*British Journal of Psychiatry* (1991), **158**, 471–474

# Evidence for Allelic Heterogeneity in Familial Early-Onset Alzheimer's Disease

CORNELIA M. VAN DUIJN, CHRISTINE VAN BROECKHOVEN, JOHN A. HARDY, ALISON M. GOATE, MARTIN N. ROSSOR, ANTON VANDENBERGHE, J-J. MARTIN, ALBERT HOFMAN and MICHAEL J. MULLAN

**Age of onset was examined for 139 members of 30 families affected by early-onset AD. Most (77%) of the variance of age of onset derived from differences between rather than within families. The constancy of age of onset within families was also observed in an analysis restricted to families derived from a population-based epidemiological study with complete ascertainment of early-onset AD. Furthermore, we observed clustering of age of onset within those families that support linkage to the predisposing locus on chromosome 21. Our data are compatible with the view that allelic heterogeneity at the AD locus may account for the similarity in age of onset within families. This finding may be of value for scientific studies of AD as well as for genetic counselling.**

There are over 100 reported families in which Alzheimer's disease (AD) segregates as an autosomal dominant disorder (Cook *et al*, 1979; Goudsmit *et al*, 1981; Nee *et al*, 1983; Farrer *et al*, 1990). Three studies have shown genetic linkage between early-onset AD and polymorphic DNA markers on the proximal long arm of chromosome 21 (St George-Hyslop *et al*, 1987; Goate *et al*, 1989; Van Broeckhoven *et al*, 1991). Although age of onset of AD may differ considerably between families, similar ages of onset of dementia have been reported previously within several large families with apparently autosomal dominant AD (Cook *et al*, 1979; Folstein *et al*, 1988; Huff *et al*, 1988). The origin of the family resemblance in age of onset of dementia is not yet clear. The age of onset within a family may be determined by one or more genetic loci. On the other hand, given a common genetic predisposition, environmental factors shared by relatives may be involved in the onset of AD. In addition, selection bias may account for the family-specific age of onset, 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 AD, we examined 30 families multiply affected by AD. These families were selected for molecular linkage studies or were ascertained for an epidemiological study with complete ascertainment of patients with early-onset AD. Thus the analysis was performed in pedigrees with known linkage to a genetic locus on chromosome 21 and in a population-based unbiased sample of early-onset AD families.

Although early-onset and late-onset AD are considered as one neuropathological entity, we have excluded late-onset patients because inaccuracies in diagnosis of AD in this group may seriously bias the analysis.

## Method

We studied age of onset of AD within and between 30 families derived from Belgium (Van Broeckhoven *et al*, 1991), Great Britain (Goate *et al*, 1989) and the Netherlands (Hofman *et al*, 1989) (Table 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 an autosomal dominant inheritance of AD.

The criteria for including a family in the study were: (a) at least three individuals with clinically diagnosed AD in two or more generations; (b) detailed medical records available on the clinical diagnosis of AD of at least two affected relatives (McKhann *et al*, 1984); (c) mean age of onset below 60 years in the Belgian and British families, and (d) age of onset of the proband before the age of 60 in the Dutch study. 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 epidemiological 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 age of onset of the relatives. The Dutch study comprised all patients diagnosed with early-onset AD during 1980–87 in two areas of the Netherlands (Hofman *et al*, 1989); 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 (Goate *et al*, 1989; Van Broeckhoven *et al*, 1991). Although in one British family, a cross-over between the disease locus and one of the markers

Table 1
Clinical characteristics of families multiply affected with Alzheimer's disease

| Family number | Country | Mean age of onset (range): years | No. of patients | No. of pathological conformations |
|---|---|---|---|---|
| ADA[1] | Belgium | 35 [26-45] | 38 | 11 |
| ADB[1] | Belgium | 35 [30-39] | 24 | 6 |
| 14 | Great Britain | 60 [55-65] | 3 | |
| 15[1] | Great Britain | 52 [45-70] | 3 | |
| 23[1] | Great Britain | 55 [51-62] | 14 | |
| 32[1] | Great Britain | 54 [47-59] | 6 | |
| 34 | Great Britain | 44 [37-52] | 3 | 1 |
| 53 | Great Britain | 52 [43-60] | 5 | |
| 74[1] | 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 | |

1. Families with evidence of linkage to chromosome 21 (see Goate *et al*, 1989; Van Broeckhoven *et al*, 1991).

(D21S1/S11) was inferred, there was no significant evidence for heterogeneity. Age of onset was 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 behaviour was first noted. This information was available for 139 (67%) of the 214 affected individuals.

Analysis of variance was used to compare age of onset among and within families (Smith, 1975). To see whether it changed over generations, the difference in age of onset was calculated for all possible combinations of sibships within a family. In the case of first-degree relatives, the difference in mean age of onset of a sibship and the age of onset of the affected parent is given. The comparison of second-degree relatives comprised the difference between the mean age of onset of a sibship and the mean age of onset of all second-degree relatives of this sibship (i.e. uncles, aunts and grandparents). In the same way, the differences were calculated between third-degree, fourth-degree, fifth-degree, sixth-degree, seventh-degree, and eighth-degree relatives.

## Results

The age of onset of AD was more similar within than between families. Most (77%) of the variance in age of onset was due to differences between families (Table 2). Restriction of the analysis to the pedigrees for whom we have previously reported linkage data 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 2). Unaffected siblings of these patients, however, are still at risk of AD; since ages of siblings tend to be similar, bias may occur towards a limited variation in age of onset of dementia. Upon exclusion of siblings of the probands, the intraclass correlation did not change materially (intraclass correlation 0.63, $P<0.005$).

Table 3 shows that the age of onset has remained constant in seventh-degree 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 not available, we compared ages of onset of relatives raised apart to see whether there was evidence for a modifying role of environmental factors. The average difference in age of onset between sibships of

https://doi.org/10.1192/bjp.158.4.471 Published online by Cambridge University Press

Table 2
Analysis of variance between and within families multiply affected with early-onset Alzheimer's disease

| Families | Source of variation | Degrees of freedom | Mean sum of squares | *P* value | Intraclass 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 | | |

Table 3
Difference (s.d.) in age of onset between sibships by degree of relationship

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

a family born more than 50 km apart (3.7 years, s.d. = 3.0, $n = 13$) was similar to the difference found for sibships born less than 50 km apart (4.4 years, s.d. = 3.0, $n = 54$).

## Discussion

These data show that age of onset of AD is more similar within multiply-affected families than between families. The analysis of the population-based data indicates that this finding is not due to ascertainment bias. Determination of age of onset may still be subject to error. Collecting data through interviews of relatives may have led to artefactual clustering of age of onset within families. However, it was always obtained from a family history from the next of kin of each patient, that is, there were multiple informants within a family. Since in the large pedigrees, ages of onset 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 age of onset. Moreover, an analysis of age of death, dates of which were always checked against independent medical records, demonstrated the same effect.

In accordance with other studies of onset of dementia in relatives raised apart, our findings do not support a strong influence of environmental factors. A constant age of onset has been reported within a large Italian pedigree, although relatives lived as far apart as France, Italy and the USA (Foncin *et al*, 1986). Similarity in environmental factors and lifestyle are likely to decrease if members of a family are more distantly related. Age of onset, however, did not differ significantly with distance in relationship or place of birth in our analysis. Although environmental neurotoxins (e.g. aluminium) may play a role in the aetiology of AD, our results show that within early-onset pedigrees there is little evidence of a predominant influence on age of onset.

The data from families which support linkage to chromosome 21 suggest that the characteristic age of onset of a family may have a genetic origin. There

https://doi.org/10.1192/bjp.158.4.471 Published online by Cambridge University Press

are two possible explanations for this: (a) other genes, which are coinherited with the locus on chromosome 21, may modify the age of onset; (b) different alleles at the same genetic locus predispose to different ages of onset, that is, there is allelic-heterogeneity. The first explanation would predict that within a family recombination between the AD locus and the putative onset-determining locus may occur. Thus the variation in age of onset would 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. Although the molecular genetic analyses were consistent with linkage to chromosome 21 in all families, significant linkage data were not obtained in each family. Therefore, we cannot exclude the possibility of non-allelic genetic heterogeneity with some families. However, the specific age of onset AD observed in the analysis of families with evidence of linkage to the predisposing locus on chromosome 21 suggests that there may be different mutations at the same locus (allelic heterogeneity).

There are several implications of our findings. Firstly, it may be possible to detect those individuals who fall outside of the expected age of onset for a particular pedigree. Patients detected in this way may represent non-genetic forms of AD, probably representing the population risk. In genetic studies, it may be of great value to distinguish these patients. Studies of these 'non-genetic' patients may also reveal the role of neurotoxins (e.g. aluminium) and environmental risk factors in AD. Secondly, these findings have relevance for genetic counsellors in assessing the risk to an individual of having inherited the predisposition to the disease. Thirdly, if allelic heterogeneity does exist between pedigrees, then it will not be possible to use linkage disequilibrium to map the genetic defect.

#### Acknowledgements

This research was funded by Eurodem EC Concerted Action, the SOOM Foundation, the Netherlands, the Organisation for Scientific Research, Innogenetics, Belgium, the Belgian National Incentive Programme 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, Caroline Valkenburg and Goedele De Winter for genealogy studies.

#### References

Cook, R. H., Ward, B. E. & Austin, J. H. (1979) Studies in aging of the brain: IV. Familial Alzheimer disease: relation to transmissible dementia, aneuploidy and microtubular defects. *Neurology*, **29**, 1402–1412.

Farrer, L. A., Myers, R. H., Crupples, L. A., *et al* (1990) Transmission and age-at-onset patterns in familial Alzheimer's disease: evidence for heterogeneity. *Neurology*, **40**, 395–403.

Folstein, M. F., Warren, A. & McHugh, P. R. (1988) Heterogeneity in Alzheimer's disease: an exercise in the resolution of a phenotype. In *Genetics and Alzheimer's Disease* (eds P. M. Sinet, Y. Lamour & Y. Christen), pp. 5–12. London: Springer-Verlag.

Foncin, J-F., Salmon, D. & Bruni, A. C. (1986) Genetics of Alzheimer's disease: a large kindred with apparent Mendelian transmission; possible implications for a linkage study. In *New Concepts in Alzheimer's Disease* (eds M. Briley, A. Kato & M. Weber), pp. 242–256. London: Macmillan.

Goate, A. M., Haynes, A. R., Owens, M. J., *et al* (1989) Predisposing locus for Alzheimer's disease on chromosome 21. *Lancet*, *i*, 352–355.

Goudsmit, J., White, B. J., Weitkamp, L. R., *et al* (1981) Familial Alzheimer's disease in two kindreds of the same geographic and ethnic origin. *Journal of the Neurological Sciences*, **49**, 79–89.

Hofman, A., Schulte, W., Tanja, T. A., *et al* (1989) History of dementia and Parkinson's disease in 1st-degree relatives of patients with Alzheimer's disease. *Neurology*, **39**, 1589–1592.

Huff, F. J., Auerbach, J., Chakravarti, A., *et al* (1988) Risk of dementia in relatives of patients with Alzheimer's disease. *Neurology*, **38**, 786–790.

McKhann, G., Drachman, D., Folstein, M., *et al* (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology*, **34**, 939–944.

Nee, L. E., Polinsky, R. J., Eldridge, R., *et al* (1983) A family with histologically confirmed Alzheimer's disease. *Archives of Neurology*, **40**, 203–208.

Smith, C. (1975) Quantitative inheritance. In *Textbook of Human Genetics* (eds G. Fraser & O. Mayo), pp. 382–391. Oxford: Blackwell Scientific.

StGeorge-Hyslop, P. H., Tanzi, R. E., Polinsky, R. J., *et al* (1987) The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science*, **235**, 885–890.

Van Broeckhoven, C., Backhovens, H., Van Hul, W., *et al* (1991) Genetic linkage analysis in early onset familial Alzheimer's dementia. In *Neuropsychopharmacology Proceedings of the XVIth CINP Congress* (eds W. E. Bunney, Jr, J. Hippius, G. Laakmann, *et al*), pp. 86–91. Berlin: Springer-Verlag.

Cornelia M. van Duijn, *Department of Epidemiology and Biostatistics, Erasmus University Medical School, Rotterdam, the Netherlands*; Christine Van Broeckhoven, *Department of Biochemistry, University of Antwerp, Belgium*; John A. Hardy, *Department of Biochemistry, St Mary's Hospital Medical School, University of London*; Alison M. Goate, *Department of Biochemistry, St Mary's Hospital Medical School*; Martin N. Rossor, *Department of Neurology, St Mary's Hospital Medical School*; Anton Vandenberghe, *Department of Biochemistry, University of Antwerp*; J-J. Martin, *Department of Neurology and Neuropathology, Born-Bunge Foundation, University of Antwerp*; Albert Hofman, *Department of Epidemiology and Biostatistics, Erasmus University Medical School, The Netherlands*; *Michael J. Mullan, *Department of Biochemistry, University of Antwerp, Department of Neurology, St Mary's Hospital Medical School*

*Correspondence: *Department of Biochemistry, St Mary's Hospital Medical School, University of London, Norfolk Place, London W2 1PG*