Key messages

- Bronchial secretions aspirated during fibreoptic bronchoscopy have been thought to have little diagnostic value and are often discarded
- In this study an accuracy of 88·4% was achieved in typing lung tumours by examining bronchial secretions
- Repeated examinations for confirmation or to resolve apparent discrepancies led to confirmation of previous findings in two thirds of cases and to the recognition of malignancy in a third of cases
- Simple aspiration of bronchial secretions is of value in diagnosing and typing lung tumours

intensive chemotherapy and sometimes radiation therapy, and non-small cell carcinomas, which are better treated surgically.[15]

As far back as 1952 Foot had shown that typing could be correctly obtained by studying sputum in 89·9%, 78·4%, and 83·5% of squamous cell carcinomas, adenocarcinomas, and small cell carcinomas respectively.[16] In 1972 Lange and Hoeg showed comparable results.[17] In 1985 Truong *et al* showed that typing of squamous cell and small cell carcinomas could be correctly obtained in more than 80% of cases regardless of the cytological method used (sputum, washings, or brushings).[18] In a recent series of 100 cases with confirmation at necropsy, the diagnostic yield of examining bronchial aspirates reached 80%.[5] The present series shows that exact concordance can be achieved in 88·4% of cases.

A correct, if not definitive, diagnosis can be obtained in almost all cases of centrally located lung tumours by examining bronchial secretions. Moreover, when a definitive histopathological diagnosis cannot be obtained because of risk of haemorrhagia, severe respiratory failure, or inconclusive sampling diagnostic procedures such as cytological examinations of bronchial aspirates become necessary.

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Apolipoprotein E genotype and association between smoking and early onset Alzheimer's disease

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Abstract

***Objective*—To investigate the hypothesis that differential survival between smokers and non-smokers leading to a decrease in the frequency of the e4 allele of the apolipoprotein E gene may explain the inverse relation between smoking history and early onset Alzheimer's disease.**

***Design*—A population based case-control study.**

***Setting*—The four northern provinces of the Netherlands and metropolitan Rotterdam.**

***Subjects*—175 patients with early onset Alzheimer's disease and two independent control groups of 159 and 457 subjects.**

***Main outcome measures*—Frequencies of the apolipoprotein e4 allele and relative risk of early onset Alzheimer's disease.**

***Results*—The inverse association between smoking history and early onset Alzheimer's disease could not be explained by a decrease in the frequency of the apolipoprotein e4 allele. Among carriers of this allele with a family history of dementia subjects with a history of smoking had a strongly reduced risk of early onset Alzheimer's disease (odds ratio 0·10 (95% confidence interval 0·01 to 0·87)).**

***Conclusions*—The results suggest that the inverse relation between smoking history and early onset Alzheimer's disease cannot be explained by an increased mortality in carriers of the apolipoprotein e4 allele who smoke. The association is strongly modified by the presence of the apolipoprotein e4 allele as well as by a family history of dementia.**

Introduction

In several case-control studies an inverse association between smoking and Alzheimer's disease has been observed.[1-3] This observation has led to debate about whether smoking exerts a neuroprotective effect or whether the relation can be explained by bias related to reduced survival among smokers.[1-8] Riggs has argued that differential survival between smokers and non-smokers leading to a shift in gene pools may underlie the inverse relation between smoking and Alzheimer's disease.[4] This hypothesis assumes that among smokers mortality is higher in those who are susceptible to Alzheimer's disease than in those who are not and therefore predicts a decreased frequency of a susceptibility gene among smokers.

Apolipoprotein E is a polymorphic protein that plays

a central part in the metabolism of cholesterol and triglycerides.[9] The e4 allele of the apolipoprotein E gene has been associated with an increased risk of late onset[10 11] and early onset[12 13] Alzheimer's disease. It has been suggested that the frequency of the apolipoprotein e4 allele may be decreased among smokers owing to an increased risk of early death from cardiovascular disease and that this may explain the inverse association between smoking and Alzheimer's disease.[5]

We studied the role of the apolipoprotein e4 allele in the association between smoking history and early onset Alzheimer's disease. Firstly, we tested whether a low frequency of the apolipoprotein e4 allele among smokers can explain the relation. Secondly, we examined whether the presence of the apolipoprotein e4 allele modifies the association between smoking history and early onset Alzheimer's disease.

Methods

STUDY POPULATION

Patients were derived from a population based case-control study of early onset Alzheimer's disease.[1 14] That study aimed at a complete ascertainment in four northern provinces of the Netherlands and in metropolitan Rotterdam of all patients with Alzheimer's disease in whom onset was at or before the age of 65 years. For this study, the clinical diagnosis of Alzheimer's disease was independently confirmed with a standardised protocol according to the criteria for Alzheimer's disease set by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.[15] Of the 201 eligible patients, 198 (99%) participated in the study. Blood samples for typing of apolipoprotein E were available for 175 (88%) patients. Table I shows the general characteristics of the patients.

In the original case-control study a control subject was selected for each patient and matched for sex, age (within five year age groups), and place of residence.[1 14] These original controls were drawn randomly from the population register of the municipality of the patient at the time of diagnosis; consent was given by the first person to be asked in 103 cases, the second person in 68, the third person in 23, and the fourth person in four. All controls had a score on the modified short portable mental status questionnaire that was higher than 20 (out of 30) and none showed symptoms of dementia.[14] Blood samples for apolipoprotein E typing were available for 159 (80%) controls from the case-control study (table I). In 32 of the 159 matched case-control pairs patients and controls were of the same age; in 23 the patient was one year older than the control, and in 33 pairs the patient was two to three years older; in 29 the control was one year older than the patient, and in 42 the control was two to three years older.

To examine whether the frequency of the apolipoprotein e4 allele was decreased among subjects with a history of smoking we added a second, independently ascertained control group to the study population to increase the statistical power. The second control group (n=457) consisted of a random sample of subjects aged 55 to 70 years from the general population. These additional controls were taken from another population based study conducted in Rotterdam, the Rotterdam Study.[16] The overall rate of participation in this study was 80%. These controls were screened for dementia with the mini-mental state examination[17]; none of them had a score lower than 26 (out of 30) or showed symptoms of dementia at the time of the study. The distribution of sex, age, and family history of dementia was similar to that of the original control group (table I).

DATA COLLECTION

For all cases and controls detailed data on family history of dementia, medical history, and history of cigarette smoking were collected.[1 14 16] For participants of the original case-control study, data for cases and controls were collected symmetrically. A next of kin was interviewed for patients and controls[1 14]; for 154 (88%) patients and 151 (95%) controls the next of kin was the spouse or an adult child. The data for the additional controls were obtained by interviewing the participants themselves.[16] In the original case-control study, questions addressed the patient's medical history and history of smoking before the age of onset of dementia.[1] Age of onset was defined as the age at which memory failure or changes in behaviour were first noted. To make valid case-control comparisons a "reference" age was defined for controls on the basis of the age of onset of Alzheimer's disease in the matched case.

For smokers the number of cigarettes smoked daily was assessed for each period they had smoked.[1 14 16] To measure the life time exposure to cigarettes the number of cigarettes smoked a day (in number of packs) was multiplied by the duration (in years) of cigarette smoking. Subjects were considered to have no history of smoking if they had smoked for less than one pack year before the onset of Alzheimer's disease (patients) or before the reference age (original controls) or if they had ever smoked (additional controls). Subjects were considered to have a history of smoking if they had smoked for one pack year or more.

In the original case-control study family history of dementia was assessed in first, second, and third degree relatives. To increase the validity the family data collected were always verified by a second informant who was a sibling of the participant.[1 14] For the additional control group family history of dementia in first degree relatives was obtained direct from the participant.[16] Patients and controls with no first degree relatives with dementia were considered to have no family history, and those with at least one first degree relative with dementia were considered to have a family history.

Apolipoprotein E typing for patients and original controls has been described earlier.[18] For 100 patients genomic DNA was used for genotyping as described by Wenham *et al.*[18] For the remaining 75 patients and the 159 original controls apolipoprotein E phenotyping was performed in stored serum samples frozen at −80°C as described by Havekes *et al.*[19] For

TABLE I—*Description of study population*

	Patients			Original controls			Additional controls		
	Total	Men	Women	Total	Men	Women	Total	Men	Women
No (%) of subjects	175 (100)	59 (34)	116 (66)	159 (100)	64 (40)	95 (60)	457 (100)	196 (43)	261 (57)
Mean (SD) age (years):									
At onset	57 (5·0)	56 (5·5)	57 (4·6)	NA	NA	NA	NA	NA	NA
At diagnosis	61 (4·2)	61 (4·3)	63 (4·2)	NA	NA	NA	NA	NA	NA
At time of study	63 (4·4)	63 (4·7)	63 (4·1)	63 (4·4)	63 (5·0)	63 (3·8)	63 (4·3)	63 (4·1)	63 (4·4)
No (%) with history of dementia in first degree relatives	107 (61)	35 (59)	72 (62)	44 (28)	18 (28)	26 (27)	118 (26)	46 (23)	72 (28)

TABLE II—*Frequency of apolipoprotein e4 allele by history of cigarette smoking in subjects with early onset Alzheimer's disease and controls. Values are percentages (proportion of total number of alleles) unless stated otherwise*

	Frequency of e4 allele		Difference (95% confidence interval)
	In subjects with no history of cigarette smoking	In subjects with history of cigarette smoking	
Patients:			
Total	38 (67/178)	31 (54/172)	−7 (−17 to 3)
No family history of dementia	27 (17/64)	24 (17/72)	−3 (−18 to 12)
Family history of dementia	44 (50/114)	37 (37/100)	−7 (−20 to 6)
Original controls:			
Total	10 (13/128)	18 (35/190	8 (1 to 16)
No family history of dementia	12 (12/98)	15 (20/132)	3 (−6 to 12)
Family history of dementia	3 (1/30)	26 (15/58)	23 (10 to 36)
Additional controls:			
Total	12 (30/260)	19 (123/654)	7 (2 to 12)
No family history of dementia	13 (23/182)	16 (78/496)	3 (−3 to 9)
Family history of dementia	9 (7/78)	28 (45/158)	19 (10 to 29)

TABLE III—*Association between early onset Alzheimer's disease and history of cigarette smoking by apolipoprotein E genotype*

Apolipoprotein e4 allele	Patients	Controls	Odds ratio (95% confidence interval) Crude†	Adjusted‡
All subjects:				
Allele present:				
No history of cigarette smoking	47	12		
History of cigarette smoking	45	31	0·37 (0·17 to 0·81)	0·25 (0·07 to 0·87)§
Allele absent:				
No history of cigarette smoking	42	52		
History of cigarette smoking	41	64	0·79 (0·45 to 1·40)	0·70 (0·30 to 1·66)§
Subjects with family history of dementia:				
Allele present:				
No history of cigarette smoking	35	1		
History of cigarette smoking	30	13	0·07 (0·01 to 0·53)	0·10 (0·01 to 0·87)
Allele absent:				
No history of cigarette smoking	22	14		
History of cigarette smoking	20	16	0·79 (0·31 to 2·03)	0·62 (0·19 to 2·07)
Subjects without family history of dementia:				
Allele present:				
No history of cigarette smoking	12	11		
History of cigarette smoking	15	18	0·76 (0·26 to 2·22)	0·47 (0·10 to 2·28)
Allele absent:				
No history of cigarette smoking	20	38		
History of cigarette smoking	21	48	0·83 (0·39 to 1·75)	0·77 (0·17 to 3·42)

*Reference group is those with no history of cigarette smoking.
†Unmatched analysis.
‡Adjusted for sex, age, and place of residence.
§Also adjusted for family history of dementia.

the 457 additional controls genomic DNA was used for genotyping as described by Wenham *et al.*[18]

STATISTICAL ANALYSIS

The frequency of alleles for patients and controls was assessed by counting alleles and calculating sample proportions. The z statistic was used to compare the frequency of alleles in those with a history of smoking with the frequency of alleles in those without.[20] Given a history of smoking in about 50% of the participants, a frequency of the apolipoprotein e4 allele of 35% in patients and 15% in controls, a significance level of 5%, and a statistical power of 80%, the smallest detectable increase in the frequency of apolipoprotein e4 was twofold in cases and 2·5 fold in controls.[21]

As the data for the additional control group were collected by interviewing the participants themselves, systematic bias may occur when comparisons are made with the patients for whom the data were collected by interviewing a next of kin. Case-control comparisons were therefore limited to the 175 patients and 159 controls of the original case-control study. The strength of association between a history of smoking and Alzheimer's disease was estimated as the odds ratio, which is presented with a 95% confidence interval.[21] We used conditional logistic regression analysis to take into account the possible confounding by the matching variables.[21] Given the matching scheme, a history of smoking in about 50% in controls, a significance level of 5%, and a statistical power of 80%, the smallest detectable decrease in risk of developing Alzheimer's disease was twofold (corresponding odds ratio 0·50) when studying 118 matched pairs, fourfold (0·25) when studying 36 pairs, and 10-fold (0·10) when studying 18 pairs.[21]

We found previously that significant evidence ($P<0·02$) existed for interaction between a history of smoking and family history of dementia[1] and therefore analyses were conducted stratified for family history. In the diagnosis of early onset Alzheimer's disease, the original Hachinski score (cut off 7) was used to exclude patients with vascular dementia.[14,22] This scale preferentially excludes patients with evidence of atherosclerotic cardiovascular disease. As atherosclerotic cardiovascular disease is associated with smoking, this may have resulted in artificially lower rates of smoking in patients with Alzheimer's disease. To control for this possible bias we performed an 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 II shows the frequencies of the apolipoprotein e4 allele by history of cigarette smoking. In patients the frequency tended to be higher among non-smokers, in particular among those with a family history of dementia. Although the effect was strongest among patients with a family history who had smoked for 11 pack years or more (difference −13% (95% confidence interval −34% to 8%)), a significant difference in the frequency of the apolipoprotein e4 allele was not found in any of the analyses. In the original as well as in the additional control group the frequency of the apolipoprotein e4 allele was significantly higher among those who had smoked than among those who had never smoked. In both control groups this difference in frequency could be attributed largely to those with a family history of dementia. Again, the effect was strongest among those who had smoked longer. The frequency of apolipoprotein e4 allele was 27% (9% to 46%) higher in controls with a family history of dementia who had smoked 11 pack years or more.

Table III shows that evidence exists for modification of the effect of the relation between history of smoking and early onset Alzheimer's disease by apolipoprotein E status. Among carriers of the apolipoprotein e4 allele the risk of early onset Alzheimer's disease for subjects with a history of smoking was 0·25 (0·07 to 0·87) times that for those without a history of smoking. No significant association between history of smoking and the risk of early onset Alzheimer's disease was found in those who did not carry an apolipoprotein e4 allele. Further stratification by family history of dementia showed that a significant association between history of smoking and the risk of early onset Alzheimer's disease could be shown only in those carriers of the apolipoprotein e4 allele with a family history of dementia (odds ratio 0·10; 0·01 to 0·87).

When we excluded all patients and controls with a history of atherosclerotic cardiovascular disease, the estimates for the odds ratios did not change appre-

ciably. Among carriers of the apolipoprotein e4 allele, the risk of early onset Alzheimer's disease for subjects with a history of smoking was 0·25 (0·06 to 1·11) times that for those without a history of smoking. For carriers of the apolipoprotein e4 allele the odds ratio for history of smoking was 0·09 (0·01 to 0·96) for those with a family history of dementia and 1·06 (0·18 to 6·11) for those without a family history. No significant association between history of smoking and early onset Alzheimer's disease was found for subjects with no apolipoprotein e4 allele (odds ratio 0·66; 0·23 to 1·91).

Among carriers of the apolipoprotein e4 allele the risk of Alzheimer's disease decreased as the number of pack years of smoking increased. The risk of early onset Alzheimer's disease was 0·45 (0·09 to 2·15) times lower in those who had smoked 1-10 pack years and 0·12 (003 to 0·54) times lower in those that had smoked $\geqslant 11$ pack years than in those who had never smoked. The number of subjects in our study was too small to stratify further by family history of dementia.

Discussion

BIAS

We have considered various types of bias that may explain the inverse association between history of smoking and Alzheimer's disease. Although non-response may have been associated with history of smoking or relatives of patients with Alzheimer's disease may have underreported smoking habits, we consider these types of bias unlikely. To explain our findings this would have had to occur only in those with the apolipoprotein e4 allele and a family history of dementia, which is improbable.

It has been suggested that the frequency of the apolipoprotein e4 allele may be decreased among smokers owing to an increased risk of early death from cardiovascular disease and that this may bias the association with Alzheimer's disease.[5] We found in two independently ascertained control populations that subjects with a history of smoking had a higher frequency of the apolipoprotein e4 allele than non-smokers. These findings contradict the hypothesis that a low frequency of the apolipoprotein e4 allele among smokers may explain the association between smoking history and Alzheimer's disease in our population. Further, the stratified analysis by atherosclerotic cardiovascular disease suggests that selection bias related to these disorders is unlikely to explain the association between history of smoking and early onset Alzheimer's disease.

At present we cannot exclude the possibility that the frequency of another, still unknown, susceptibility allele for early onset Alzheimer's disease that is in disequilibrium with the e4 allele may be decreased among smokers in the case series that we have studied. To explain the association between smoking and Alzheimer's disease, however, this locus should be tightly linked with the apolipoprotein E gene.

INTERPRETATION OF FINDINGS

In most case-control studies an inverse association between smoking and Alzheimer's disease has been observed.[1-3] Our study shows that the inverse relation between history of smoking and early onset Alzheimer's disease cannot be explained by an increased mortality of carriers of the apolipoprotein e4 allele among smokers. In addition, our study shows a significant inverse relation only in subjects with a family history of dementia who carry the apolipoprotein e4 allele. Although odds ratios were decreased in other subgroups, the more modest decrease suggests that the association is modified by the presence of the apolipoprotein e4 allele as well as by family history of dementia.

A protective effect of smoking in carriers of the apolipoprotein e4 allele would predict a decreased frequency of this allele among patients with a history of smoking. Although our data were compatible with this hypothesis, in particular in those with a family history of dementia, no significant difference was found in the frequency of the allele between patients with and without a history of smoking. However, the lower frequency of the allele in controls with a family history of dementia but no history of smoking is of interest. Subjects with a family history of dementia are at increased risk of carrying the susceptibility allele for Alzheimer's disease. As expected, the frequency of the apolipoprotein e4 allele is high among those with a history of smoking. In contrast, the frequency is low among control subjects with a family history of dementia who have never smoked. It is unlikely that this finding is related to survival bias, as these subjects are not exposed to cigarette smoke. Findings were very consistent in both control groups. It may be speculated, therefore, that this low frequency might be explained by a higher risk of early onset Alzheimer's disease among non-smokers with the apolipoprotein e4 allele—that is, these subjects are selected out of the control series as they are more likely to be affected with Alzheimer's disease.

MECHANISM FOR ASSOCIATION

Various possible mechanisms for the association between history of smoking and early onset Alzheimer's disease have been suggested, including nicotinic receptor density[1,2] and apoptosis.[6] The interaction with the apolipoprotein e4 allele that we found may shed new light on the association. Some evidence exists that patients with the apolipoprotein e4 allele have a lower nicotinic receptor density than other patients.[23] Nicotine has been reported as increasing the density of nicotinic receptors in the brain and may thus be implicated in the course of disease in carriers of the apolipoprotein e4 allele.[24] Another possible mechanism relates to the binding of apolipoprotein E to β amyloid, which is an oxygen mediated process.[25] In this light one of the early explanations for the inverse relation observed between Parkinson's disease and history of smoking—the increased carbon monoxide concentrations in smokers, leading to anoxia in the brain[26]—may be relevant.

The finding that the interaction with the apolipoprotein e4 allele was influenced by family history of dementia remains to be explained. Earlier, we found that among carriers of the apolipoprotein e4 allele homozygosity was sufficient by itself to increase the risk of early onset Alzheimer's disease but that in the case of heterozygosity an association with the disease could be shown only in those with a family history.[13] This suggests that other genetic or environmental factors underlying the familial aggregation may have a crucial additive role in the aetiology of early onset Alzheimer's disease. Smoking may be implicated in such a multifactorial causation.

IMPLICATIONS

A word of caution is needed in relation to the implications of our findings. Firstly, although our findings do not support the existence of bias, we cannot exclude this possibility. Secondly, we do not know whether our findings, based on a series of patients with early onset Alzheimer's disease, are applicable to patients with late onset Alzheimer's disease; the latter constitute the vast majority of patients with Alzheimer's disease. Thirdly, we recognise that smokers have an increased risk of many diseases, including cancer and cardiovascular disease. The well documented risks of cigarette smoking outweigh by far any potential benefit to individuals who might be

Key messages

- Differential survival between smokers and non-smokers with the e4 allele of the apolipoprotein E gene has been proposed to explain the inverse relation between history of cigarette smoking and Alzheimer's disease
- This study shows that the inverse association between smoking history and early onset Alzheimer's disease cannot be explained by a shift in frequency of the apolipoprotein e4 allele
- The inverse relation was significant only in subjects with a family history of dementia who carry the e4 allele
- Our study suggests that clinical trials with nicotine or nicotine derivatives have the greatest chance of success in patients with familial Alzheimer's disease who carry the apolipoprotein e4 allele

protected from Alzheimer's disease by continuing to smoke. The present study has no direct relevance, therefore, for the prevention of disease. Our findings may have implications, however, for the understanding of the pathogenesis and therapeutic strategies in Alzheimer's disease. Our study suggests that clinical trials with nicotine or nicotine derivatives have the greatest chance of success in patients with Alzheimer's disease who have a family history of dementia and carry the apolipoprotein e4 allele.

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Trends in rates and seasonal distribution of sudden infant deaths in England and Wales, 1988-92

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In the United Kingdom around half of all deaths between 1 month and 1 year of age are sudden—that is, cot death, the sudden infant death syndrome, or a similar description is recorded on the death certificate with or without any other cause. Epidemiological features suggest that infections, sleeping prone, exposure to cigarette smoke, and overheating of infants, particularly in the winter, may be associated with sudden infant deaths.[1,2] A campaign launched in October 1991 in the United Kingdom encouraged parents to avoid putting infants to sleep on their fronts, smoking near them, and overheating them. A similar campaign in New Zealand was followed by a fall in sudden infant death rates, and a noticeable decrease in the winter peak.[3] We studied the trends in rates and the seasonal distribution of sudden infant deaths in England and Wales, 1988-92.

Methods and results

We used published statistics.[4] They showed that sudden infant death rates rose more or less continuously from 1971 to a peak of 2·30 deaths per 1000 live births in 1988. Rates then fell steadily to 1·44 in 1991 and abruptly to 0·70 in 1992.

The seasonal distribution is shown in the figure. Linear regression of each quarter's rates for 1988-91 showed that all quarters except the second (April-June) had a significant negative slope (b=-0·13, 95% confidence interval -0·38 to 0·12). For deaths in July-September b=-0·19 (-0·04 to -0·35), particularly