

A Genealogical Study of Alzheimer Disease in the Saguenay Region of Quebec

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We performed an analysis of inbreeding and kinship among the ascending genealogies of 205 autopsy-confirmed Alzheimer disease (AD) subjects recruited in the Saguenay area of Québec. We hypothesized that if some traits pertaining to the disease were determined by inherited factors, and if the corresponding genes were not too frequent in the population, it might be possible to detect some clusters of patients related to common ancestors and presenting a level of kinship and/or inbreeding higher than is observed in the unaffected population of the same age. In view of the heterogeneity of the disease, we also verified if some of the factors investigated could be associated more specifically with subsets of cases based on age of onset and on apolipoprotein E (APOE) genotype. Results were compared with those obtained on 205 controls matched for gender, place and year of birth. We found that late-onset AD cases with an APOE- ϵ 4 were significantly more inbred than controls and that this increase was explained by the high level of inbreeding of a few cases whose parents were related at the first-cousin level. This could possibly indicate the implication of a recessive element in a small subset of AD cases in the Saguenay population. We also found that late-onset ϵ 4+ cases were significantly more closely related among themselves than with controls. This increase in kinship may be attributable to the

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presence of the $\epsilon 4$ allele or to some other unidentified genetic factor possibly acting in conjunction with APOE- $\epsilon 4$. *Genet. Epidemiol.* 16:412–425, 1999.

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INTRODUCTION

Investigations into the etiology of Alzheimer disease (AD) have implicated hypotheses based on the potential role of a great variety of factors including family history of dementia and related disorders [Lautenschlager et al., 1996]; exposure to toxic elements in the environment, like heavy metals [Martyn et al., 1997] and solvents [Kukull et al., 1995], or from lifestyle habits like smoking [Salib and Hillier, 1997]; components of personal medical history like head trauma [Schofield et al., 1997] and depression [Speck et al., 1995], as well as some socio-demographic characteristics like level of education and occupation [Bonaiuto et al., 1995] and parental age at birth [Whalley et al., 1995]. However, very few of these factors have offered clear evidence of an association with AD and it is conceivable that some of them could play a role among genetically predisposed individuals as has already been proposed for head trauma [Mayeux et al., 1995] and smoking [van Duijn et al., 1995]. In recent years, the field of molecular biology has greatly increased our knowledge of genetic factors involved in AD [Pericak-Vance and Haines, 1995; Morrison-Bogorad et al., 1997]. Three different genes have now been identified on chromosomes 21 [Goate et al., 1991], 14 [Sherrington et al., 1995] and 1 [Levy-Lahad et al., 1995], and mutations have been described which are responsible for the onset of the disease in approximately 50% of the families characterized by an early onset [Tanzi et al., 1996]. These genes are transmitted on a dominant mode and their action is sufficient, although not necessary, to trigger AD in a carrier subject. However, early-onset familial cases account for just around 10% of total AD cases [Van Broeckhoven, 1995].

A strong association between the allele $\epsilon 4$ of the apolipoprotein E (APOE) gene and the more common late-onset subtype of the disease has also been demonstrated [Saunders et al., 1993; Strittmatter et al., 1993]. The exact role of APOE in the etiology of AD is, however, not completely elucidated: studies tend to demonstrate that APOE genotype influences susceptibility to the disease and age of onset [Roses, 1996] but the link with the rate of progression and duration is less clear [Bennett et al., 1995; Norrman et al., 1995]. It is also likely that other factors, of genetic and/or environmental nature, are involved in the etiology of AD and remain to be identified [Jarvik et al., 1996; Tang et al., 1996]; this task, in the context of this heterogeneous and multifactorial disease, might prove to be quite a complex endeavour.

As described by Roberts [1983], one goal of genetic epidemiology is to identify the possible relation between a disease etiology, especially when it appears to be located halfway in the spectrum going from predominantly environmental to purely genetic causes, and the genetic structure and characteristics of a particular population. This approach presents a special interest in a population such as the Québec

population of French origin with a known founder effect dating around 350 years and records allowing for genealogical reconstruction.

In an attempt to contribute to a better understanding of the genetic factors involved in the etiology of AD, we performed an analysis of inbreeding and kinship among the ascending genealogies of 215 autopsy-confirmed AD subjects recruited in the Saguenay area of Québec. We hypothesized that if some traits pertaining to the disease are determined by inherited factors, and if the corresponding genes are not too frequent in the population, it might be possible to detect some clusters of patients related to common ancestors and presenting a level of kinship and/or inbreeding higher than is observed in the unaffected population of the same age. In view of the heterogeneity of the disease, we also verified if some of the factors investigated could be associated more specifically with some clinical or genetic characteristics of our AD subjects.

MATERIALS AND METHODS

Cases

The Projet IMAGE research group has been conducting a population-based study of AD in the Saguenay region since 1986 [Gauvreau et al., 1988]. An extensive field network has been set up to recruit patients and the register now contains more than 700 definite, probable, and possible cases based on the NINCDS-ADRDA criteria [McKhann et al., 1984] and on previously published morphological and morphometric criteria [Khachaturian, 1985; Tiberghien et al., 1993]. Informed consent on all research procedures, including blood collection and brain donation, was obtained from all participants or their surrogate.

We initiated our study on 221 neuropathologically-confirmed definite cases of AD deceased between 1987 et 1993. Verification of first-degree kinship indicated that our sample contained 3 pairs of sibs, 1 pair of half-sibs, and 1 group of 3 sisters. We decided to keep in our analysis only one case per sibship in order to control for any ascertainment bias and to avoid overrepresentation of some families. We randomly selected one member from each of these families, leaving us with 215 cases. We also evaluated the completeness of the genealogies of the patients; we wanted to make sure that at least 60% of the ancestors had been identified among the first seven generations. Ten more cases were thus excluded from the study. As sources used for genealogical reconstruction are complete and very precise (description below), most of the withdrawn cases were either adopted children or individuals whose parents did not originate from the French Canadian population; it is, therefore, preferable to exclude them from genetic studies.

Thus, this study was carried on a sample of 205 AD cases, which was analyzed as a whole and was subdivided into groups based on age of onset and on APOE genotype. By doing so, we aimed at improving our chances of working on groups that would be more homogeneous, based on the hypothesis that the chosen characteristics correspond to specific etiological factors.

Patients were divided according to age of onset because the genetic factors identified up until now influence the age at which carriers develop the disease [Harrington and Wischik, 1995]. Ages of onset in our sample ranged from 49 to 88 years old. Cases were classified as early if onset was before 65 and as late if onset was at 65 or

after (see Table I). We also grouped cases according to the APOE alleles they carried in their genotype: as there are three possible alleles $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$, we formed three groups that are not mutually exclusive since each person can carry two different alleles. Finally, because $\epsilon 4$ is the risk factor for AD, we also formed a group of probands lacking this allele. Our goal was to verify if inbreeding and kinship varied among the carriers of the three alleles and, moreover, to take into account the possibility that other genetic factors could operate in conjunction or in the absence of the $\epsilon 4$ allele of the APOE gene. Lastly, we ran our analyses a second time by using only those patients who were born in the Saguenay area. We wanted to verify if using a more restrictive criterion for the birthplace would modify inbreeding and kinship and have an influence on the comparison between cases and controls.

Controls

A collaborative study on health and aging was initiated, in 1994, in the Saguenay region, which has now, among other things, defined a control group composed of individuals aged over 70 years at the time of the study and free from any cognitive impairment based on neuro-psychological evaluation [Mortimer et al., 1996]. A control was matched to each one of the 205 affected individuals of our sample on the basis of gender, birthplace—within or outside the Saguenay region—and the closest year of birth. Cases and controls were not matched for APOE genotype. Following verification of first-degree kinship of each control with all the other controls and cases, we had to replace four controls. As for cases, we also made sure we identified at least 60% of the ancestors in the first 7 generations of each control's ascending genealogy. This led to replacement of 8 controls.

Characteristics of cases and controls are described in Table I for gender, place and date of birth, APOE allele frequencies and, for cases, age at onset and at death; early and late onset cases are described separately. Cases and controls presented significant differences in APOE allele distribution both among early ($\chi^2 = 24.2$, $df = 2$, $P = 0.00001$) and late onset cases ($\chi^2 = 51.2$, $df = 2$, $P < 0.00001$). Frequency of the allele $\epsilon 4$ is elevated for both early (0.43) and late (0.39) onset cases; among early onset cases, this remains true for cases with onset before 60 years of age (0.44). The frequencies were calculated on the genotypes of 175 cases and 134 controls.

Genealogical Reconstruction

Genealogies of all patients and controls were reconstructed following identical procedures at the Institut Interuniversitaire de Recherches sur les Populations (IREP). The IREP works with two computerized population databases: its Balsac file, which contains the linked records of all baptisms, marriages, and burials that took place in the Saguenay area from its opening to European settlement in 1838 up to 1971, and, the Registre de Population du Québec Ancien from the Programme de Recherche en Démographie historique (PRDH) containing linked records of baptisms, marriages and burials that took place in the whole of Nouvelle-France from the founding of the city of Québec in 1608 up to 1800. Genealogical dictionaries as well as marriage repositories were also consulted. On average, in each genealogy, more than 65% of the ancestors were traced back up to the tenth generation. However, for the purpose of this study, we decided to look at inbreeding and kinship within 7 generations of ancestry. We suspected that if we investigated further back in time, it would become

TABLE I. Sample Description Statistics[†]

	All subjects		Early onset (<65)		Late onset (≥65)	
	Cases	Controls	Cases	Controls	Cases	Controls
Number	205	205	40	40	165	165
Male		33%		35%		33%
Female		67%		65%		67%
Saguenay-born		80%		75%		81%
Year of birth	1,911 ± 7.3 (1895–1933)	1,911 ± 6.2 (1897–1923)	1,920 ± 6.4 (1908–1933)	1,918 ± 4.6 (1903–1923)	1,909 ± 5.6 (1895–1924)	1,909 ± 5.2 (1897–1922)
Age of onset	71.4 ± 8.2 (49–88)		59.3 ± 4.2 (49–64)		74.4 ± 5.9 (65–88)	
Age at death	79.5 ± 7.4 (58–95)		70.5 ± 6.6 (58–85)		81.7 ± 5.8 (69–95)	
APOE-ε2	0.08	0.12	0.08	0.10	0.08	0.12
APOE-ε3	0.52	0.79	0.49	0.85	0.53	0.78
APOE-ε4	0.40	0.09	0.43	0.05	0.39	0.10
No. alleles	350	268	72	58	278	210

[†]For year of birth, age of onset, and age at death values represent mean ± SD (range).

increasingly difficult to find differences between cases and controls because of the population structure. A recent study by Heyer and Tremblay [1995] has shown this to be true for the founders of various genetic diseases in the Saguenay area.

Analysis of Consanguinity

The coefficient of consanguinity of every subject was computed using the SYGAP software [Poulard et al., 1991] based on the following formula:

$$F_1 = \Sigma (1/2)^i (1 + F_A)$$

where the summation is over all the possible consanguinity paths through all common ancestors, i is the number of individuals in each path, and A is the common ancestor in each path [Hartl, 1988]. Here, $1+F_A$ is always equal to 1 because we did not take into account the possibility that some ancestors could be inbred themselves; this leads to a slight underestimation of the inbreeding coefficients of our subjects. In each study group, we calculated the proportion of inbred individuals as well as the mean coefficient. Results were compared using the paired Student's t -test [Daniel, 1991]. We used a one-sided test since our alternative hypothesis states that inbreeding would be higher among cases than controls.

Analysis of Kinship

We investigated within-group (among cases) and between-groups (between cases and controls) kinship. First, we computed for each study group, the highest number of kinship pairs that could possibly be found. For within-group kinship, this is equal to $(n*(n - 1))/2$ where n is the number of individuals in the group; for between-groups analysis, it is equal to n_1*n_2 where n_1 and n_2 are the number of individuals in the first and second groups, respectively. Then, we identified, for the whole group, all the pairs that were actually related, that is who shared at least one common ancestor within 7 generations. This allowed us to determine, for each study group, the proportion of related pairs. In order to estimate if the mean distance of the kinship paths—defined as the number of generations separating the closest common ancestor from the two probands—was different among the case-case pairs than among the case-control pairs, we calculated the coefficients corresponding to these distances using the following formula taken from Jacquard [1974]:

$$\phi_{XY} = (1/2)^{n+p+1}$$

where n and p are the number of generations separating X and Y from the common ancestor. Statistical comparison of the results obtained in kinship studies is complicated because of the lack of independence among kinship pairs [Jorde et al., 1990]. To minimize this problem, we used the method proposed by Hauck and Martin [1984]. As suggested by these authors, we compared the differences in the mean coefficients of the case-case pairs and of the case-control pairs; the observed kinship among cases and controls is considered to be representative of the kinship level pertaining to the population structure. Therefore, if kinship is higher within the group of cases than between cases and controls, it could be explained by some characteristic shared uniquely by the cases and related to the disease under study. To perform a statistical

evaluation of the results, Hauck and Martin worked with the mean of the distribution of the differences between case-case and case-control coefficients and they used the jackknife method to obtain a variance for this distribution. A one-sided t-type test for paired samples is then used to compare the results.

RESULTS

Analysis of Consanguinity

Results on inbreeding measurements are in Table II. The proportion of inbred individuals in all groups is high: between 75 and 85%. However, this appears to be a feature of the population as a whole, since it is similar for both cases and controls, the proportion being actually slightly higher among the latter. The mean inbreeding coefficient is more elevated among cases than controls but in both groups it is close to 0.0039, which corresponds to parents of subjects being related on average at the third cousin level. Subdividing cases according to age of onset introduces important variations: although late-onset cases remain somewhat more inbred on average than their matched controls, we notice the opposite trend for early-onset cases who have a lower mean coefficient than the controls. However, none of these differences reaches significance at the 0.05 level.

Results on the level of inbreeding for groups based on the three alleles at the APOE locus indicate a higher mean coefficient among cases than controls in the $\epsilon 3$ and $\epsilon 4$ groups; conversely, cases that have an $\epsilon 2$ allele and those who do not have an $\epsilon 4$ allele are less inbred than their matched controls.

The results for Saguenay-born cases (data not shown) are similar to the ones obtained for the whole group: most coefficients are slightly elevated for both cases and controls but the differences between these two groups remain essentially the same.

Because we observed considerable variations in the results among subgroups based on age of onset and on presence or absence of APOE- $\epsilon 4$, we decided to look at inbreeding in subsets of cases based on a combination of these two criteria. Hence, four new groups were analyzed: late onset $\epsilon 4+$, late onset $\epsilon 4-$, early onset $\epsilon 4+$ and early onset $\epsilon 4-$; the results of the calculations are in Table III. Late onset $\epsilon 4+$ cases are more inbred than controls and this difference reaches significance ($t = 2.21$, $df = 91$, $P = 0.02$) but early onset $\epsilon 4+$ cases are less inbred than controls. Cases without the $\epsilon 4$ allele are less inbred than controls independently of their age of onset. Once again, results for Saguenay-born subjects (data not shown) are very similar to the

TABLE II. Inbreeding Among Cases and Controls at a 7-Generation Depth

Group	Number of case/control pairs	Proportion of inbred individuals		Mean coefficient (*0.0001)		
		Cases	Controls	Cases	Controls	Difference
All cases	205	0.77	0.81	43.8	37.4	6.4
Early-onset cases	40	0.75	0.80	31.2	51.8	-20.6
Late-onset cases	165	0.78	0.81	46.9	33.8	13.1
Cases $\epsilon 4+$	118	0.78	0.80	52.5	33.7	18.8
Cases $\epsilon 3+$	137	0.78	0.79	44.0	37.1	6.9
Cases $\epsilon 2+$	28	0.75	0.86	15.6	38.7	-23.1
Cases $\epsilon 4-$	57	0.77	0.82	26.5	40.2	-13.7

TABLE III. Inbreeding Among Cases and Controls According to Age of Onset and Presence or Absence of APOE- ϵ 4 Among Cases

Group	Number of case/control pairs	Proportion of inbred individuals		Mean coefficient (*0.0001)		
		Cases	Controls	Cases	Controls	Difference
Early-onset cases ϵ 4+	26	0.77	0.85	23.4	61.1	-37.7
Early-onset cases ϵ 4-	10	0.70	0.70	12.9	39.9	-27.0
Late-onset cases ϵ 4+	92	0.78	0.78	60.8	25.9	34.9 ^a
Late-onset cases ϵ 4-	47	0.79	0.85	29.4	40.2	-10.8

^a $t = 2.21$, $df = 91$, $P = 0.02$ (one-tail).

ones obtained working on the whole group: inbreeding is also significantly higher for late-onset ϵ 4+ cases ($t = 2.22$, $df = 73$, $P = 0.02$). We compared the distribution of inbreeding coefficients among late-onset ϵ 4+ cases and their matched controls to see how the difference in the coefficients could be explained. Figure 1 demonstrates clearly that this difference is attributable to a few high coefficients corresponding to parents being related as first-degree cousins.

Analysis of Kinship

Results of the kinship analysis are in Table IV. This table indicates the number of individuals included in each study group as well as the number of pairs that could possibly be formed, the number that was actually related within 7 generations of ancestry, and the ratio of these two values. The corresponding kinship coefficients are indicated, as well as the mean and standard deviation of the observed differences between case-case and case-control kinship coefficients. In a few instances, these differences do not correspond exactly to the subtraction of the two coefficients found in Table IV because these differences are those obtained using the method devised

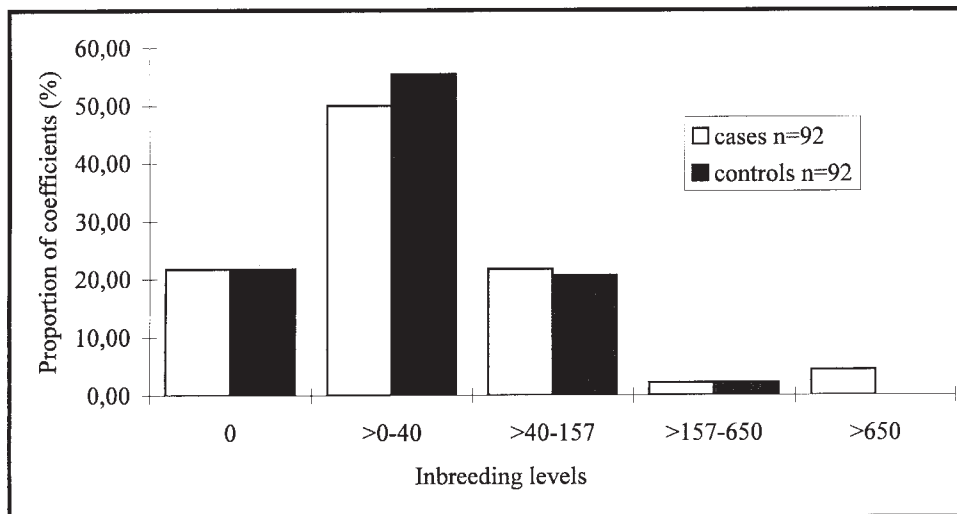


Fig. 1. Distribution of inbreeding coefficients among late-onset ϵ 4+ cases and their matched controls.

TABLE IV. Kinship at a 7-Generation Depth Among Case-Case Pairs and Among Case-Control Pairs

Group ^a	n	Number of pairs			Coefficient (*0.0001) ^e	Different (mean ± SD) (*0.0001) ^f
		Related ^b	Total ^c	Ratio ^d		
All cases	205	16,716	20,910	0.80	3.4	0.17 ± 0.21
	410	33,914	42,025	0.81	3.2	
Early-onset cases	40	553	780	0.71	1.9	-0.67 ± 0.39
	80	1,192	1,600	0.75	2.6	
Late-onset cases	165	11,157	13,530	0.82	3.6	0.27 ± 0.25
	330	22,366	27,225	0.82	3.3	
Cases ε4+	118	5,387	6,903	0.78	3.4	0.62 ± 0.32*
	236	10,834	13,924	0.78	2.8	
Cases ε3+	137	7,518	9,316	0.81	3.4	0.26 ± 0.28
	274	15,309	18,769	0.82	3.1	
Cases ε2+	28	299	378	0.79	3.7	0.78 ± 2.52
	56	652	784	0.83	2.9	
Cases ε4-	57	1,299	1,596	0.81	3.3	-0.67 ± 0.64
	114	2,817	3,249	0.87	3.9	

^aFor each group, the first row refers to case-case pairs and the second to case-control pairs.

^bNumber of pairs of related individuals.

^cTotal number of possible pairs: $(n*(n-1))/2$ for case-case pairs; n_1*n_2 for case-control pairs.

^dNumber of pairs of related individuals/Total number of possible pairs.

^eMean kinship coefficient based on closest ancestor within 7 generations for each pair.

^fDifference between case-case and case-control kinship coefficient.

* $t = 1.92$, $P = 0.03$ (one-tail).

by Hauck and Martin [1984], which does not consider kinship between a case and his matched control in the estimation of the case-control kinship whereas we did for the calculation of the coefficients. This introduces slight variations especially when sample size is smaller.

All cases and controls are related with at least one other subject, and, for the whole group, at least one common ancestor was found within 7 generations in 80% of the pairs. Cases are slightly more related among themselves than with their matched controls; however, more important variations are observed in subsets of cases based on age of onset. Early-onset cases are less related than late-onset cases; they are also less related among themselves than with their matched controls; kinship among late-onset cases is higher than with the controls but the difference does not reach significance. Kinship coefficients do not vary much among the carriers of the three APOE alleles but are all higher than the corresponding case-control coefficients; the difference reaches significance for the group of ε4 carriers ($P = 0.02$). Lastly, cases without an APOE-ε4 are more closely related to their controls than among themselves.

As we noticed for inbreeding, the level of kinship for individuals born in the Saguenay region is elevated (data not shown). On average, the proportion of pairs composed of related individuals is about 10% higher with small variations among the various groups. However, we observe substantial variations in the mean distance of the kinship paths, as indicated by the computed coefficients. The increase is more important among case-case than among case-control coefficients leading to a marked increase in the difference between those groups.

As we had done for inbreeding, our results prompted us to evaluate kinship in

subsets of cases based on a combination of age of onset and presence or absence of the APOE- ϵ 4. Results of this analysis are in Table V. They indicate that, when they have an APOE- ϵ 4, early-onset cases are less related among themselves than with their matched controls. However, it is interesting to notice that early-onset cases who lack an APOE- ϵ 4 are slightly more related among themselves than they are with controls, although the difference does not reach significance. We observe the opposite situation among late-onset cases where the difference between case-case and case-control coefficients is positive and reaches significance for cases with an APOE- ϵ 4 and is negative for those without. Thus, among APOE- ϵ 4 carriers, the higher kinship level among cases characterizes, in fact, only the late-onset cases. The results are similar, although once again more marked, for the Saguenay-born subjects (data not shown).

DISCUSSION

In this study, we have found that:

1. Cases are slightly more inbred than controls and they are also relatively more closely related among themselves than with their matched controls. However, these results do not reach significance.
2. Grouping of cases according to age of onset (early < 65, late \geq 65) and according to presence or absence of the APOE- ϵ 4 allele introduces important variations in the inbreeding and kinship values leading to the following observations:
 - a. In the late-onset ϵ 4+ group, cases are significantly more closely related among themselves than they are with controls. Since controls were not matched to cases for APOE genotype, our comparison bore upon AD cases who were carriers of at least one APOE- ϵ 4 allele and controls whose

TABLE V. Kinship at a 7-Generation Depth for Subsets of Cases Based on Age of Onset and Presence or Absence of APOE- ϵ 4 Among Cases

Group ^a	n	Number of pairs			Coefficient (*0.0001) ^e	Different (mean \pm SD) (*0.0001) ^f
		Related ^b	Total ^c	Ratio ^d		
Early-onset cases ϵ 4+	26	239	325	0.74	2.0	-0.31 \pm 0.59
	52	498	676	0.74	2.3	
Early-onset cases ϵ 4-	10	25	45	0.56	2.7	0.06 \pm 3.04
	20	72	100	0.72	2.5	
Late-onset cases ϵ 4+	92	3,322	4,186	0.79	3.6	0.79 \pm 0.39*
	184	6,664	8,464	0.79	2.9	
Late-onset cases ϵ 4-	47	949	1,081	0.88	3.6	-0.79 \pm 0.81
	94	2,010	2,209	0.91	4.4	

^aFor each group, the first row refers to case-case pairs and the second to case-control pairs.

^bNumber of pairs of related individuals.

^cTotal number of possible pairs: $(n*(n-1))/2$ for case-case pairs; n_1*n_2 for case-control pairs.

^dNumber of pairs of related individual/Total number of possible pairs.

^eMean kinship coefficient based on closest ancestor within 7 generations for each pair.

^fDifference between case-case and case-control kinship coefficient.

* $t = 2.03$, $P = 0.02$ (one-tail).

allele frequency at the APOE locus is taken to be representative of the population aged over 70 years, free of cognitive problems and living in the Saguenay area of Québec. Therefore, our results raise the following question: are the late onset $\epsilon 4+$ cases more related because they have an APOE- $\epsilon 4$ allele or because they carry some other as yet unidentified heritable factor acting or not in conjunction with APOE- $\epsilon 4$? Implication of additional genetic factors in the etiology of AD is expected [Levy-Lahad and Bird, 1996; Mayeux, 1996; Lendon et al., 1997]. Some research suggests the existence of genes operating as modifiers to the risk associated to the APOE- $\epsilon 4$ allele [Kamboh et al., 1995; Okuizumi et al., 1995] whereas other investigations point towards the presence of factors acting independently of APOE- $\epsilon 4$ [Wragg et al., 1996]. In our sample, the frequency of APOE- $\epsilon 4$ is significantly elevated among both early- and late-onset cases (see Table I), indicating an involvement of $\epsilon 4$ as a risk factor in both groups; however, early-onset cases, who are carriers of an APOE- $\epsilon 4$ allele, do not show any increase in within-group kinship. Lastly, we cannot exclude the possibility that this result constitutes a type I error. This probability is increased due to multiple testing.

- b. Late-onset $\epsilon 4+$ cases are also significantly more inbred than controls and this increase is explained by the high level of inbreeding of a few cases whose parents were related at the first-cousin level. When a recessive effect of rare genes is suspected in a disease etiology, it can be investigated through the study of consanguineous matings among parents of affected individuals [Khlal and Khoury, 1991]. In segregation studies performed on AD, a recessive inheritance model has consistently been rejected [Rao et al., 1994; Jarvik et al., 1996] although a recent analysis has found that a recessive factor could be involved in the transmission of AD among families of probands lacking $\epsilon 4$ [Rao et al., 1996]. We cannot discard the possibility that, in our population, a recessive component plays a role among a small subset of cases, possibly interacting with the APOE- $\epsilon 4$ allele; in such a case, it could be difficult to detect it until we have been able to define this subgroup more accurately. We must also consider the possibility of a type I error in our analysis which is increased due to multiple testing.
3. Lastly, working only with subjects born in the Saguenay area increases the proportion of inbred and related individuals in most groups of cases and controls; however, for kinship, the increase is somewhat more marked among case-case pairs than among case-control pairs. We believe this observation is of interest for molecular and epidemiological studies because it indicates that the capacity to detect potential differences between cases and controls might be considerably influenced by the choice of criteria for geographical origin of subjects even at the regional level.

To our knowledge, this is the first study of this type to be conducted on AD; therefore, the results must be regarded with caution. Similar analyses carried out on disorders with complex etiologies have yielded interesting clues on the role of genetic factors. Genealogical analysis of neural tube defects [Jorde et al., 1983] and of

autism [Jorde et al., 1990] based on the Utah genealogical database indicated that familial clustering was confined to sib pairs and that recessive inheritance was unlikely. Investigation of multiple sclerosis in Orkney [Roberts, 1991] and of Down's syndrome in Shetland [Roberts et al., 1991] showed that parents of cases were more closely related than parents of controls.

In conclusion, the present study indicates a higher level of inbreeding and kinship among late-onset AD cases who are APOE- ϵ 4 carriers. This increase in kinship may be attributable to the presence of the ϵ 4 allele or to some other unidentified genetic factor. Moreover, the possibility of a recessive element playing a role among a small subset of these cases cannot be excluded. Further investigation will be needed to clarify these matters. Meanwhile, we believe that the use of genealogical data can contribute positively to case selection in epidemiological and molecular studies of AD.

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REFERENCES

- Bennett C, Crawford F, Osborne A, Diaz P, Hoyne J, Lopez R, Roques P, Duara R, Rossor M, Mullan M. 1995. Evidence that the APOE locus influences rate of disease progression in late onset familial Alzheimer's disease but is not causative. *Am J Med Genet* 60:1-6.
- Bonaiuto S, Rocca WA, Lippi A, Giannandrea E, Mele M, Cavarzeran F, Amaducci L. 1995. Education and occupation as risk factors for dementia: a population-based case-control study. *Neuroepidemiology* 14:101-109.
- Daniel W. 1991. *Biostatistics: a foundation for analysis in the health sciences*, 5th ed. New York: John Wiley & Sons, pp 218-220.
- Gauvreau D, Bouchard R, Gauthier S, Mathieu J, Boily C, Cholette A, Robitaille Y, Bouchard P, Bouchard N, Doyon L-P, Gaudreault M, Ouellet A, Dumont M, Kishka P, Fournier C, Nalbantoglu J, Lacoste-Royal G, Gaurin D, Froda S, de Braekeleer M, Bouchard G, Mortimer J. 1988. The IMAGE Project: a geographical laboratory for the investigation of multidisciplinary data. In: Sinet PM, Lamour Y, Christen Y, editors. *Research and perspective in Alzheimer's disease*. Heidelberg: Springer-Verlag, pp 40-50.
- Goate A, Chartier-Harlin M, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J. 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 349:704-706.
- Harrington CR, Wischik CM. 1995. The impact of genetic and environmental factors on the pathobiology of Alzheimer's disease: a multifactorial disorder? *Int Rev Psychiatry* 7:361-384.
- Hartl DL. 1988. *A primer of population genetics*, 2nd ed. Sunderland: Sinauer Associates, pp 59-62.
- Hauck WW, Martin AO. 1984. A statistical test for detection of ancestral genetic contributions to disease occurrence in finite populations. *Genet Epidemiol* 1:383-400.
- Heyer E, Tremblay M. 1995. Variability of the genetic contribution of Quebec population founders associated to some deleterious genes. *Am J Hum Genet* 56:970-978.
- Jacquard A. 1974. *Génétique des populations humaines*. Paris: Presses universitaires de France.
- Jarvik GP, Larson EB, Goddard K, Kukull WA, Schellenberg GD, Wijsman EM. 1996. Influence of apolipoprotein E genotype on the transmission of Alzheimer disease in a community-based sample. *Am J Hum Genet* 58:191-200.

- Jorde LB, Fineman RM, Martin RA. 1983. Epidemiology and genetics of neural tube defects: an application of the Utah genealogical data base. *Am J Phys Anthropol* 62:23–31.
- Jorde LB, Mason-Brothers A, Waldmann R, Ritvo ER, Freeman BJ, Pingree C, McMahon WM, Petersen B, Jenson WR, Mo A. 1990. The UCLA-University of Utah epidemiologic survey of autism: genealogical analysis of familial aggregation. *Am J Med Genet* 36:85–88.
- Kamboh MI, Sanghera DK, Ferrell RE, DeKosky ST 1995. APOE*4-associated Alzheimer's disease risk is modified by alpha1-antichymotrypsin polymorphism. *Nature Genet* 10: 486–488.
- Khachaturian ZS. 1985. Diagnosis of Alzheimer's disease. *Arch Neurol* 42:1097–1105.
- Khlat M, Khoury M. 1991. Inbreeding and diseases: demographic, genetic, and epidemiologic perspectives. *Epidemiol Rev* 13:28–41.
- Kukull WA, Larson EB, Bowen JD, McCormick WC, Teri L, Pfanschmidt ML., Thompson JD, O'Meara ES, Brenner DE, van Belle G. 1995. Solvent exposure as a risk factor for Alzheimer's disease. *Am J Epidemiol* 141:1059–1071.
- Lautenschlager NT, Cupples LA, Rao VS, Auerbach SA, Becker R, Burke J, Chui H, Duara R, Foley EJ, Glatt SL, Green RC, Jones R, Karlinsky H, Kukull WA, Kurz A, Larson EB, Martelli K, Sadovnick AD, Volicer L, Waring SC, Growdon JH, Farrer LA. 1996. Risk of dementia among relatives of Alzheimer's disease patients in the MIRAGE study: What is in store for the oldest old? *Neurology* 46:641–650.
- Lendon CL, Ashall F, Goate AM. 1997. Exploring the etiology of Alzheimer disease using molecular genetics. *JAMA* 277:825–831.
- Levy-Lahad E, Bird TD. 1996. Genetic factors in Alzheimer's disease: a review of recent advances. *Ann Neurol* 40:829–840.
- Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Yu C, Jondro PD, Schmidt SD, Wang K, Crowley AC, Fu Y-H, Guenette SY, Galas D, Nemens E, Wijsman EM, Bird TD, Schellenberg GD, Tanzi RE. 1995. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* 269:973–977.
- Martyn CN, Coggon DN, Inskip H, Lacey RF, Young WF. 1997. Aluminum concentrations in drinking water and risk of Alzheimers-disease. *Epidemiology* 8: 281–286.
- Mayeux R. 1996. Understanding Alzheimer's disease: expect more genes and other things. *Ann Neurol* 39: 689–690.
- Mayeux R, Ottman R, Maestre G, Ngai C, Tang MX, Ginsberg H, Chun M, Tycko B, Shelanski M. 1995. Synergistic effects of traumatic head injury and apolipoprotein-ε4 in patients with Alzheimer's disease. *Neurology* 45:555–557.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human services task force on Alzheimer's disease. *Neurology* 34:939–944.
- Morrison-Bogorad M, Phelps C, Buckholtz N. 1997. Alzheimer disease research comes of age. The pace accelerates. *JAMA* 277:837–840.
- Mortimer JA, Fortier I, Pepin EP, Gauvreau D. 1996. Sensory loss as a predictor of dementia and questionable dementia in a community study of elderly persons. Paper presented at the Fifth International Conference on Alzheimer's disease and related disorders, Osaka, Japan, July 24–29.
- Norrman J, Brookes AJ, Yates C, St-Clair D. 1995. Apolipoprotein E genotype and its effect on duration and severity of early and late onset Alzheimer's disease. *Br J Psychiatry* 167:533–536.
- Okuizumi K, Onodera O, Namba Y, Ikeda K, Yamamoto T, Seki K, Ueki A, Nanko S, Tanaka H, Takahashi H, Oyanagi K, Mizusawa H, Kanazawa I, Tsuji S. 1995. Genetic association of the very low density lipoprotein (VLDL) receptor gene with sporadic Alzheimer's disease. *Nature Genet* 11: 207–209.
- Pericak-Vance MA, Haines JL. 1995. Genetic susceptibility to Alzheimer disease. *Trends Genet* 11:504–508.
- Poulard S, Heyer E, Guillemette A, Brunet G, Bideau A, Lègaré J. 1991. Sygap- Système de gestion et d'analyse de population. Villeurbanne: Programme pluriannuel en sciences humaines Rhône-Alpes, pp 198–227.
- Rao VS, van Duijn CM, Connor-Lacke L, Cupples LA, Growdon JH, Farrer LA. 1994. Multiple etiologies for Alzheimer disease are revealed by segregation analysis. *Am J Hum Genet* 55:991–1000.
- Rao VS, Cupples A, van Duijn CM, Kurz A, Green RC, Chui H, Duara R, Auerbach SA, Volicer L,

- Wells J, van Broeckhoven C, Growdon JH, Haines JL, Farrer LA. 1996. Evidence for major gene inheritance of Alzheimer disease in families of patients with and without apolipoprotein E ϵ 4. *Am J Hum Genet* 59:664–675.
- Roberts DF. 1983. Genetic epidemiology. *Am J Phys Anthropol* 62:67–70.
- Roberts DF. 1991. Consanguinity and multiple sclerosis in Orkney. *Genet Epidemiol* 8:147–151.
- Roberts DF, Roberts MJ, Johnston AW. 1991. Genetic epidemiology of Down's syndrome in Shetland. *Hum Genet* 87:57–60.
- Roses AD. 1996. Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu Rev Med* 47:387–400.
- Salib E, Hillier V. 1997. A case-control study of smoking and Alzheimers disease. *Int J Geriatr Psychiatry* 12:295–300.
- Saunders AM, Strittmatter WJ, Schmechel D, St. George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ, Hulette C, Crain B, Goldbager D, Roses AD. 1993. Association of Apolipoprotein E allele ϵ 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 43:1467–1472.
- Schofield PW, Tang M, Marder K, Bell K, Dooneief G, Chun M, Sano M, Stern Y, Mayeux R. 1997. Alzheimers disease after remote head injury: an incidence study. *J Neurol Neurosurg Psychiatry* 62:119–124.
- Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin J-F, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov Y, Pollen D, Wasco W, Haines JL, Da Silva R, Pericak-Vance M, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St. George-Hyslop PH. 1995. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 375:754–760.
- Speck CE, Kukull WA, Brenner DE, Bowen JD, McCormick WC, Teri L, Pfanschmidt ML, Thompson JD, Larson EB. 1995. History of depression as a risk factor for Alzheimer's disease. *Epidemiology* 6:366–369.
- Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance MA, Enghild J, Salvesen GS, Roses AD. 1993. Apolipoprotein E: high-avidity binding to β -amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer's disease. *Proc Natl Acad Sci USA* 90:1977–1981.
- Tang MX, Maestre G, Tsai WY, Liu XH, Feng L, Chung WY, Chun M, Schofield P, Stern Y, Tycko B, Mayeux R. 1996. Relative risk of Alzheimer disease and age-at-onset distributions, based on APOE genotypes among elderly African Americans, Caucasians, and Hispanics in New York city. *Am J Hum Genet* 58:574–584.
- Tanzi RE, Kovacs DM, Kim T-W, Moir RD, Guenette SY, Wasco W. 1996. The presenilin genes and their role in early-onset familial Alzheimer's disease. *Alzheimer Dis Rev* 1:90–98.
- Tiberghien D, Robitaille Y, Laroche-Cholette A, Houde L, Grenon M, Gauvreau D. 1993. Retrospective assesment of relative risks of coronary arteriosclerosis and myocardial infarct in autopsy confirmed dementias of the AD and non-AD types. In: Corain B, Iqbal K, Nicolini M, Winblad B, Wisniewski H, Zatta P, editors. *Alzheimer's disease: advances in clinical and basic research*. New York: John Wiley & Sons, pp 121–127.
- Van Broeckhoven C. 1995. Presenilins and Alzheimer disease. *Nat Genet* 11:230–232.
- van Duijn CM, Havekes LM, Van Broeckhoven C, de Knijff P, Hofman A. 1995. Apolipoprotein E genotype and association between smoking and early onset Alzheimer's disease. *BMJ* 310:627–631.
- Whalley LJ, Thomas BM, McGonigal G, McQuade CA, Swingler R, Black R. 1995. Epidemiology of presenile Alzheimer's disease in Scotland 1974-88) II. Exposures to possible risk factors. *Br J Psychiatry* 167: 728–731.
- Wragg M, Hutton M, Talbot C, Alzheimer's Disease Collaborative Group. 1996. Genetic association between intronic polymorphism in presenilin-1 gene and late-onset Alzheimer's disease. *Lancet* 347:509–512.