Familial clustering and genetic risk for dementia in a genetically isolated Dutch population


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Summary
Despite advances in elucidating the genetic epidemiology of Alzheimer’s disease and frontotemporal dementia, the aetiology for most patients with dementia remains unclear. We examined the genetic epidemiology of dementia in a recent genetically isolated Dutch population founded around 1750. The series of 191 patients ascertained comprised 122 probable Alzheimer’s disease patients with late onset and 17 with early onset, and 22 with possible Alzheimer’s disease. It further included 10 patients with vascular dementia, nine with Lewy body dementia and six with frontotemporal dementia. All patients, except those with vascular dementia, were more closely related than healthy individuals from the same area. Clustering was strongest for patients with early-onset Alzheimer’s disease or Lewy body dementia. Although 14% of late-onset Alzheimer’s disease patients had evidence of autosomal dominant disease, consanguinity was found in three late-onset Alzheimer’s disease patients, suggesting a recessive or polygenic model underlying the trait. We found no clustering of vascular dementia, implying a difference in genetic risk for late-onset Alzheimer’s disease and vascular dementia. Mutations in known genes could not explain the occurrence of dementia, but the population attributable proportion of apolipoprotein E gene (APOE*4) was high (45%) due to a high frequency of APOE*4 carriers. Earlier identified regions on chromosomes 10 and 12, nor the effect of the alpha-2-macroglobulin (A2M) I/D polymorphism on Alzheimer’s disease could be confirmed in our study. We did find evidence for association between the A2M D-allele and Lewy body dementia. Our data showed a strong familial clustering of various forms of dementia in this isolated Dutch population. A high percentage of late-onset Alzheimer’s disease could be explained by APOE*4, but 55% of its origin is still unknown.

Keywords: dementia; familial aggregation; genetically isolated population

Abbreviations: Aβ = amyloid beta protein; A2M = alpha-2-macroglobulin; A2P = amyloid precursor protein; APOE = apolipoprotein E; EOAD = early-onset Alzheimer’s disease; FTD = frontotemporal dementia; I/D = insertion/deletion; IDE = insulin-degrading enzyme; LBD = Lewy body dementia; LOAD = late-onset Alzheimer’s disease; K = kinship coefficient; LPR = low-density lipoprotein receptor-related protein; MAPT = microtubule-associated protein tau; PSEN = presenilin; STR = short tandem repeat; VaD = vascular dementia.
Introduction

Dementia is known to aggregate in families (van Duijn et al., 1991; Rao et al., 1994). First-degree relatives of patients with Alzheimer’s disease have a 3.5× increased risk of developing Alzheimer’s disease. The risk of disease for relatives decreases with increasing age at onset of disease of the proband. Despite substantial evidence for familial aggregation, the mode of inheritance is not clear for late-onset Alzheimer’s disease (LOAD) (Rao et al., 1994). Although the evidence is strongest for autosomal dominant and multifactorial segregation, there is recent evidence for recessive inheritance (Farrer et al., 2003). For other dementia subtypes, findings on familial clustering are less consistent. Several families have been reported in which frontotemporal dementia (FTD) or Lewy body dementia (LBD) segregated as an autosomal dominant trait (Ohara et al., 1999; Morris et al., 2001; Galvin et al., 2002; Tsuang et al., 2002). Little is known about the familial aggregation of vascular dementia (VaD), the most common form of dementia following Alzheimer’s disease.

Various genes are involved in the familial forms of dementia. Three major genes were identified which are involved in early-onset Alzheimer’s disease (EOAD), i.e. amyloid precursor protein (APP) gene (Goate et al., 1991), presenilin 1 (PSEN-1) (Mullan et al., 1992; Schellenberg et al., 1992; St George-Hyslop et al., 1992; Van Broeckhoven et al., 1992; Sherrington et al., 1995) or presenilin 2 (PSEN-2) (Levy-Lahad et al., 1995; Rogaev et al., 1995). Although these genes may be important in some families with early onset of the disease, mutations are rare in the general population and explain <0.1% of all Alzheimer’s disease patients (Tol et al., 1999). For sporadic late-onset Alzheimer’s disease, the most common genetic risk factor is the E4 allele of the apolipoprotein E gene (APOE*4) (Strittmatter et al., 1993), which explains up to 20% of all patients. Individuals homozygous for APOE*4 are estimated to have a 6–15 times increased risk for Alzheimer’s disease, while carrying one allele is associated with a 1.5-fold increased risk (Farrer et al., 1997; Tol et al., 1999).

Numerous other loci were suggested to be associated with late-onset Alzheimer’s disease, but the associations found were often not replicated in other studies. However, there is consistent evidence for Alzheimer’s disease loci on chromosome 10, including the insulin-degrading enzyme (IDE) gene and α-T catenin (Bertram et al., 2000; Kehoe et al., 1999; Ertekin-Taner et al., 2000, 2003; Myers et al., 2000; Prince et al., 2003) and on chromosome 12, including alpha-2 macroglobulin (A2M) (Pericak-Vance et al., 1997; Rogaeva et al., 1998; Wu et al., 1998). Various genes are involved in other forms of dementia. Among those is the microtubule-associated protein tau (MAPT) gene, in which mutations are predominantly involved in frontotemporal dementia (Morris et al., 2001).

Here, we aimed to study familial aggregation of dementia in a population-based study in a recent genetically isolated population in the south-west of the Netherlands. This approach offered several advantages. Not only could we use genealogical data available through municipal and church records rather than family history, we were also able to verify the diagnoses in all patients. Further, we could obtain DNA from all patients in this study. This allowed us to examine the extent to which familial aggregation could be explained by known genes involved in various forms of dementia.

Material and methods

Study population

The study is based in a genetically isolated population in the south-west of the Netherlands (featured in the Genetic Research in Isolated Populations (GRIP) research programme at the Erasmus Medical Centre, Rotterdam). The population was founded in the middle of the 18th century, when a group of ~150 people settled in the area. The descendants of these founders lived in relative isolation until the middle of the 20th century. From the middle of the 19th century, the population started to expand considerably from 700 inhabitants in 1848 to >20 000 inhabitants by the end of the 20th century.

Patients

In cooperation with local general practitioners, neurologists and nursing home physicians, we asked patients with any dementia syndrome and their relatives to participate in our study. Written informed consent was obtained. The Medical Ethical Committee of the Erasmus Medical Centre Rotterdam, The Netherlands, approved the study protocol. All ascertained patients (n = 191) were examined by one of two research physicians to re-evaluate the clinical diagnosis. Examination consisted of neurological examination and brief neuropsychological testing. A standard interview was performed with close relatives concerning presenting symptoms, disease course, medical, social and family history. The degree of dementia was classified using the Clinical Dementia Rating Scale (CDR). In addition, we reviewed all available medical records, neuropsychological test results and hard copies of CT or MRI scans to establish a diagnosis according to National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer’s Disease and Related Disorders Association (NINCDS–ADRDA) criteria (McKhann et al., 1984). Two neurologists (W.A.vG, J.C.vS) independently assessed all available information and made a clinical diagnosis. In case of discrepancy, the final diagnosis was established in a consensus meeting.

Genealogy

Genealogical data comprising name, date and place of birth of parents and grandparents were collected at a home interview with a close relative. This genealogical information was extended up to 22 generations using municipal registers and data from a large genealogy database that holds genealogical information on ~60 000 individuals from the GRIP region. Genealogical relationships between patients were expressed as the kinship coefficient (K).

Family and medical history

We obtained information on family history of dementia by means of interviews with first-degree relatives. Family history was defined...
positive if at least one first-degree relative had dementia. When at least three relatives in two generations were affected, independent of the number of first-degree relatives, we classified the disease as an autosomal dominant form of dementia.

History of cardiovascular disease was defined based on report of hypertension or treatment for hypertension, stroke or transient ischaemic attack, myocardial infarction, angina pectoris, atrial fibrillation and/or hypercholesterolaemia in heteroanamnesis or medical records. History of diabetes mellitus was defined based on report of diabetes mellitus or when patients used anti-diabetic medication, or when increased fasting glucose levels or HbA1C were mentioned in the medical records.

**Laboratory analysis**

Blood was drawn from patients, spouses and offspring, or siblings. DNA was extracted from peripheral leucocytes according to a standard protocol (Miller et al., 1988). Given the tight relations between patients in this study, we limited mutation screening in APP, PSEN-1 and PSEN-2 to a representative subset of 80 patients. Basically, for each nuclear family one of the patients was selected. Exons 16 and 17 of APP and coding exons 3 to 12 of PSEN-1 and PSEN-2 were analysed for mutations. Mutations were analysed in 283 control individuals randomly selected from the general Dutch population (Netherlands and Flanders-Belgium) by pyrosequencing. Patients diagnosed with frontotemporal dementia who had a positive family history for dementia were screened for mutations in MAPT, using protocols described previously (Rizza et al., 1999), sequencing all exons of MAPT except exon 6 and 8.

We genotyped two markers flanking APOE (D19S420, D19S902; Applied Biosystems, Foster City, CA, USA) for linkage analysis. APOE genotyping was successfully performed in 190 patients and a control set consisting of 156 spouses, spouses of siblings and spouses of patients with diabetes mellitus or Parkinson’s disease originating from the same area, following previously described protocols (Wenham et al., 1991; van Duijn et al., 1994).

For linkage studies, a sample of 73 closely related patients was genotyped for eight short tandem repeat (STR) markers on chromosome 12 (D12S99, D12S336, D12S310, D12S1617, D12S345, D12S85, D12S368 and D12S83 from the Applied Biosystems version 2 MD-10 marker set) spanning ~60 cM and covering regions that showed evidence for linkage to Alzheimer’s disease in other studies (Pericak-Vance et al., 1997; Rogaeva et al., 1998; Wu et al., 1998). On chromosome 10, seven STR markers (D10S196, D10S1652, D10S537, D10S1686, D10S185, D10S192 and D10S597) were genotyped, spanning an ~65 cM region that showed evidence for linkage to Alzheimer’s disease in previous studies (Bertram et al., 2000; Ertekin-Taner et al., 2000; Myers et al., 2000). Polymerase chain reactions (PCR) were performed according to the manufacturer. PCR products were pooled and subsequently analysed on an ABI377 or ABI3100 automated sequencer (Applied Biosystems). Based on the results obtained in the linkage analysis, patients and 120 controls were genotyped for the A2M insertion-deletion (I/D) polymorphism (Matthijs and Marynen, 1991) and three STR markers on chromosome 10 (D10S192, D10S538 and D10S1686) surrounding the location of IDE.

**Statistical analysis**

We performed general descriptive statistics using \( \chi^2 \) statistics for dichotomous variables and a general linear model for continuous variables. The markers on chromosome 10 and 12 were analysed by means of Dislamb (Terwilliger, 1995), with disease allele frequency set to 0.01. Hardy Weinberg equilibrium was tested using the GENEPOP-package (Raymond M. & Rousset F., 1995. GENEPOP version 3.1c). We calculated pairwise kinship coefficients (K) using PEDIG software (http://dga.jouy.inra.fr/sqga/diffusions.htm) based on a pedigree of the total population, consisting of 56 693 subjects. Distributions of K were compared between dementia subtypes using \( \chi^2 \) statistics. Average kinship coefficients of patients were compared with those of their cognitively healthy spouses.

**Results**

**Patients**

We ascertained 191 patients (73.6% females, 26.4% males) with dementia in the isolated Dutch population (see Fig. 1). Of those, 122 (63.9%) had clinically probable late-onset Alzheimer’s disease and 17 (8.9%) had probable Alzheimer’s disease with an onset before 65 years (EOAD). In 13 patients, the clinical picture was compatible with Alzheimer’s disease but included symptoms suggestive of Lewy body dementia (n = 7), frontotemporal dementia (n = 3) or vascular dementia (n = 3). We therefore classified those patients as having possible Alzheimer’s disease. The diagnostic group of possible Alzheimer’s disease further included nine patients for whom available clinical data were not sufficient to fulfil criteria for probable Alzheimer’s disease. Ten patients were diagnosed with probable vascular dementia (5.2%), nine patients with Lewy body dementia (4.7%), six patients with frontotemporal dementia (3.1%) and five (2.6%) patients had an unspecified type of dementia.

The general characteristics for all groups are summarized in Table 1. The patients with late-onset Alzheimer’s disease had a mean onset age of 75 years (SD 5.3), with a mean duration of 7.2 years (SD 3.9). At examination, most patients had progressed into severe stages of dementia (median
clinical dementia rating scale 3). More than half of all probable LOAD patients (64%) had at least one first-degree relative with dementia based on reported family history. The family history was compatible with that of an autosomal dominant disease in 14% of probable LOAD patients and in 39% of possible LOAD patients. The percentage of EOAD patients with at least one affected first-degree relative was 77%, while 29% of the families showed evidence for an autosomal dominant pattern of inheritance. Overall, familial aggregation of Lewy body dementia resembled that of late-onset Alzheimer’s disease, the percentage of patients with an apparent autosomal dominant form being higher for Lewy body dementia. For vascular and frontotemporal dementia, we did not identify patients with a family history suggestive of autosomal dominant disease. Of note is that only 20% of the patients with vascular dementia had a positive family history of dementia.

Diabetes mellitus was present in 19% of the LOAD patients and a history of cardiovascular disease was found in 60%. Compared with patients with late-onset Alzheimer’s disease, vascular co-morbidity was lower in patients with early-onset Alzheimer’s disease and frontotemporal dementia, but not in those with Lewy body dementia.

**Genealogy**

In Fig. 2, a simplified pedigree is shown connecting 134 dementia patients to a common ancestor within 11 generations. Only the shortest connections to a common ancestor are shown.

**Table 1 Characteristics of the patients with dementia**

<table>
<thead>
<tr>
<th></th>
<th>Sex (%)</th>
<th>Age at examination (years)</th>
<th>Age at onset (years)</th>
<th>Duration (years)</th>
<th>Family history* (%)</th>
<th>Family history* (% autosomal dominant)</th>
<th>DM² (%)</th>
<th>CVD³ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probable Alzheimer’s disease LOAD (122)</td>
<td>78.7</td>
<td>82.2 (5.3)</td>
<td>75.0 (5.3)</td>
<td>7.2 (3.9)</td>
<td>63.6</td>
<td>14.3</td>
<td>19.3</td>
<td>59.7</td>
</tr>
<tr>
<td></td>
<td>EOAD (17)</td>
<td>76.5</td>
<td>70.9 (5.8)</td>
<td>60.1 (5.0)</td>
<td>10.4 (3.9)</td>
<td>76.5</td>
<td>29.4</td>
<td>0</td>
</tr>
<tr>
<td>Possible Alzheimer’s disease LOAD (18)</td>
<td>77.8</td>
<td>83.2 (4.8)</td>
<td>77.8 (5.9)</td>
<td>5.4 (2.9)</td>
<td>77.8</td>
<td>38.9</td>
<td>16.7</td>
<td>61.1</td>
</tr>
<tr>
<td></td>
<td>EOAD (4)</td>
<td>75.0</td>
<td>67.0 (6.9)</td>
<td>61.0 (3.6)</td>
<td>6.0 (3.9)</td>
<td>75.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LBD (9)</td>
<td>33.3</td>
<td>83.8 (8.1)</td>
<td>77.1 (6.1)</td>
<td>6.7 (2.6)</td>
<td>66.7</td>
<td>25.0</td>
<td>12.5</td>
<td>62.5</td>
</tr>
<tr>
<td>VaD (10)</td>
<td>50.0</td>
<td>79.0 (5.7)</td>
<td>69.6 (6.8)</td>
<td>9.4 (5.2)</td>
<td>20.0</td>
<td>0</td>
<td>55.6</td>
<td>88.9</td>
</tr>
<tr>
<td>FTD (6)</td>
<td>83.3</td>
<td>76.2 (10.6)</td>
<td>70.3 (10.5)</td>
<td>5.8 (4.8)</td>
<td>50.0</td>
<td>0</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>Other (5)</td>
<td>40.0</td>
<td>66.0 (10.9)</td>
<td>60.2 (13.7)</td>
<td>9.0 (4.6)</td>
<td>25.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values presented are percentages or mean (SD). *Positive family history: at least one affected first degree relative; autosomal dominant: at least three affected relatives in two generations. ‡Report of diabetes mellitus type II, anti-diabetic treatment or report of increased fasting glucose or HbA1C. §Report of treatment for hypertension, stroke, transient ischaemic attack, myocardial infarction, angina pectoris, arrhythmia, hypercholesterolemia, hyperlipidaemia.
Dementia in a genetic isolate

Table 2 Proportions of pairs in each kinship coefficient (K) category shown by diagnosis

<table>
<thead>
<tr>
<th>K</th>
<th>LOAD</th>
<th>EOAD</th>
<th>LBD</th>
<th>FTD</th>
<th>VaD</th>
<th>Spouse*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 1/3</td>
<td>0.001 (7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1/3 - 1/6</td>
<td>0.02 (131)</td>
<td>0.009 (1)</td>
<td>0.047 (1)</td>
<td>0</td>
<td>0</td>
<td>0.006 (25)</td>
</tr>
<tr>
<td>1/6 - 1/9</td>
<td>0.08 (554)</td>
<td>0.14 (15)</td>
<td>0.09 (2)</td>
<td>0</td>
<td>0.03 (1)</td>
<td>0.03 (111)</td>
</tr>
<tr>
<td>1/9 - 1/12</td>
<td>0.13 (918)</td>
<td>0.34 (36)</td>
<td>0.28 (6)</td>
<td>0.20 (2)</td>
<td>0.17 (6)</td>
<td>0.02 (60)</td>
</tr>
<tr>
<td>1/12 - 1/15</td>
<td>0.23 (1646)</td>
<td>0.12 (13)</td>
<td>0</td>
<td>0.10 (1)</td>
<td>0.19 (7)</td>
<td>0.01 (55)</td>
</tr>
<tr>
<td>&lt; 1/15</td>
<td>0.28 (2016)</td>
<td>0.23 (24)</td>
<td>0.28 (6)</td>
<td>0</td>
<td>0.39 (14)</td>
<td>0.03 (121)</td>
</tr>
<tr>
<td>0</td>
<td>0.26 (1868)</td>
<td>0.15 (16)</td>
<td>0.28 (6)</td>
<td>0.70 (7)</td>
<td>0.22 (8)</td>
<td>0.90 (3544)</td>
</tr>
</tbody>
</table>

Values are proportions of pairs per diagnosis, with absolute numbers between brackets. *Spouses of cases with LOAD.

Proportions of pairs by K are shown in Table 2. If K is zero, at least one of the pair of patients descends from another population. For EOAD patients, the proportion of pairs with K = 0 was smallest (15%), differing significantly from that of late-onset Alzheimer’s disease (26%; P = 0.01). Seventy percent of the pairs of patients with frontotemporal dementia had a kinship coefficient equal to zero. However, the sample size for this group was small.

A kinship coefficient of (1/2)^9 or larger was found in 15% of the EOAD pairs, being very similar to the 14% of patients with Lewy body dementia in this category. The proportion of K > (1/2)^9 was slightly lower for LOAD pairs (10%), although the difference in distribution of K was only borderline statistically significant between LOAD and EOAD pairs (P = 0.06). Kinship for LBD pairs did not differ significantly from those with late-onset Alzheimer’s disease (P > 0.1). When comparing subgroups of patients with late-onset Alzheimer’s disease based on presence or absence of cardiovascular disease, the proportion with K > (1/2)^9 was significantly higher for LOAD patients without cardiovascular disease than for those with cardiovascular disease (14% versus 8%; P < 0.00001). Patients with vascular dementia were even more distantly related; for only 3% K was larger than (1/2)^9. This was statistically significantly different from EOAD pairs (P < 0.05). The percentage of vascular dementia patients (3%) with K > (1/2)^9 was very similar to that seen in spouses of LOAD patients (3%).

Only for patients with late-onset Alzheimer’s disease, K was found to be significantly increased compared with their cognitively healthy matched spouses (P < 0.0001). For none of the other forms of dementia, K differed significantly from the K of their controls. However, given that the K of EOAD patients (as well as the K of patients with Lewy body dementia) was higher than that of LOAD patients, this is most likely explained by the small number of subjects.

APP, PSEN-1 and PSEN-2

Since patients were closely related, we selected a sample consisting of 80 possible or probable LOAD patients as a representative group to screen for autosomal dominant mutations in APP, PSEN-1 and PSEN-2. No mutations were found in APP or PSEN-1. In three probable LOAD patients, we identified a single base change that predicted a missense mutation in PSEN-2 (G34S, R62H and R71W). The variations did not segregate with disease in the families of these three patients. We detected the R71W variation in one of 283 healthy controls (566 chromosomes) as well [relative risk 3.56; 95% confidence interval (CI): 0.22–57.72; P > 0.1]. We did not observe G34S and R62H in controls, but we did identify a R62C variation in one of the controls. Since PSEN-2 includes a substantial number of polymorphisms that do not affect functionality (Lleo et al., 2002), we tested if these missense mutations were associated with an increased secretion of 42-residue amyloid beta protein (Aβ42) proportional to 40-residue amyloid beta protein (Aβ40) (Scheuner et al., 1996; Citron et al., 1997). No evidence was obtained that the amino acid changes altered the Aβ42/Aβ40 ratio (J. Theuns, M. Couts, B. Dermaut, W. Anneant, K. Sleegers, K. Vennekens et al, unpublished data).

Chromosomes 10 and 12

With the exception of D12S336 (P = 0.03), the chromosomes 10 and 12 STR markers did not show significant association with late-onset Alzheimer’s disease. Since D12S336 is located close to A2M, we genotyped the I/D polymorphism in this gene in the entire sample. The genotype and allele proportions did not deviate significantly from Hardy Weinberg equilibrium. The frequency of the A2M genotypes did not differ significantly between patients with either probable or possible late-onset Alzheimer’s disease, and controls (Table 3) making it unlikely that A2M explained the association between D12S336 and late-onset Alzheimer’s disease. Two (22.2%) of the patients with Lewy body dementia were homozygous for the D-allele, one was heterozygous and six were homozygous for the I-allele. This distribution differed significantly from controls, although the number of patients was very small compared with the control sample (Fisher’s exact P-value = 0.009).

APOE

Linkage analysis yielded non-significant LOD scores for both flanking markers of APOE [LOD score 0.21; P = 0.16 (D19S420) and 0.35; P = 0.10 (D19S902)]. APOE genotype
frequencies were in Hardy Weinberg equilibrium proportions. The frequency of carrying at least one allele APOE*4 is shown in Fig. 3. Of the LOAD disease patients, 53.9% were heterozygous for APOE*4 and 10.5% were homozygous for APOE*4, compared with 30.1% heterozygous for APOE*4 and 2.6% homozygous in controls. Odds ratios were 3.34 (95% CI 2.14–5.36) for heterozygous and 7.72 (95% CI 2.53–23.56) for homozygous individuals. The population-attributable proportion was 45% of the 16 early-onset Alzheimer’s disease patients for whom APOE genotyping was available, eight were heterozygous and five were homozygous for the E4 allele. None of the nine patients with Lewy body dementia were homozygous and five were heterozygous for APOE*4. Of the 10 patients with vascular dementia, only one was heterozygous for APOE*4 and none were homozygous. Four of the six patients with frontotemporal dementia were heterozygous, and none were homozygous for APOE*4.

**MAPT**

We screened MAPT in six patients with frontotemporal dementia. None of the patients carried a known mutation. We found no evidence for haplotype association. In one patient, we found a rare polymorphism in the intron following exon 9 (IVS9 +40 C/T). No DNA was available from affected relatives to test segregation of the polymorphism with affection status. This polymorphism was not found in 384 Dutch control chromosomes tested (Rizzu et al., 1999). Three different splice prediction programs (NetGene2, Splice Site Prediction by Neural Networks and SpliceSiteFinder) predicted no change in splicing due to the +40 C/T base change, making it unlikely that this mutation explains the disease.

**Discussion**

In this paper, we describe the occurrence of dementia in a recent genetically isolated Dutch population. As expected, late-onset Alzheimer’s disease was the most common type of dementia (122 out of 191 patients), and its clinical picture resembled that of Alzheimer’s disease in the general Dutch population, including a high percentage of cardiovascular disease and diabetes mellitus (Hofman et al., 1997). We observed strong familial aggregation of dementia in this genetically isolated population. Clustering was strongest in patients with early-onset Alzheimer’s disease and Lewy body dementia. Although a relatively large portion of LBD patients descended from different populations, the pairs that were related were as closely related as EOAD pairs. Vascular dementia showed weakest evidence for familial clustering. Of interest, in three out of four sib pairs with late-onset Alzheimer’s disease, kinship was >0.25, indicating consanguinity in this patient group. This finding suggests a recessive mutation in a (small) subset of patients. Until now, no mutations causing autosomal recessive Alzheimer’s disease are known, but evidence for recessive inheritance of disease has also been found in an inbred Israeli–Arab community (Farrer et al., 2003).

**Table 3 Genotype distribution of A2M insertion/deletion (I/D) polymorphism in patients and controls**

<table>
<thead>
<tr>
<th></th>
<th>I/I (%)</th>
<th>I/D (%)</th>
<th>D/D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>73 (60.8%)</td>
<td>45 (37.5%)</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>Probable LOAD</td>
<td>83 (71.6%)</td>
<td>30 (25.9%)</td>
<td>3 (2.6%)</td>
</tr>
<tr>
<td>Possible LOAD</td>
<td>12 (66.7%)</td>
<td>5 (27.8%)</td>
<td>1 (5.6%)</td>
</tr>
</tbody>
</table>

Values are presented as absolute numbers, with percentages between brackets.
Overall, we observed that patients were more closely related than a group of cognitively healthy spouses from the same sampling area. One might argue that the observed difference is based on the sex difference between patients and spouses, as patients in our study were predominantly female. However, we did not find significant evidence for a difference between males and females in patients, or between males and females in controls, arguing against a bias because of sex difference.

Having access to a database holding the genealogy of almost 60,000 individuals from the genetically isolated population allowed a thorough investigation of the clustering of dementia in seemingly unrelated patients. A finding of interest was the strong familial clustering in late-onset Alzheimer’s disease. In 14% of the patients, the family history was compatible with autosomal dominant disease. A high percentage of patients with possible Alzheimer’s disease also had a family history compatible with autosomal dominant disease. Possibly, they shared a genetic risk factor conferring a distinct phenotype.

Epidemiological studies suggested that cardiovascular disease is a risk factor for late-onset Alzheimer’s disease (Hofman et al., 1997; de la Torre, 2002). Indeed, patients diagnosed with late-onset Alzheimer’s disease in this present study had a high frequency of cardiovascular disease and diabetes mellitus. This raises the question whether similar genes are involved in the risk of vascular dementia and late-onset Alzheimer’s disease. Although only a few patients had vascular dementia in our study, we found no evidence for familial clustering of vascular dementia. This suggested that genes play only a minor role in vascular dementia, and that there is a difference in genetic etiology for late-onset Alzheimer’s disease and vascular dementia. Patients with late-onset Alzheimer’s disease who did not have a history of cardiovascular disease showed a significantly higher degree of familial aggregation than those with cardiovascular disease. This might indicate a stronger genetic factor in those without cardiovascular disease. But still, patients with late-onset Alzheimer’s disease and cardiovascular disease have a higher kinship than patients with vascular dementia, indicating separate genetic etiologies.

In our analysis of genetic risk factors already known in dementia, we detected three missense mutations in PSEN-2 in three patients with probable late-onset Alzheimer’s disease, and an intronic polymorphism in MAPT in a patient with frontotemporal dementia. We cannot fully exclude the relevance of these missense mutations for Alzheimer’s disease although: (i) the sequence variations in PSEN-2 did not segregate with affection status in relatives; (ii) they did not affect the \( A\beta_{1-42} / A\beta_{1-40} \) ratio in an in vitro assay; and (iii) one of the missense mutations in PSEN-2 (R71W) was detected in a control. New missense mutations continue to be reported more often in patients than in controls, but their frequency is so low that statistical evidence for association can hardly be shown. To exclude functionality of the MAPT polymorphism, we relied on splice prediction programs since no affected relatives were available for further study of the segregation of this polymorphism with the clinical phenotype. Definitive evidence arguing against functionality should come from in vitro studies, but until now, polymorphisms located this far outside the functional loop have not been shown to affect splicing (Hutton et al., 1998).

Despite increasing evidence for a locus on chromosome 10 from other studies (Bertram et al., 2000; Ertekin-Taner et al., 2000, 2003; Myers et al., 2000; Prince et al., 2003) we found no association between markers in this chromosomal region and late-onset Alzheimer’s disease in the isolated population. Inherent to the genetic structure of an isolated population, genetic factors that do have considerable impact on disease in an outbred population might not be present or only with undetectable frequencies in a genetically isolated population. As have others, we found some evidence for association with Alzheimer’s disease on chromosome 12 (Pericak-Vance et al., 1997; Rogaeva et al., 1998; Wu et al., 1998) close to the location of A2M. However, evidence was weak and the I/D polymorphism in A2M could not explain this association, which is consistent with other studies that have been negative for A2M (Koster et al., 2000). Some studies reported a possible association between Lewy body dementia and the region on chromosome 12 and the A2M D-allele (Singleton et al., 1999; Scott et al., 2000). Correspondingly, we found evidence for excess homozygosity of the A2M D-allele in patients with Lewy body dementia. Since the deletion in A2M appears not to have biological consequences (Blennow et al., 2000), this polymorphism might be located close to a true disease locus elsewhere on chromosome 12. Another candidate gene on chromosome 12 is low-density lipoprotein receptor-related protein (LRP) (Kang et al., 1997; Sanchez-Guerra et al., 2001), but LRP is located at a distance of 50 cM from the marker suggestive of linkage on chromosome 12. Further, none of the markers close to LRP conferred evidence for linkage. We therefore excluded LRP.

The frequency of APOE*4 was slightly higher, both in LOAD cases and controls from the isolated population compared with the general Caucasian population. Although the odds ratios associated with APOE*4 were similar to those seen in Caucasian populations, the risk of Alzheimer’s disease in the isolated population attributable to APOE*4 is higher (45%) due to the high frequency of the allele (Farrer et al., 1997).

We found a high degree of relationship between patients in this isolated population, especially in early-onset Alzheimer’s disease and Lewy body dementia. More importantly, patients with late-onset Alzheimer’s disease also showed strong evidence for familial aggregation as opposed to vascular dementia. Consanguinity in several patients suggested an underlying recessive mutation. Mutations in known genes causing autosomal dominant disease were unlikely to explain dementia in this population. We found evidence for an association between Lewy body disease and A2M. The frequency of APOE*4 was high, resulting in an attributable risk of 45% for Alzheimer’s disease. This suggested that this
allele is important as a determinant and/or a modifier of disease in the isolate. However, a large portion of Alzheimer’s disease remains unexplained, demanding further research on the genes involved in this type of dementia.

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