cases that was performed in the re-analysis of case-control studies did not alter any of the conclusions (chapter 2.2), we cannot fully exclude the possibility of bias. Another putative source of bias concerns the diagnosis of Alzheimer's disease, which was in most cases based on clinical data without pathological confirmation. Therefore

misclassification may have occurred. This type of misclassification will most likely attenuate a true association.

- *Secondly*, bias may have occurred in the selection of controls in several ways. Spurious associations may occur if the control subjects have been drawn from a different study-base or study-population than the cases. In some of the studies included in the re-analysis of case-control studies, the case-series was hospital-based whereas the control series was drawn from the general population. However, a separate analysis in which cases were compared to hospital controls gave essentially similar results and did not change any of our conclusions (chapter 2.2). Among control subjects, the non-response may have been associated with other risk factors than among cases. Such bias can not be adjusted for in the data analysis.
- *Thirdly*, there is ample opportunity for information bias in studies of Alzheimer's disease. There may have been non-differential as well as differential measurement error of either the disease outcome or the exposure. Non-differential misclassification, i.e., bias operating similar in cases and controls, is often considered to be a minor problem in epidemiology as it will most likely lead to an underestimation of the strength of association rather than create a spurious association. One may argue, however, that such bias introduced by the use of surrogate informants in retrospective studies of Alzheimer's disease may have reduced the statistical power to show an association considerably. Moreover, non-differential misclassification may be a serious problem if the degree of the misclassification varies across subgroups, thus creating a pattern that may give rise to erroneous speculations about heterogeneity of the disease.[23] Misclassification in the diagnosis of Alzheimer's disease when studying effect modification by onset age may serve as an example. It is well recognized that the diagnosis of Alzheimer's disease becomes less accurate with increasing onset age[22] and this must be considered as a possible explanation for a weaker association of a risk factor for late-onset Alzheimer's disease as compared to early-onset Alzheimer's disease. Differential misclassification between cases and controls, which may create spurious associations, is also a problem in studies of Alzheimer's disease. Most obvious in this case may be the possibility of recall bias as exposures may have occurred decades before the onset of disease and informants of cases may be more willing to recollect such historical data than informants of controls. In studies of Alzheimer's disease differential bias may operate also in the measurement of disease outcome, as the probability of being diagnosed with Alzheimer's disease may vary within a population according to, for example, socio-economic status, education or gender.[23]

• *Fourthly*, a point of criticism concerns the possibility of confounding. As little is known of the etiology of Alzheimer's disease, it is difficult to exclude the possibility that an unmeasured confounder may explain the relationship to a risk factor. Residual confounding may have occurred when putative confounders, e.g. genetic susceptibility, have been measured with so much error that effective control has been impossible in the data analysis phase.[24]

Meta-analysis

Case-control studies of Alzheimer's disease have not yielded consistent evidence for risk factors. A major concern in the interpretation of these findings has been the relatively small sample size of the individual studies. The low statistical power of such studies to detect a significant association may explain some of the apparently conflicting results. To tackle this problem, we conducted a pooled analysis of all formal case-control studies of Alzheimer's disease conducted before January 1, 1990.

Meta-analysis has become an important feature in modern scientific research and has already been applied successfully in therapeutic trials.[26] However, unlike a meta-analysis of blinded randomized trials, there may be large differences in methodology in case-control studies making a meta-analysis not straightforward. In order to compare results, a re-analysis of the raw data is in practice inevitable. There are two ways in which case-control studies may be pooled into one single effect estimate: (1) the risk estimates from the individual studies, analyzed in a comparable way, may be pooled into a single risk estimate; the rationale of this analysis is that the effect measures may vary across studies, and (2) the raw data of the studies may be pooled and analyzed; thus we assume there is a fixed effect. We have pooled the studies according to the latter approach (chapter 2.2).

A re-analysis of observational studies must be judged on its own merits.[25] Indeed, it is clear that the large sample size of a pooled analysis will increase the statistical power to detect risk factors conferring only moderately increased risks. Furthermore, pooling of the data will result in more precise risk estimates and will enable subgroup analyses. However, the quality of the pooled analysis is fully determined by the quality of the individual studies, which, as discussed earlier, may be criticized in the case of the case-control studies of Alzheimer's disease. Considerations with regard to the validity may be withdrawn if consistency of findings across studies conducted with different methodology

by different research groups can be established. Although it is possible that all studies have been biased in the same direction, the finding of consistency does support the evidence for a true relationship with Alzheimer's disease. Therefore, an important goal of the re-analysis of case-control studies has been to test whether odds ratios differed across studies. If consistency can be assessed, or heterogeneity can be explained, pooling of the data of single studies may be considered more valuable.

Gene-environment interaction

Some additional comments should be made with regard to misclassification in genetic susceptibility based on family history of dementia when studying gene-environment interaction as suggested by Ottman.[27] This approach assumes that the disease under investigation has multiple causes, which may be of genetic or environmental origin. Table 6.2.1 shows how a gene may interact with other risk factors and what associations are to be expected in a case-control study for each mechanism. In the first model, both the presence of the risk factor and the genotype are necessary to increase the risk of disease. Such a mechanism predicts an increased risk of the disease only for those exposed to both factors. In the second model, the risk factor modifies the risk of a disorder primarily of genetic origin. Thus, one would expect the risk for those exposed to the risk factor to be increased only in genetically susceptible individuals. In the third model, the gene does not cause the disease directly, but is involved in the expression of the risk factor. In this case, one would expect that the gene does not increase the risk of the disease within the strata of the risk factor, while the risk associated with the risk factor is expected to be similar regardless of the absence or presence of the gene. The fourth model shows a mechanism in which the genetic factor modifies the risk of a disorder primarily of environmental origin. In this circumstance, one would expect an increased relative risk for the genetic factor only among those exposed to the risk factor, while the risk associated with the risk factor is expected to be stronger among subjects who are genetically susceptible. In the fifth model, the genotype and the risk factor increase the risk of Alzheimer's disease independently of each other. This model predicts that genetic susceptibility and risk factor will each increase the risk of Alzheimer's disease if the other is absent.

Ottman has suggested that family history may be used as an indicator of genetic susceptibility.[27] As a result of non-differential misclassification, the strength of association

Table 6.2.1 Gene-environment interaction: predicted observations for the odds ratio in case-control studies as suggested by Ottman[27]*

Model	Genetic susceptibility + Risk factor +	Genetic susceptibility + Risk factor -	Genetic susceptibility - Risk factor +	Genetic susceptibility - Risk factor -
1. Gene and risk factor both required Gene ‖⟹ Disease Risk factor	+	1	1	1
2. Risk factor exacerbates gene Gene ⟹ Disease ↑ Risk factor	++	+	1	1
3. Gene increases expression risk factor Risk factor ⟹ Disease ↑ Gene	+	1	+	1
4. Gene exacerbates risk factor Risk factor ⟹ Disease ↑ Gene	++	1	+	1
5. Gene and risk factor increase in risk in the absence of each other Gene ⟹ Disease Risk Factor ⟹ Disease	+?-	+	+	1

* 1 denotes no association with the disease, + denotes an increase in odds ratio whereas ++ denotes a stronger association due to effect modification

for genetic factors as well as for other risk factors is likely to be underestimated.[28] Therefore, less extreme results are expected than outlined in table 6.2.1. However, as pointed out in chapter 5.4, not only will misclassification in genetic susceptibility based on family history data result in a reduction of the statistical power, it will also distort studies of gene-environment interaction when the disease is genetically heterogeneous, as may be the case for Alzheimer's disease. If we assume that there exists at least one dominant gene, which increases the risk of Alzheimer's disease in the absence of other

risk factors, it follows that the risk associated with family history of dementia will always be increased. In the situation that a risk factor interacts with another gene, it will be difficult to distinguish the models 1 and 2 and the models 3, 4 and 5, as in models 1, 3 and 4 the predicted lack of association for those with this gene but without the risk factor is likely to be blurred by the increase in risk for Alzheimer's disease for those with a positive family history due to the dominant gene. Thus, in the circumstance of genetic heterogeneity, the finding that the strength of association is similar among those with and without a positive family history may be compatible with model 3 and model 5. Similarly, the finding of a stronger association among those with a positive family history in such a situation may be compatible with model 4 and model 5.

It is obvious that studies of gene-environment interaction will strongly gain value when biologic markers for genetic susceptibility become available. However, when studying interaction with risk factors conferring only small or more moderately elevated relative risks, very large studies are necessary to achieve sufficient power to detect interaction,[29] even if biologic genetic markers are available. In such case, risk estimates may be compared rather than to test for statistical interaction. To overcome the problem of statistical power, twin studies may be used not only to investigate risk factors for Alzheimer's disease,[30] but in addition they may serve as a vehicle for studies of gene-environment interaction. The difference in onset age between concordant affected identical twins may be linked to environmental factors. To date, a limited number of twin studies have been performed, although a number of studies are in progress. As a corollary of such twin studies, it is suggested in this thesis that this approach may be extended to families in which early-onset Alzheimer's disease is apparently inherited as an autosomal dominant disorder (chapter 5.2). As the onset age of Alzheimer's disease within decades appears to be genetically determined within a family (chapter 5.2), it is conceivable that environmental factors may have been implicated in patients that fall outside the family-specific age of onset range. These patients may represent phenocopies, that is patients with the disease who do not have the genotype. It is also possible that environmental factors have merely altered the genetically determined age of onset in these patients. To distinguish between these mechanisms, studies of interaction between genetic and environmental factors should preferably be conducted among families in which linkage to genetic markers or the underlying mutation is known.

Finally, two points are to be addressed here concerning the relative risk as a measure of strength of association between a risk factor and the disease. The first point concerns the use of the odds ratio, which may be considered an estimate for the relative risk when

the disease under study is rare. The rare disease assumption is likely to break down in a study among a high risk population such as carriers of a major dominant gene for Alzheimer's disease. Therefore, the odds ratio as derived from a case-control study may be used as a measure of association, but will most likely overestimate the relative risk because the rare disease assumption is not met unless the study is limited to incident cases diagnosed in a short period of time.[31] Secondly, as the relative risk is dependent on the prevalence of the disease,[25] bias may occur when comparing the relative risk for a risk factor between two populations with a different baseline risk, such as gene carriers and non-gene carriers.

Future approach

A case-control study can be considered an efficient way to study risk factors for a chronic disease as compared to the following up a complete population.[25] This design is widely used in scientific disciplines other than epidemiology. However, case-control studies are rather susceptible for recall bias and exposure suspicion bias, when exposures are assessed by interviews. Therefore, there is a need to confirm risk factors that have emerged in case-control studies of Alzheimer's disease conducted to date in prospective follow-up studies, in which exposure to the risk factor is measured before the onset of disease. Future studies also have to resolve the problem of selection bias, in particular related to the inclusion of prevalent cases. A feasible way to overcome both types of bias may be to incorporate studies of risk factors in incidence studies.[24] Such studies are already in progress.[32] Although data collection of events that occurred in the past (e.g. medical history) may still be subject to measurement error, an important advantage is that bias will no longer be associated with case-control status. An evident drawback of this design is that it only allows to study risk factors acknowledged at baseline measurement, unless exposure can be measured in stored blood samples. Therefore, in order to test a new hypothesis, a retrospective case-control study of incident cases may be a more efficient design.

There are several methodologic issues to be anticipated in follow-up studies of Alzheimer's disease. As the onset of Alzheimer's disease is insidious,[1] cases are always diagnosed some time after the disease onset. Thus, selection bias may occur in studies of incident cases with a late-onset disorder like Alzheimer's disease due to mortality or migration during follow-up, albeit to a lesser extent than in a study of prevalent cases.[23]

In particular, this may occur in the old age category, in which the highest percentage of new cases will emerge while mortality in this group is highest and may be associated with putative risk factors as well as with Alzheimer's disease. The magnitude of this problem will be determined mainly by the time between the baseline measurement and the follow-up examination. To enable a complete follow-up, this period is to be as short as possible. Yet, as the disease progresses slowly in most cases, one may also plea for a longer period of follow-up in order to exclude the possibility of the presence of sub-clinical disease at the baseline measurement. A rather costly and time-consuming solution may be to examine the population several times with short periods of follow-up between the examinations. Since there is a high likelihood that mortality will occur during the period of follow-up, a complete ascertainment of incident cases requires consideration of the occurrence of Alzheimer's disease among those who died before the follow-up examination. As death certificates have proven to be unreliable to assess Alzheimer's disease,[33] formal attention should be given to criteria for the diagnosis of Alzheimer's disease based on informant interviews of relatives and of physicians. Another point of concern is that bias may occur when the disease is diagnosed earlier in those exposed to certain risk factors (e.g. head trauma or other medical conditions).[23] An important aspect of the diagnosis of disease to be considered is therefore the definition of onset age.

A different approach that may be fruitful to follow in prospective follow-up studies may be to study risk factors associated with cognitive decline. Clearly, a study of cognitive decline will overcome problems related to the diagnosis of Alzheimer's disease and the definition of onset age, which limit at present the interpretation of studies conducted in the elderly. Although such a design will not yield information on the risk of any type of dementia specifically, such studies may be relevant from a public health point of view. Potential pitfalls, however, result from the measurement of the level of cognitive function as well as cognitive decline, which may be determined by cohort effects and factors such as pre-morbid intelligence, education and socio-economic status. Furthermore, the association between baseline level and change in cognitive function requires attention in the study design as well as in the data-analysis.

There are two strong points to link studies of heredity to epidemiologic incidence studies. Firstly, in order to study the extent of genetic involvement, an unselected population of cases should be investigated and this condition is met in a study of patients derived from a population-based incidence study. Secondly, the growing body of evidence for a role of non-genetic factors in familial aggregation of Alzheimer's disease (see chapter 3.3) implies that the power of studies of inheritance may be increased

considerably by taking into account the influence of other risk factors as putative confounders. Regressive models may be used to conduct such analyses.[34]

Studies of segregation of Alzheimer's disease conducted to date have been limited to first degree relatives. In future research, larger pedigrees including more distant relatives should preferably be studied to provide evidence on the number of major genes involved[35] and to disentangle polygenic and environmental effects. A specific methodologic issue to be addressed in genetic-epidemiologic studies is the use of family history of dementia as an indicator for genetic susceptibility in the absence of a biologic genetic marker. To increase the reliability, one would prefer to assign a probability to each case that the disease is of genetic origin varying from 0 to 1 rather than to use a dichotomous outcome such as family history positive or negative. To establish such a measurement requires an extensive study of pedigree data and risk factors simultaneously. Farrer and coworkers are at present conducting such a study (personal communication). In light of the evidence for genetic heterogeneity (see chapter 3.3), it may be argued that at present studies of gene-environment interaction should preferably be conducted among families in which linkage to genetic markers or the underlying mutation is known, as interaction may be specific to the mutation involved. Age of onset may be an important measure of outcome in such studies.

It may be concluded that long term follow-up studies, preferably population-based, of families well characterised from an epidemiologic and genetic point of view, are needed to study the genetic transmission of Alzheimer's disease.

Conclusions

The validity of the case-control studies of risk factors for Alzheimer's disease conducted to date is limited. Therefore the risk factors that have emerged from these studies need to be confirmed in prospective follow-up studies of incident cases in which exposure to the risk factor is measured at baseline. Although this design may resolve the problems of recall bias and exposure suspicion bias, difficulties related to selective mortality of cases require consideration in follow-up studies. To clarify the mode of inheritance, segregation of Alzheimer's disease should be studied in extensive pedigrees including several generations. At present, population studies on the interaction between hereditary and environmental factors are limited by the lack of a biologic genetic marker suitable for population studies.

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Genetic and environmental risk factors for Alzheimer's disease

The scope of this chapter is to place the evidence for risk factors for Alzheimer's disease provided in this thesis in a perspective of the current knowledge of the etiology of Alzheimer's disease. The evidence will be discussed for: (1) genetic factors, including family history of dementia and related disorders as well as the possible underlying genetic mechanism and origin; (2) environmental risk factors, including all factors that are not inherited such as parental age, medical history, smoking, alcohol consumption and occupational exposures; and (3) gene-environment interaction.

Genetic factors

Family history of dementia

There is little doubt that Alzheimer's disease aggregates within families. There has been debate, however, on whether the strength of familial aggregation of Alzheimer's disease may vary with age of onset. It has been suggested that familial aggregation of Alzheimer's disease may be specific to patients with early-onset of disease.[1]

As expected, the collaborative re-analysis of case-control studies of Alzheimer's disease supported familial aggregation of dementia (chapter 3.2) as did the study of risk of possible Alzheimer's disease in first degree relatives of patients with early-onset Alzheimer's disease (chapter 3.3). The observations of the re-analysis of case-control studies presented in chapter 3.2, showed an association between family history of dementia and early-onset as well as late-onset Alzheimer's disease. Among patients with an onset of disease after 80 years, there were significantly more subjects with one or more first degree relatives with dementia as compared to controls. However, the risk decreased with increasing onset age. The lower risk of late-onset Alzheimer's disease was due to a lower prevalence of dementia among parents of cases with late-onset Alzheimer's disease. As environmental exposures are likely to be more similar among

siblings than for parents and siblings, it may be speculated that familial aggregation of late onset Alzheimer's disease may in part be of non-genetic or multifactorial origin. Although there is concern that such a pattern may also be created by selective censoring (chapter 3.2), the view of genetic heterogeneity derives support from a study of risk in first degree relatives of patients with familial Alzheimer's disease.[2] This study showed that when the censoring age is taken into account, the risk among relatives of patients with late-onset Alzheimer's disease was significantly higher than expected for an autosomal dominant disorder, suggesting that environmental factors may play a role in familial aggregation.

Family history of Down's syndrome

There is much evidence for a link between Alzheimer's disease and Down's syndrome, since neuropathologic changes of the Alzheimer type as well as dementia are frequently found in patients with Down's syndrome.[3-5] Another finding suggesting chromosome 21 may be implicated in Alzheimer's disease is the higher frequency of patients with Down's syndrome that has been observed in the family of patients with Alzheimer's disease.[1] As reviewed in chapter 3.2, studies of family history of Down's syndrome have yielded equivocal results, which may in part be explained by the relatively low rate of occurrence of Down's syndrome. A significant increase in risk of Alzheimer's disease for those with a first degree relative with Down's syndrome was shown in the re-analysis of case-control studies (chapter 3.2). A previous study of familial aggregation of Down's syndrome and Alzheimer's disease suggested that the risk of early-onset Alzheimer's disease was only increased.[1] This hypothesis was rejected by the re-analysis; there was evidence for familial aggregation in early-onset as well as late-onset patients. This finding is of particular interest because it suggests a link with chromosome 21 for both early-onset as well as late-onset Alzheimer's disease. To date there is debate whether the late-onset form is linked to chromosome 21.[6] A potential source of bias which needs to be addressed in future studies is the age of the mother at birth of the relative with Down's syndrome, which was not available in the present studies.

Familial aggregation of Down's syndrome and Alzheimer's disease was observed in the absence of a first degree relative with dementia (chapter 5.4). The risk of Alzheimer's disease for those with a family history of Down's syndrome tended to be higher for those with a positive family history of dementia (chapter 5.4). This finding is compatible with a genetic link between the disorders. The association with the sporadic form may be explained by misclassification of gene-carriers. Another explanation may be inferred from

a hypothesis put forward by Potter,[7] suggesting that accumulation of trisomy 21 cells during life may underlie familial as well as sporadic Alzheimer's disease.[7] According to this view, there may be an increased frequency of nondisjunction of chromosome 21 during mitosis in patients with Alzheimer's disease resulting in trisomy 21 in somatic cells. This may lead to the Alzheimer's disease pathology through the same mechanism by which patients with Down's syndrome develop the disease. Nondisjunction during meiosis in these patients would generate trisomy 21 germ cells, thus increasing the risk of Down's syndrome in the offspring. Such a process may occur sporadically, perhaps induced by factors such as late maternal age or neurotoxins.[7] Non-disjunction may also be influenced by genetic factors, e.g., by mutations in or near the centromere,[7] resulting in a stronger association among those with a positive family history of dementia. Patients with Alzheimer's disease whose lymphocytes were mosaic for trisomy 21 but who did not have the phenotype of Down's syndrome have been reported and in one case, the patient had a Down's child.[8,9] Cytogenetic studies of aneuploidy in Alzheimer's disease have yielded contradicting results,[10-15] however, non-disjunction does not need to occur in all cells and may therefore easily go undetected.[7] Future cytogenetic studies, using interphase techniques and various tissue sections of patients from families in which Alzheimer's disease and Down's syndrome aggregate, may be used to test this hypothesis.

Family history of Parkinson's disease

Alzheimer's disease and Parkinson's disease share several neuropathologic characteristics.[16] It has been suggested that these disorders may have a common pathogenesis.[17] This hypothesis was supported by the re-analysis of case-control studies, that suggested familial aggregation of Alzheimer's disease and Parkinson's disease (chapter 3.2). The association tended to be stronger in men as compared to women. Although the risk of Alzheimer's disease for those with a positive family history for Parkinson's disease was increased in the absence of a first degree relative with dementia, the risk tended to be higher for those who had a positive family history of dementia as well (chapter 5.4). The stronger association with family history of Parkinson's disease among men and among those with a positive family history of dementia may be explained by the hypothesis that Parkinson's disease may be of multifactorial origin, meaning that genetic as well as environmental factors are required to increase the risk of the disease.[18] The stronger association in those with a positive family history of dementia may be explained by a genetic link between Alzheimer's disease and Parkinson's disease, while the stronger association in men may be explained by environmental factors to which men

in particular are exposed, for example occupational exposures. Such a mechanism remains to be resolved in genetic-epidemiologic studies.

Genetic mechanism

As presented in chapter 3.2, the re-analysis of case-control studies showed a difference in risk for those with two or more first degree relatives with dementia as compared to those with one affected relative. Such a trend was observed for early- as well as late-onset Alzheimer's disease and may point at heterogeneity. The view of heterogeneity in early-onset Alzheimer's disease was supported by a segregation analysis using data of first degree relatives of 198 patients with early-onset Alzheimer's disease (chapter 3.3). This analysis confirmed a role of a major dominant gene in early-onset Alzheimer's disease. Our data suggested, however, that it is unlikely that a major dominant gene effect accounts for the total variance in transmission of Alzheimer's disease. Other genetic mechanisms or environmental factors may account for the familial aggregation of disease in a considerable number of cases. Similar findings have been reported for a population comprising patients with early-onset as well as late-onset Alzheimer's disease.[19]

Genetic origin

Linkage between early-onset Alzheimer's disease and markers on the proximal long arm of chromosome 21 was first reported in 1987.[20] In the same year the gene coding for the amyloid precursor protein (APP), a precursor of the A4 protein deposited in the brains of patients with Alzheimer's disease and Down's syndrome, was located to the same region of chromosome 21.[21] As two studies excluded the APP gene as the cause of Alzheimer's disease[22,23] and since genetic heterogeneity in early-onset Alzheimer's disease was not yet considered at that time, molecular genetic research concentrated on the search for other mutations on chromosome 21. Although in the following years a number of studies confirmed linkage to chromosome 21, linkage was excluded by others.[24-28] After pooling the data of 48 families in which Alzheimer's disease was apparently inherited as an autosomal dominant disorder, linkage to chromosome 21 was confirmed for early-onset Alzheimer's disease but excluded for late-onset Alzheimer's disease.[28] For the latter patients, linkage to chromosome 19 has been reported.[29] At present it is unclear what proportion of the families with Alzheimer's disease is linked either to chromosome 19 or to chromosome 21.

In view of the growing evidence for genetic heterogeneity, the possibility that a mutation in the APP gene may lead to Alzheimer's disease in a subset of families with

early-onset Alzheimer's disease was reconsidered by Goate and coworkers.[30] In 1991 they reported a mutation in exon 17 (transcript APP770) of the APP-gene in two families that showed linkage to APP on chromosome 21. This mutation was also found in four other families in which early-onset Alzheimer's disease was inherited as an autosomal dominant disorder.[30,31] To assess the role of the APP_{717} mutation as a cause of early-onset Alzheimer's disease in the general population, we screened 100 patients with familial and sporadic Alzheimer's disease but failed to show the mutation reported by Goate et al (chapter 3.4). Our finding suggests that this APP_{717} mutation is not a common cause of early-onset Alzheimer's disease in the Dutch population. However, it cannot be excluded that other mutations in the APP gene may be involved in Alzheimer's disease. To date, two other mutations at codon 717 of the APP-gene were reported.[32,33]

For Hereditary Cerebral Haemmorhages With Amyloidosis of the Dutch type (HCHWA-D) a mutation has been detected in exon 17 of the APP-gene, at residue 693 (APP_{770}).[34,35] HCHWA-D brains are characterized by ß-amyloid angiopathy, but amorphous plaques have been shown as well. Since a great number of patients with Alzheimer's disease have ß-amyloid angiopathy, it has been suggested that the ß-amyloid in the senile plaques may be of vascular origin.[36] It is of interest that in one of the Rotterdam families (family 1302), intra-cerebral bleedings and dementia appeared to segregate together (unpublished findings). In this family, there was one subject with intra-cerebral haemmorhage (age 40 years) due to amyloid angiopathy confirmed at biopsy. In this patient, amyloid reactive senile plaques were found in addition to the amyloid angiopathy. None of the mutations in the APP gene reported to date were detected in this patient. In this five generation family, one other living patient was known with intra-cerebral bleedings, while four others suffered from a slowly progressive dementia, probably of the Alzheimer type. No massive bleedings were observed in the patients with dementia. As the intra-cerebral bleedings and the dementia appeared to segregate together in this family, it is tempting to speculate that in this family cerebral haemmorhage and dementia may have the same genetic origin. However, in the absence of pathologic confirmation, it is at present impossible to distinguish whether the patients with dementia suffer from dementia due to ß-amyloid angiopathy or from Alzheimer's disease.

DNA repair

Another possible mechanism implicated in the pathogenesis of Alzheimer's disease is DNA repair. Increased levels of DNA-damage have been found in cortex tissue of patients with Alzheimer's disease.[37] This finding may be the result of an increased level

of exposure to DNA damaging agents in patients, but it is also conceivable that patients with Alzheimer's disease may have a decreased DNA repair capacity. As reviewed in chapter 3.5, studies of DNA repair using fibroblast or lymphoid cell lines of patients with Alzheimer's disease have yielded equivocal results.[38-43] We observed a delay in DNA repair of single-strand breaks induced by N-ethyl-N-nitrosourea in freshly isolated lymphocytes of patients from families in which Alzheimer's disease is apparently inherited as an autosomal dominant disorder (chapter 3.5). There was no evidence for a decrease in DNA repair capacity in the other patients, suggesting DNA-repair may only be implicated in inherited early-onset Alzheimer's disease. However, as pointed out in chapter 3.5, our study must be considered as exploratory and our findings remain to be confirmed by independent studies.

Environmental factors

Parental age

The role of parental age in Alzheimer's disease is debated.[44] To date 12 studies have reported on this issue, yielding contradicting results.[45-56] Four studies have reported a significant association to late maternal age,[45,46,51-53] while two studies reported a significant increase in risk for young maternal age as well as young paternal age.[50,56] Of the latter studies, the most recent one showed that the association with young maternal age disappeared when adjusting for paternal age, while the association with paternal age was specific for late-onset Alzheimer's disease.[56] As a corollary of these contradicting findings, there are two competing hypotheses on the underlying mechanism. For late maternal age, the association has been explained by the link with Down's syndrome. This hypothesis predicts that the risk of Alzheimer's disease follows the risk of Down's syndrome, which increases slowly with increasing maternal age until age 30 years and rapidly thereafter.[44] According to the second more speculative hypothesis, the association of late-onset Alzheimer's disease with young paternal age may be explained by genetic imprinting, i.e., patients have inherited an increased predisposition to the disease through a particular parent.[56]

The findings of the re-analysis of case-control studies as presented in chapter 4.2 did not show an association with paternal age, when maternal age and paternal age were studied simultaneously and data were analyzed stratified by gender and onset age. The findings provided weak support for an association between Alzheimer's disease and late

maternal age. Although the risk of Alzheimer's disease was significantly increased among subjects whose mother was 40 years or older at birth, the association was mainly determined by one study. At the same time, there was also evidence for an increased risk of early-onset Alzheimer's disease for young maternal age (19 years or younger), although this association could also be attributed to the strong relation observed in one study. In the pooled analysis, there was no evidence for an increase in risk of Alzheimer's disease for maternal age at birth between 20 and 40 years.

The persistent discrepancies between the findings on this putative risk factor give rise to the hypothesis that, as opposed to the idea of two competing hypotheses on the role of parental age in Alzheimer's disease, the association may be determined by two different mechanisms: (1) young parental age may be a risk factor for Alzheimer's disease, through an unknown mechanism, perhaps related to genetic imprinting and (2) late maternal age may be associated with an increase in risk of Alzheimer's disease, perhaps due to a chromosome 21 linked mechanism.[7] These two potential mechanisms may outbalance each other and therefore it is conceivable that their effects can only be shown at both extremes of the parental age distribution, which in most studies comprised only a limited number of subjects. In such a situation, comparison of means of parental age will be of little use and a large number of patients need to be studied to show a significant association in the extremes of the distribution of parental age. Another issue to address in future research is related to the assessment of the parental age at birth. Although agreement between the next of kin interview and direct interview was shown to be high (88%),[57] the informants could not provide us with data on parental age for 17% of the cases and 11% of the controls. Finally, studies of the role of the sex of the transmitting parent on age of onset in offspring (imprinting) and changes in age of onset in offspring (anticipation) may be of interest in light of the contradicting findings on the role of maternal and paternal age in Alzheimer's disease.

Head trauma

The similarity in pathology has led to the hypothesis of a common pathogenesis of Alzheimer's disease and dementia pugilistica (punch drunk syndrome).[58-60] Head trauma was studied in chapter 4.2, based on the re-analysis of case-control studies, and in chapter 4.3, based on the Dutch case-control study. In the re-analysis of case-control studies, an association with Alzheimer's disease could be shown only in men. Although the association was strongest for head trauma that occurred within ten years before the disease onset, a significant elevation in risk was also observed in the re-analysis for head

trauma that occurred more than ten years before the onset of disease. By contrast, the Dutch study suggested that the risk of Alzheimer's disease was increased only within the ten year period before the disease onset, in men and women. These findings suggest a short lag-time between the head trauma and the occurrence of disease. No association was found with head trauma that occurred early in life. This finding is compatible with the view that head trauma may interact with a sub-clinical disease process. It is also conceivable that head trauma may interact with an age-related process, e.g. a decrease in regeneration capacity of the brain at old age.

Despite the apparently consistent findings of epidemiologic studies (reviewed in chapter 4.3), there are several reasons for challenging the interpretation of a causal relationship. There is considerable scope for recall bias for events that occurred long before the disease onset. For head trauma occurring close to the disease onset, we cannot exclude the possibility that the head trauma may be a consequence of an early stage of the dementia. Furthermore, the only prospective follow-up study based on data obtained from medical records of the Rochester register showed only a slight non-significant elevation in risk (chapter 4.2). Therefore, to determine the etiologic significance of the relation between head trauma and Alzheimer's disease, the association remains to be confirmed in a prospective follow-up study.

Medical history

A great variety of disorders have been linked with Alzheimer's disease, but many of these associations appeared in one or two studies and were not replicated in others. As pointed out in chapter 4.2, caution is warranted when interpreting the findings of the re-analysis of case-control studies on medical history. Bias, multiple testing and data dredging apply particularly to these analyses.[30] Moreover, as exposures are usually rare and the precision in assessing the disease history or previous treatment is generally low, it may be argued that a perhaps more fruitful approach to study medical history may be to conduct a prospective follow-up study based on historical cohorts defined within a morbidity register, e.g. using the medical records of the Rochester register (described in chapter 2.2).

There is some evidence for an association between Alzheimer's disease and history of hypothyroidism. In the Rochester study, an increase in risk was observed for history of hypothyroidism, albeit non-significant. However, exposure frequency was low in cases (10/392) and controls (6/392). The re-analysis of case-control studies confirmed this finding (chapter 4.2). The association between Alzheimer's disease and hypothyroidism

may be of particular interest because of the role of the thyroid hormone on maturation of the nervous system and on neuritic outgrowth.[61-63] The thyroid hormone is furthermore related to other hormones and trophic factors that have been implicated in Alzheimer's disease.[64] There are several arguments which plea for cautious interpretation: (1) earlier studies yielded contradicting results,[61] (2) the classification for type of thyroid disorder may be criticized as it was based on functional status as reported by informants, who, with the exception of the Rochester study, were not medically trained, (3) although an association could be shown to hypothyroidism, from a statistical point of view skepticism on this relationship results from the lack of an association to all thyroid diseases combined, and (4) hypothyroidism can be a cause of secondary dementia and such cases may not have been recognized. Therefore, it is acknowledged that this finding is to be considered as tentative. The association with hypothyroidism must be confirmed preferably in a prospective follow-up study of well-defined, non-demented cases with hypothyroidism.

Increased levels of acute-phase proteins have been reported in patients with Alzheimer's disease.[65-67] It has been demonstrated that the production of acute-phase proteins is in part mediated by lymphokines such as interleukin-1 and interleukin-6.[68] As presented in chapter 4.4, serum levels of interleukin-6 were not associated with Alzheimer's disease. However, this finding does not exclude the possibility that interleukin-6 is locally elevated within the brain and may in this way be related to Alzheimer's disease.

Psychiatric history

In the re-analysis of case-control studies, history of depression that was medically treated emerged as a risk factor for Alzheimer's disease, in particular for the late-onset form (chapter 4.2). There were two findings that overruled concern of bias with regard to this relationship. Firstly, the association was present in the Rochester study, which overcomes the problem of recall bias as this prospective study is based on medical records. Secondly, a significant association was observed for episodes of depression that occurred more than ten years before the disease onset, suggesting a true association rather than the depression being the result of the dementia.

There are several possible explanations for the association between history of depression and Alzheimer's disease possible.[69] Anti-depressant treatment is to be considered as an explanation, as it may alter neurotransmitter functioning. Because in our study there were only limited data available on type of medication and duration of use,

we can not exclude this possibility. Another explanation may be a joint etiology of both disorders. Systems disrupted in depression may also be involved in Alzheimer's disease. It is also conceivable that the depression is an early symptom of Alzheimer's disease, that may occur before the first symptoms of dementia. As patients with depression may already have subtle cognitive deficits, it is also possible they may reach more quickly the threshold for the diagnosis of dementia. These explanations are not exclusive. They all assume heterogeneity in the pathogenesis of early- and late-onset Alzheimer's disease in order to explain for the association to late-onset Alzheimer's disease specifically.

Despite the evidence for an association to history of depression, further studies on incidence of Alzheimer's disease in confirmed cases with depression are needed to study the relation with specific types of depression (bipolar, unipolar). Furthermore, the role of electro-convulsive therapy and prior medication, in particular those with anticholinergic effects, need to be addressed. The difference between early- and late-onset Alzheimer's disease requires further investigation in light of the possibility of genetic heterogeneity between the early- and late-onset form.

Smoking, alcohol and occupational exposures

There is some evidence from clinical trials that nicotine may improve information processing and attention in Alzheimer patients.[70,71] The mechanism underlying this association may be related to the decreased nicotinic receptor binding, which has been linked to the Alzheimer type pathology.[72,73] Nicotine has been reported to increase the density of nicotinic receptors in the brain.[74] We may speculate that nicotine from cigarette smoke may compensate the loss of nicotinic receptors in Alzheimer's disease and may thus delay the progression of Alzheimer's disease. Such a mechanism would predict an inverse association between smoking and Alzheimer's disease. Indirectly, this hypothesis derives support from the fact that a decrease in nicotinic receptor binding has also been observed in patients with Parkinson's disease, while the majority of studies of Parkinson's disease have reported an inverse association with smoking.[75]

The first evidence for a possible inverse association with smoking history was obtained in the Dutch case-control study of early-onset Alzheimer's disease (chapter 5.3). In this study, we were able to show that it was unlikely to explain the relation by (1) selection bias due to the exclusion of patients with evidence of atherosclerotic cardiovascular disease in the clinical diagnosis of Alzheimer's disease, and (2) by differential survival of smoking and non-smoking patients. The findings of the Dutch study were supported by the re-analysis of case-control studies (chapter 4.2). Furthermore, in the re-analysis

(chapter 4.2) as well as in the Dutch study (chapter 5.3) there was evidence for a dose-response relationship when smoking was analyzed stratified according to the number of pack-years smoked. An inverse relationship between smoking and Alzheimer's disease could only be shown among patients with a positive family history of dementia. There was no association among those with no first degree relatives with dementia in the Dutch case-control study (chapter 5.3) or in the re-analysis of case-control studies (chapter 5.4), suggesting that smoking may interact with a genetically determined process.

The role of smoking in Alzheimer's disease requires further study. To fully overcome the problem of bias due to mortality related to smoking, a preferably population-based study of incident cases has to be conducted. Although the association we observed is compatible with a protective effect of smoking for familial Alzheimer's disease, it has no relevance for prevention of Alzheimer's disease because of the adverse health effects of smoking. Moreover, we cannot exclude the possibility that preclinical changes in for instance nicotinic receptors may have resulted in a loss of desire to smoke in patients with familial Alzheimer's disease.

There was no evidence in the re-analysis of case-control studies for an increase in risk of Alzheimer's disease for alcohol intake or for occupational exposure to lead or solvents (chapter 4.2). These findings should be treated with caution. Cases with high alcohol intake may have been excluded when applying the criteria for possible or probable Alzheimer's disease and this may have led to an underestimation in risk.[76] The frequency of exposure to lead and solvents was low, even when the studies were pooled, and exposure definition has been imprecise for these putative neurotoxins.[77]

Aluminum

Aluminumsilicates are found in the cores of senile plaques and in neurones containing neurofibrillary tangles.[78] However, it remains to be established if the presence of aluminum is a cause or a consequence of the disease. Case reports of subjects exposed to high doses of aluminum which led to high concentrations in the brain suggest that the exposure does not lead to pathologic changes specific for Alzheimer's disease.[79-81] Yet, several studies reported an association between aluminum intake through drinking water and the risk of Alzheimer's disease, despite the fact that water contributes only a small percentage of aluminum intake.[82-84] However, there is considerable scope for bias in these observational studies. In the earliest study, dementia was assessed by death certificates,[82] a method which has been shown to be unreliable.[85,86] The study by Martyn et al[83] may be criticized because the diagnosis of Alzheimer's disease was based on CT-scan readings

without clinical examination of the patients. Further, the association was mainly due to an increase in risk for the highest exposure category, without showing convincing evidence for a dose-response relationship. Finally, the findings of a relationship in the most recent study[84] could not be replicated after re-measurement of the aluminum content of the drinking water (personal communication).

A role of aluminum in Alzheimer's disease was supported by three other findings. Firstly, a higher risk of Alzheimer's disease was observed for miners who were treated with aluminum powder.[87] Secondly, a case-control study based on informant interviews reported an increase in risk of Alzheimer's disease for subjects using aluminum containing antiperspirants.[88] Thirdly, a slower progression of the disease in patients with Alzheimer's disease treated with aluminum binding chemicals was reported, suggesting aluminum may be involved in the progression of the disease.[89] On the other hand three case-control studies that have investigated the role of aluminum containing antacids failed show an association.[51,90,91]

Although a number of studies provided evidence for a relationship of aluminum with Alzheimer's disease, one cannot escape the conclusion that the role of aluminum in Alzheimer's disease remains to be clarified. In particular, the interaction with genetic susceptibility or other risk factors may be of interest, as it remains to be resolved how aluminum is able to pass the blood-brain barrier. It is possible that genetic factors and environmental factors (e.g. head trauma) may enable aluminum to enter the brain. On the other hand, it is also conceivable that aluminium merely exacerbates the effect of other risk factors, as there is evidence that aluminium is implicated in the progression of Alzheimer's disease.

Education

Education may be related to Alzheimer's disease through several mechanisms.[92] It is conceivable that highly educated subjects have greater cognitive or neuronal reserves than poorly educated subjects and therefore can lose more neurons due to Alzheimer's disease before showing symptoms of the disease. It is also possible that the highly educated practice their cognitive skills more intensively during their lives than those with a low education and it has been suggested that lack of intellectual stimulation may lead to an increased risk of neuronal loss and Alzheimer's disease.[93] A third possibility is that low education may merely be related to socio-economic status and that lifestyle and occupational exposures may be underlying the association with Alzheimer's disease.

Although education has been linked to cognitive decline and dementia in a large

number of studies, the interpretation of these findings has been hampered by the possibility of assessment bias.[92] When the ascertainment of patients is accomplished through screening for cognitive impairment, an association with education may result from the fact that the scores of those screening tests may in part be determined by the subject's level of education. As an example of the difficulties encountered when interpreting findings on the relation between Alzheimer's disease and education, may serve the findings of a population-based prevalence study conducted in Shanghai China.[94] This study showed an association between education and Alzheimer's disease, which was mainly determined by women in the old age category. The interpretation of this finding is not straightforward as education-dependent cut-off points for the screening instrument were used and effect modification by gender may be explained by inadequacy of the screening test in women. Another example may be the findings of the Dutch case-control study, as reported in chapter 2.1. The findings were compatible with the view of an inverse association between Alzheimer's disease and education, as there was an increased risk for those who received less than seven years of education (chapter 2.1). However, we cannot rule out the possibility that bias has occurred due to selective non-response among control subjects with low education.

It may be concluded that the evidence for an association of education with Alzheimer's disease is still weak. The major difficulty to overcome is to deal with methodologic problems, in particular that of assessment bias in population-based prevalence and incidence studies. Furthermore, the hypothesis that life-style, occupational or medical exposures may explain the relationship needs to be examined in future studies.

Gene-environment interaction

For family history of Down's syndrome, family history of Parkinson's disease, late maternal age, history of head trauma and history of depression, the association with Alzheimer's disease was observed regardless of the absence or presence of a first degree relative with dementia (chapter 5.4). An additional analysis using the data of the re-analysis of case-control studies showed that hypothyroidism and young maternal age were also associated with an increased risk of Alzheimer's disease for those with and without a positive family history of dementia (unpublished data). Because family history of dementia remained strongly associated with Alzheimer's disease in the absence of other risk factors, these findings are consistent with a model in which genetic and

environmental risk factors may increase the risk of Alzheimer's disease independently of each other. For late maternal age, history of head trauma, history of depression and history of hypothyroidism, the strength of association did not vary when stratifying for family history of dementia, suggesting there is no interaction with a genetic factor. However, as pointed out in chapter 6.2, due to misclassification resulting from the use of family history of dementia as an indicator for genetic susceptibility, the possibility that genetic factors may underlie these environmental factors cannot be excluded. Such a mechanism is conceivable in particular for history of depression and history of hypothyroidism. The risk for family history of Down's syndrome, for family history of Parkinson's disease and for young maternal age tended to be higher for those who had a positive family history of dementia. However, in all cases the interaction was not statistically significant, but this may be due to the small number of exposed patients and controls. There is some evidence suggesting that smoking may merely interact with a genetically determined process. An association was found only among those with one or more first degree relatives with dementia (chapter 5.3 and 5.4). The re-analysis of case-control studies showed that the risk associated with family history of dementia tended to be lower for smokers as compared to non-smokers (chapter 5.4). Although no significant differences could be established, this effect was most pronounced for those with two or more affected relatives. The Dutch genetic-epidemiologic study of early-onset Alzheimer's disease showed that the disease onset was significantly later in smoking patients as compared to non-smoking patients from families in which the disease is apparently inherited as an autosomal dominant disorder (chapter 5.3). Because there is familial aggregation of Alzheimer's disease and Parkinson's disease and Parkinson's disease has been found to be inversely associated with smoking as well, it may be speculated that smoking may modify the risk of a genetic factor that is involved in Alzheimer's disease as well as Parkinson's disease.

Conclusions

The findings of this thesis may be summarised as follows:

1. There is evidence for familial aggregation of early-onset as well as late-onset Alzheimer's disease.
2. Not all patients with early-onset Alzheimer's disease can be explained by autosomal

dominant inheritance, i.e., there may be other genetic or environmental factors implicated.

3. There is some evidence for a genetic link between Alzheimer's disease and Down's syndrome and between Alzheimer's disease and Parkinson's disease.
4. In all likelihood, there is more than one mutation involved in Alzheimer's disease, the one in exon 17 of the amyloid precursor protein gene[30] being a rare cause.
5. Decreased DNA repair may be implicated in inherited Alzheimer's disease.
6. Parental age may be involved in Alzheimer's disease through several mechanisms.
7. History of head trauma, history of depression and history of hypothyroidism may increase the risk of Alzheimer's disease regardless of the genetic susceptibility.
8. Smoking may be inversely related to Alzheimer's disease, through a mechanism of genetic origin.

Case-control studies have shown a great variety of risk factors to be associated with Alzheimer's disease. Many of these associations appeared only in one or two studies and were not replicated by others. Given the exploratory design of the epidemiologic studies conducted to date, at present there appears to be a need for studies designed to test specific hypotheses. As evidenced by the recent findings of several mutations in the amyloid precursor protein gene that are associated with Alzheimer's disease, it is likely that Alzheimer's disease may be divided into many smaller subgroups based on genetic characteristics. As a consequence, interaction between genetic and environmental factors may become an important feature in epidemiologic studies and special attention should be paid to genetic aspects in the design of the study. An important assumption underlying current research on Alzheimer's disease is that we are dealing with a single disorder. One of the key questions to be answered is whether this assumption holds. To resolve this issue, a strong link between the genetic-epidemiologic and molecular biologic approach is likely to be profitable.

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Chapter 7

Summary

Summary

The aim of the investigations presented in this thesis was to study the role of various genetic and environmental factors in Alzheimer's disease, as well as the interaction between genetic and environmental risk factors (*chapter 1*). Risk factors for Alzheimer's disease were studied in a Dutch genetic-epidemiologic study of 198 patients with early-onset Alzheimer's disease (diagnosis before 70 years) and 198 age- and gender-matched controls, and in a re-analysis of all formal case-control studies conducted to January 1, 1990, comprising a total of 1385 cases and 1739 age- and gender-matched controls. The design of the studies was described in (*chapter 2*).

In *chapter 3*, genetic factors were addressed. Familial aggregation of Alzheimer's disease and related disorders was studied in the re-analysis of case-control studies (*chapter 3.2*). When pooling the raw data of the studies, the risk of Alzheimer's disease for those with at least one first degree relative with dementia was significantly increased. Stratification according to age of onset of Alzheimer's disease showed that the risk decreased with increasing onset age. However, among patients with an onset of disease after 80 years, there were still significantly more subjects with one or more first degree relatives with dementia as compared to controls. The risk of early-onset as well as late-onset Alzheimer's disease was lower for patients who had one first degree relative with dementia as compared to those who had two or more affected relatives. Furthermore, the re-analysis showed an increased risk of Alzheimer's disease for those with a positive family history of Down's syndrome as well as for those with a positive family history of Parkinson's disease. The risk of possible Alzheimer's disease and the genetic transmission of disease were studied in first degree relatives of the 198 Dutch patients with early-onset Alzheimer's disease (*chapter 3.3*). Among first degree relatives of patients, the risk of Alzheimer's disease (0.39) was lower than expected for an autosomal dominantly inherited disorder, although the risk estimate did not differ significantly from the expected risk (i.e., 0.50). Segregation analysis suggested that familial aggregation of Alzheimer's disease is most likely in part explained by a dominant major gene effect. Other genetic or environmental factors may play a role in the etiology as well. A sample of 100 cases out of the 198 Dutch cases with early-onset Alzheimer's disease was

screened for a mutation within exon 17 (transcript APP770) of the amyloid precursor protein gene (*chapter 3.4*). The study comprised 14 unrelated cases from families in which Alzheimer's disease was apparently inherited as an autosomal dominant disorder. None of the patients showed the mutation. This finding suggests that the mutation in the amyloid precursor protein gene is not a common cause of early onset Alzheimer's disease in the general population. As increased levels of DNA damage have been reported in Alzheimer's disease, DNA repair may be implicated in the pathogenesis. DNA repair after treatment of lymphocytes with alkylating agents was studied in 43 patients with early-onset Alzheimer's disease and 48 controls (*chapter 3.5*). Our study showed a delay in DNA repair of single-strand breaks induced by N-ethyl-N-nitrosourea in patients from families in which Alzheimer's disease was apparently inherited as an autosomal dominant disorder. There was no evidence for a decrease in DNA repair capacity in the other patients, suggesting DNA-repair may only be implicated in inherited Alzheimer's disease. However, our study must be considered as exploratory.

Environmental risk factors were addressed in *chapter 4*. The evidence for non-inherited risk factors for Alzheimer's disease was reviewed in *chapter 4.2*, based on the findings of the re-analysis of case-control studies. There was weak evidence for an association of late maternal age at birth (40 years or older) with Alzheimer's disease. Although overall a significant elevation in risk was shown, this finding was mainly determined by one study. In addition, there was evidence for a relation between young maternal age (19 years or younger) and early-onset Alzheimer's disease, but also this finding could be attributed to one study. No association was shown with paternal age, when adjusting for maternal age. In the re-analysis of case-control studies concerning medical history, history of hypothyroidism and history of depression emerged as two possible risk factors for Alzheimer's disease. An association between head trauma and Alzheimer's disease could be shown only in men. Although the association was strongest for head trauma that occurred within ten years before the disease onset, a significant elevation in risk was observed also for head trauma that occurred more than ten years before the onset of disease. By contrast, the Dutch study of early-onset Alzheimer's disease suggested that the risk of Alzheimer's disease was increased only for head trauma that occurred within the ten year period before the disease onset, in men and women. No association was found for head trauma that occurred early in life (*chapter 4.3*). In the re-analysis, an inverse relationship between smoking and Alzheimer's disease was observed (*chapter 4.2*). An inverse relationship could only be shown among those with a positive family history of dementia. Furthermore, there was evidence for a dose-response relationship when

subjects were classified according to the number of pack-years they had smoked. The findings of the Dutch case-control study of early-onset Alzheimer's disease were very similar (*chapter 5.3*). Increased levels of acute-phase proteins have been reported in patients with Alzheimer's disease. It has been demonstrated that the production of acute-phase proteins is in part mediated by interleukin-6. Serum levels of interleukin-6 were not associated with Alzheimer's disease in a study of 97 patients with early-onset Alzheimer's disease and 79 controls (*chapter 4.4*), but this finding does not exclude the possibility that interleukin-6 is locally elevated within the brain.

Gene-environment interaction was discussed in *chapter 5*. In *chapter 5.2*, age at onset was examined for 139 members of 30 families in which early-onset Alzheimer's disease (onset before 60 years) was apparently inherited as an autosomal dominant disorder. Age of onset was more similar within than between families. Although onset age within decades is most likely genetically determined in patients from such families, the relatively wide spread in onset age observed within these families as well as within sets of identical twins suggests that environmental factors may influence the family-specific onset age. The issue of interaction between genetic and environmental risk factors was addressed in the re-analysis of case-control studies (*chapter 5.4*). For late maternal age, history of head trauma and history of depression, an association with Alzheimer's disease was observed regardless of the absence or presence of a first degree relative with dementia. Since family history of dementia remained strongly associated with Alzheimer's disease in the absence of other risk factors, these findings are consistent with a model in which genetic and environmental risk factors may increase the risk of Alzheimer's disease independently. The risk of Alzheimer's disease for family history of Down's syndrome and for family history of Parkinson's disease tended to be higher for those who also had a positive family history of dementia. These findings support the view of a genetic link between Alzheimer's disease and Down's syndrome and between Alzheimer's disease and Parkinson's disease. However, in both cases the interaction was not statistically significant. There was some evidence that smoking may be related specifically to a genetically determined process. The risk of Alzheimer's disease associated with family history of dementia tended to be lower in smokers as compared to non-smokers. This effect was most pronounced for those with two or more affected relatives. When comparing smoking patients to non-smoking patients from families in which the disease was apparently inherited as an autosomal dominant disorder, the onset of disease was significantly later in smokers (*chapter 5.3*).

The inference that can be drawn upon our findings was discussed in *chapter 6.2*

(methodologic issues) and *chapter 6.3* (formal aspects). The quality of the case-control studies conducted to date may be criticized. In order to determine the etiologic significance, risk factors that have emerged in these studies remain to be confirmed, preferably in prospective follow-up studies. At present, research on the interaction between hereditary and environmental factors is limited by the lack of biologic genetic markers suitable for population studies.

Samenvatting

Samenvatting

Het doel van de onderzoeken beschreven in dit proefschrift was mogelijke risicofactoren voor de ziekte van Alzheimer op te sporen (*hoofdstuk 1*). Erfelijke en omgevingsfactoren, alsmede hun onderlinge interactie, zijn bestudeerd in twee onderzoeken. Het eerste was een genetisch-epidemiologisch onderzoek bij 198 patiënten bij wie de ziekte van Alzheimer op jonge leeftijd (voor het 70ste levensjaar) werd gediagnostiseerd en 198 op leeftijd en geslacht gematchte controle personen. De patiënten en controles waren afkomstig uit de vier noordelijke provincies en de regio Rotterdam. Het tweede onderzoek betrof een her-analyse van alle patiënt-controle onderzoeken naar risicofactoren voor de ziekte van Alzheimer, die voor 1 januari 1990 zijn uitgevoerd. Deze analyse betrof zowel patiënten bij wie de ziekte van Alzheimer op jonge leeftijd begon als patiënten bij wie de ziekte zich pas op oudere leeftijd manifesteerde. De opzet van de twee onderzoeken werd besproken in *hoofdstuk 2*.

In *hoofdstuk 3* werd de rol van erfelijke factoren onderzocht. Het vóórkomen van de ziekte van Alzheimer in de familie werd nagegaan in de her-analyse van patiënt-controle onderzoeken (*hoofdstuk 3.2*). Het risico op de ziekte van Alzheimer was duidelijk verhoogd voor personen met één of meer eerste graads verwanten met dementie. Het risico nam af met het toenemen van de leeftijd waarop de ziekte begon. Echter ook patiënten bij wie de ziekte na het 80ste levensjaar begon hadden vaker eerste graads verwanten met dementie dan controle personen. Het risico op de ziekte van Alzheimer was lager bij patiënten met één familielid met dementie dan bij patiënten met twee of meer eerste graads familieleden met dementie. Daarnaast kwamen bij eerste graads familieleden van patiënten met de ziekte van Alzheimer vaker familieleden met het syndroom van Down en met de ziekte van Parkinson voor dan bij eerste graads verwanten van de controle personen (*hoofdstuk 3.2*). De wijze van erfelijke overdracht van de ziekte van Alzheimer werd onderzocht bij de eerste graads familieleden van de 198 Nederlandse patiënten bij wie de ziekte van Alzheimer voor het 70ste levensjaar werd vastgesteld (*hoofdstuk 3.3*). Bij de eerste graads verwanten was het risico op de ziekte van Alzheimer (0.39) lager dan verwacht voor een aandoening die autosomaal dominant wordt overgedragen (0.50), hoewel de geobserveerde waarde niet significant van de verwachte waarde verschilde. Met behulp van segregatie analyse kon worden aangetoond dat het clusteren van de ziekte van Alzheimer in families slechts ten dele te verklaren was door een dominant overgedragen gen. Daarnaast spelen andere erfelijke

of niet erfelijke factoren mogelijk een rol. Bij een aantal families is een mutatie in exon 17 (transcript APP770) van het amyloid precursor eiwit gen aangetoond, die mogelijk de ziekte van Alzheimer veroorzaakt. Bij 100 patiënten uit het Nederlandse onderzoek werd deze mutatie nagegaan (*hoofdstuk 3.4*). De onderzoekspopulatie bevatte ondermeer 14 niet verwante patiënten afkomstig uit families waarin het overervingspatroon erop wees dat de ziekte van Alzheimer mogelijk erfelijk is bepaald. Bij geen van de 100 patiënten kon de mutatie worden aangetoond. Deze bevinding suggereert dat het een zeldzame oorzaak van de ziekte van Alzheimer betreft. Bij patiënten met de ziekte van Alzheimer zijn verhoogde niveaus van DNA-schade waargenomen. Het is mogelijk dat het DNA-herstel mechanisme een rol speelt in de ziekte van Alzheimer. DNA-herstel werd nagegaan bij 43 patiënten bij wie de ziekte voor het 70ste levensjaar begon en bij 48 controle personen (*hoofdstuk 3.5*). Een verminderd herstel van DNA-breuken geïnduceerd door N-ethyl-N-nitrosourea werd waargenomen bij patiënten afkomstig uit families waarin de ziekte leek te worden overgedragen als een autosomaal dominante aandoening. Bij de overige patiënten waren er geen aanwijzingen voor een verminderd DNA-herstel. De resultaten van dit exploratieve onderzoek dienen bevestigd te worden door onafhankelijke onderzoeken.

In *hoofdstuk 4* werd de rol van omgevingsfactoren onderzocht. In *hoofdstuk 4.2* werd een overzicht gegeven van de rol van niet-erfelijke en omgevingsfactoren, zoals gevonden in de her-analyse van patiënt-controle onderzoeken. Een verhoogd risico op de ziekte van Alzheimer kon worden aangetoond voor personen van wie de moeder 40 jaar of ouder was bij de geboorte, hoewel dit effect voornamelijk werd bepaald door de resultaten van één onderzoek. Tevens was er een verband met een jonge leeftijd van de moeder bij de geboorte (19 jaar of jonger), maar ook deze relatie kon worden toegeschreven aan het effect waargenomen in één onderzoek. In de medische voorgeschiedenis kwamen schildklierziekte (hypothyroïdie) en depressie als mogelijke determinanten van de ziekte van Alzheimer naar voren. Een verband tussen de ziekte van Alzheimer en ongevallen met het hoofd kon alleen worden aangetoond bij mannen. Hoewel het verband het sterkst was voor ongevallen met het hoofd die binnen een periode van tien jaar voor het begin van de ziekte optraden, werd in deze analyse ook een significant verhoogd risico gevonden voor ongelukken met het hoofd die meer dan tien jaar voor de eerste ziekteverschijnselen optraden. In het Nederlandse onderzoek kon daarentegen geen verband worden aangetoond met ongelukken met het hoofd die meer dan tien jaar voor het begin van de ziekte optraden (*hoofdstuk 4.3*). Tenslotte werd er een inverse relatie gevonden tussen roken en de ziekte van Alzheimer in de her-analyse van patiënt-controle

onderzoeken (*hoofdstuk 4.2*). Een inverse relatie kon alleen worden aangetoond bij patiënten met een positieve familie-anamnese voor dementie. Verder waren er aanwijzingen voor een dosis-effect relatie. De bevindingen van het Nederlandse onderzoek waren zeer vergelijkbaar (*hoofdstuk 5.3*). Verhoogde waarden van acute-fase eiwitten zijn waargenomen bij patiënten met de ziekte van Alzheimer. De aanmaak van deze eiwitten is deels bepaald door interleukine-6. Serum waarden voor interleukine-6 waren echter vergelijkbaar bij 97 patiënten en 79 controle personen (*hoofdstuk 4.4*).

In *hoofdstuk 5* werd de interactie tussen erfelijke en omgevingsfactoren nagegaan. De leeftijd bij eerste ziekteverschijnselen werd onderzocht bij 139 familieleden afkomstig uit 30 families waarin de ziekte autosomaal dominant leek te worden overgedragen (*hoofdstuk 5.2*). Alleen families waarin de eerste ziekteverschijnselen reeds voor het 60ste levensjaar optraden werden bestudeerd. De leeftijd bij eerste ziekteverschijnselen was meer vergelijkbaar binnen families dan tussen families. Deze bevinding suggereert dat het begin van de ziekte waarschijnlijk erfelijk is bepaald. De aanzienlijke spreiding in de leeftijd bij eerste ziekteverschijnselen tussen patiënten uit dezelfde familie en tussen identieke tweelingen doet echter vermoeden dat ook omgevingsfactoren een rol spelen. Tenslotte werd de interactie tussen erfelijke en omgevingsfactoren onderzocht in de her-analyse van patiënt-controle onderzoeken (*hoofdstuk 5.4*). De leeftijd van de moeder bij de geboorte van de patiënt, ongelukken met het hoofd, depressie en hypothyroïdie waren geassocieerd met de ziekte van Alzheimer bij zowel patiënten met als zonder een positieve familie-anamnese voor dementie in eerste graads familieleden. Een positieve familiegeschiedenis voor dementie was geassocieerd met de ziekte van Alzheimer onafhankelijk van deze risicofactoren. Deze bevindingen suggereren dat erfelijke en omgevingsfactoren afzonderlijk het risico op de ziekte van Alzheimer kunnen beïnvloeden. Het risico op de ziekte van Alzheimer voor personen met een positieve familiegeschiedenis voor het syndroom van Down en voor de ziekte van Parkinson was hoger voor personen die tevens een positieve familiegeschiedenis voor dementie hadden. Hoewel in beide gevallen geen significant verschil tussen personen met en zonder een positieve familie-anamnese voor dementie kon worden aangetoond, suggereren deze bevindingen een gemeenschappelijke, wellicht erfelijk bepaalde, etiologie. Er waren aanwijzingen dat roken specifiek een rol speelt in een erfelijk bepaald proces. De her-analyse van patiënt-controle onderzoeken liet zien dat het risico op de ziekte van Alzheimer voor personen met een positieve familie-anamnese voor dementie lager was voor rokers dan voor niet rokers. Dit effect was het sterkst bij personen met twee of meer eerste graads verwanten met dementie. In de Nederlandse families was het begin

van de ziekte later bij patiënten die in het verleden hadden gerookt dan bij patiënten die nooit hadden gerookt (*hoofdstuk 5.3*).

De implicaties van de onderzoeken beschreven in dit proefschrift werden besproken in *hoofdstuk 6*. Hierbij werd ingegaan op methodologische aspecten (*hoofdstuk 6.2*) en de rol van erfelijke en omgevingsfactoren (*hoofdstuk 6.3*). Geconcludeerd werd dat gezien de kwaliteit van de tot nu toe uitgevoerde patiënt-controle onderzoeken, de risicofactoren bevestigd dienen te worden bij incidente patiënten, bij voorkeur in een prospectief opgezet onderzoek. Onderzoek naar interactie tussen erfelijke en omgevingsfactoren wordt vooralsnog beperkt door het ontbreken van biologische genetische merkers die geschikt zijn voor bevolkingsonderzoek.

Nawoord

Dit proefschrift is het resultaat van een proces waaraan een groot aantal mensen een bijdrage heeft geleverd. In dit slotwoord wil ik een ieder die heeft bijgedragen aan de totstandkoming van dit werk bedanken. Een aantal personen ben ik in het bijzonder erkentelijk. Dit is in de eerste plaats mijn promotor Prof. Dr A. Hofman. Zijn nooit aflatend optimisme en enthousiasme hebben mij zeer gestimuleerd en ik ben hem met name dankbaar voor de *onbegrensde* mogelijkheden die hij mij geboden heeft. Prof. Dr M.F. Niermeijer, mijn tweede promotor, ben ik zeer erkentelijk voor zijn bijdrage aan het proefschrift. Zijn constructieve commentaar was voor mij bijzonder waardevol. Prof. Dr H.A. Valkenburg dank ik voor de steun en stimulans die hij mij in de begin periode op de afdeling Epidemiologie gegeven heeft. Dr C. Van Broeckhoven was nauw betrokken bij de opzet en de uitvoering van het familie-onderzoek en toonde mij de verschillende aspecten van het genetisch onderzoek. Hiervoor ben ik haar bijzonder dankbaar.

I am greatly indebted to Dr L.A. Farrer for his contribution to the genetic studies. It was a great pleasure to learn from and work with him. I thank Dr J.A. Hardy, who instigated my interest in genetics. I owe very much to those directly involved in the EURODEM re-analysis of case-control studies: Dr M.M.B. Breteler, Dr V. Chandra, D.G. Clayton, Dr L. Fratiglioni, Dr A.B. Graves, Dr A. Heyman, Dr A. Hofman, Dr A.F. Jorm, Dr E. Kokmen, Dr K. Kondo, Dr J.A. Mortimer, Dr W.A. Rocca, Dr S.L. Shalat, Dr H. Soininen, Dr T. Stijnen. My gratitude is due to all of them.

Veel dank ben ik verschuldigd aan de leden van de werkgroep EPIDEM: Dr R. Haaxma, Dr A.J. Lameris, Drs V. Otten, Dr R.J. Saan, Dr W. Schulte, Dr T.A. Tanja. Zij hebben een essentiële bijdrage geleverd aan verschillende onderdelen van het onderzoek. Ir W. Hop ben ik zeer dankbaar voor zijn adviezen op methodologisch en statistisch gebied. Dr M.E.T.I. Boerrigter, Dr F. van Harskamp, Dr L. Hendriks, Dr L. Nagelkerken, Dr E. Nijhuis, en Dr J. Vijg wil ik bedanken voor de plezierige samenwerking.

Het onderzoek kon mede worden uitgevoerd door de grote inzet van verschillende medewerkers van de afdeling Epidemiologie en Biostatistiek. In de opeenvolgende perioden waren Helen de Bruijn en Caroline Valkenburg, Hanneke van Meurs,

Micheline de Haes en Jeanette Kamman bij het onderzoek betrokken. Allen hebben op voortreffelijke wijze zorggedragen voor het slagen van de verschillende deelprojecten. Ook Els de Buck, Iteke Herweijer en Irene van Koppen hebben als stagiaires een belangrijke bijdrage geleverd aan het onderzoek. Van veel waarde was ook de ondersteuning door Peter Boerlage, die zorgde voor een uitstekende infrastructuur. Voor wat betreft de meta-analyse waren de inspanningen van Marcel Eijgermans, die alle wettelijke normen voor kantoortijden overschreden, zeer belangrijk. Ik wil hem bedanken voor de plezierige samenwerking. Jeanette Kamman ben ik zeer dankbaar voor de extra ondersteuning gedurende deze periode. Zonder haar zou een groot deel van het onderzoek niet afgerond zijn. Monique Breteler, Jan-Willem Coebergh, Cuno Uiterwaal en Jacqueline Witteman waren dierbare collegae en kamergenoten, die altijd bereid waren wetenschappelijke en niet-wetenschappelijke beslommeringen te delen. Ook alle niet met name genoemde medewerkers van de afdeling Epidemiologie en Biostatistiek wil ik hierbij bedanken voor hun steun.

Veel waardering heb ik voor de deelnemers aan het onderzoek en hun familieleden. Ik wil hen bedanken voor hun vertrouwen en bereidwillige medewerking. Mijn dank geldt ook alle artsen die door de directe zorg voor de patiënten met de ziekte van Alzheimer betrokken waren bij het onderzoek. Zonder hun steun was dit onderzoek onmogelijk geweest.

Frans Huisman wil ik bedanken voor zijn artistieke inbreng in het proefschrift.

Rest mij, in de laatste maar daarom niet minder belangrijke plaats, Henk te bedanken.

Curriculum vitae

Cornelia van Duijn was born on April 11, 1962 in Katwijk aan Zee, The Netherlands. She passed secondary school in 1980 at the "Pieter Groen College" in Katwijk. She studied at the Agricultural University of Wageningen and obtained her doctoral degree in human nutrition in 1987. In the same year, she started her training in epidemiology at the Department of Epidemiology and Biostatistics of the Erasmus University Rotterdam (Head: Prof. Dr A. Hofman). She was involved in a study of risk factors for childhood leukemia (Auspices: Dutch Childhood Leukaemia Study Group). From 1987 to 1990, she conducted a genetic-epidemiologic study of Alzheimer's disease in collaboration with the Department of Clinical Genetics of the Erasmus University Rotterdam (Prof. Dr M.F. Niermeijer). In 1990, she was one of the coordinators of a meta-analysis of case-control studies of Alzheimer's disease and was co-editor of the proceedings on the collaborative work, which were published as a supplement to the International Journal of Epidemiology (Auspices: EC Concerted Action on the epidemiology of dementia, the National Institute on Aging of the US National Institutes of Health, and the World Health Organisation Program on Aging). In 1991 she became a member of the Amyloid Precursor Protein (APP) consortium.

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