ORIGINAL ARTICLE

ApoE genotype as risk factors for Alzheimer's disease in the population of Lublin region, Poland

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Abstract: There is much data concerning ApoE in the pathology of Alzheimer's disease. This study examines how ApoE genotype contributes to a risk for Alzheimer's disease in the population of the Lublin region in Poland. The data were obtained as part of a population based BERCAL (Epidemiologic study of Alzheimer's diseases and other forms of dementia in the population of Lublin region) study. Odds ratios (ORs) and 95% confidence intervals (CIs) for AD, adjusted for age, were calculated for ApoE genotypes. Epsilon3/epsilon3 genotype was the most prevalent genotype in both examined groups. Epsilon2/epsilon2 and epsilon4/epsilon4 were the least prevalent. Among subjects, the risk of AD was significantly reduced for people with genotypes epsilon2/epsilon2. The same significant result was noticed where results were recalculated so that the presence of at least one epsilon2 allele was taken into account, without any epsilon 4 allele in each person. Increased risk with occurrence of epsilon 4 allele is not shown. Increased risk in epsilon 4 carriers was observed. The test did not reach the level of power required. This study suggests that the well known genetic factor, mainly ApoE, even if of noticeable importance, may not be a main risk factor among the population of the Lublin region in Poland.

Key words: apolipoprotein, Alzheimer's disease, risk assessment

INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive disorder in which neurons deteriorate, resulting in the loss of cognitive functions, mainly memory, judgment and reasoning, and perception. The disease primarily affects the cerebral cortex and the hippocampus through a number of pathological processes [1, 2] One such process involves the formation of amyloid plaques. This occurs through the abnormal production and deposition of Amyloid β in the brain and its vasculature [3-5]. Amyoid β is a 42-amino acid polypeptide, believed to be toxic to neurons and nerve terminals [6, 7].

Another pathological feature of AD is the presence of neurofibrillary tangles which are intraneuronal accumulations of paired helical filaments. This process is related to the neuronal loss associated with AD [8].

Over 160 mutations on three genetic loci have been identified as being responsible for most cases of familial early onset autosomal dominant AD (with onset before the age of 65) Chromosome 14, presenilin 1; Chromosome 1, presenilin 2; chromosome 21, β-amyloid precursor protein (β APP). However, these early onset forms are responsible for less than 1-2% of cases [9, 10].

The discovery of these genetic loci linked to AD has led to important insights into the disease. The β APP gene on chromosome 21 is cleaved by 3 enzymes, known as the α, β and γ secretases, to produce amyloidogenic (Aβ or nonamyloidogenic breakdown products. The activity of these alternative pathways of β APP catabolism, as well as the rate of β APP expression, is controlled by genotype and the environment [9].

The most important genetic risk factor for sporadic AD is the apolipoprotein E (ApoE) e4 allele located on chromosome 19 [10-13]. The ApoE4 allele is associated with a 2-3-fold increased risk of contracting the disease when one copy is present, and when there are two copies the risk is increased by as much as 12 times (the ApoE gene is co-dominant) [14]. However, the ApoE4 alleles not necessary or sufficient for AD, and perhaps 50% of the inherited risk of contracting the disease is currently unknown. The strong association of ApoE genotype to AD is a potent indicator of the importance of lipid metabolism and diet in the pathogenesis of the disease [15, 16].

Studies have shown that genetics play a role in the extracellular deposition of amyloid. Individuals afflicted with AD carrying the ApoE4 isoforms have shown a greater number of amyloid β plaques when compared to ApoE3 carriers, and inheritance of an ApoE4 allele increases the risk of AD when compared to ApoE2 and ApoE3 carriers [17, 18].

Apolipoprotein E is a polymorphic glycoprotein that plays an essential part in the binding to receptors for the uptake of chylomicrons and VLDL remnants and of LDL. The three major isoforms are ApoE3 (Cys112/Arg158), -E4 (Arg112/Arg158) and -E2 (Cys112/Cys158). The ApoE genetic variation has a great impact. Apo E polymorphism is an essential determinant in the inter-individual variations of lipids in healthy subjects in various populations. Its influence can be significant on the efficacy of nutritional or therapeutic interventions [19, 20]. The allele epsilon 4 appears to be associated with an increased risk of premature atherosclerosis. Apo E polymorphism contributes to the lipid disorders in diabetes and obesity [21]. It has been demonstrated that Epsilon 4 is associated with the lowering

of age for the onset of Alzheimer's disease, but seems to be a major risk factor in late onset AD [18, 19].

In this study, ApoE isoforms were studied as independent risk factors for AD in a group of people selected from the population of the Lublin region in Poland.

METHODS

Population based sampling design. The current study is a part of a large population-based study called BERCAL (Epidemiologic study of Alzheimer's diseases and other forms of dementia in the population of Lublin region).

The participants of the BERCAL study were randomly selected from a population-based sample within the Lublin Region (2,182,191 inhabitants) in 2005 [22]. The main aim of the project was to assess the prevalence of dementia and its risk factors. As a result of the project, the prevalence of Alzheimer's disease in the Lublin region of Poland was calculated as 1634,6 /100,000 inhabitants. The screening was repeated three times in order to strengthen the power of the prevalence testing and to obtain more samples for analysis. Since the BERCAL study design has described elsewhere in more detail [22], suffice to mention here that dementia was diagnosed by the Diagnostic and Statistical Manual of Mental Disorders (4th ed.)(DSM-IV) criteria [23], and AD was diagnosed in accordance with the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [24].

Normal control subjects were recruited from the same population. Control participants had normal cognition, excluding mild cognitive impairment (MCI).

The study was carried out in accordance with the local IRB agreement. Written informed consent was obtained from each patient (if possible), the caregiver, and the patient's representative (if applicable), before beginning detailed screening activities.

Finally, the AD group (n = 52) and 50 healthy, age and gender matched control were recruited for further genetic analysis.

DNA sampling and analysis methods. A fasting morning blood sample was collected from every subject. Plasma total cholesterol (TC), LDL and HAD was determined. Genomic DNA was extracted from buffy coat leukocytes by standard method. DNA was amplified by polymerase chain reaction (PCR) in a thermocycler (ABI 9700) (Forward primer 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3'; Reverse primer 5'-TAAGCTTGGCACGGCTGTCCAAGGA-3'), as described by Hixson and Vernier [25, 26].

Statistical analyses. Descriptive analyses were carried out for all variables. Age, sex, blood pressure, and total cholesterol, HDL and LDL levels, and MMSE, scores were compared among the dementia group and normal controls, using one-way ANOVA. Odds ratios (ORs) and 95% confidence intervals (CIs) for AD, adjusted for age, was the main outcome measurement in this study. Statistical significance was marked when Power of the test was > 0.8 for alpha=0.05.

Sigma Stat 3.0 for Windows was used for statistical analyses. All p values <0.05 were regarded as statistically significant.

RESULTS

Odds ratios (ORs) and 95% confidence intervals (CIs) for AD, adjusted for age, were calculated for ApoE genotypes epsilon2/epsilon2, epsilon2/epsilon3, epsilon2/epsilon4, epsilon3/epsilon4, epsilon4/epsilon4 and epsilon3/epsilon3 group.

Epsilon3/epsilon3 genotype was the most prevalent genotype in both examined groups. Epsilon2/epsilon2 and epsilon4/epsilon4 were the least prevalent (Table 1).

Table 1   Occurrence of ApoE in the population of Lublin region.

ApoE genotype	AD		Control	
Epsilon2/epsilon2	3	5.8%	15	30%
Epsilon2/epsilon3	11	21.2%	14	28%
Epsilon2/epsilon4	7	13.5%	2	4%
Epsilon3/epsilon3	20	38.5%	15	30%
Epsilon3/epsilon4	8	15.4%	3	6%
Epsilon4/epsilon4	3	5.8%	1	2%
Total	52	100%	50	100%

Among BERCAL subjects, the risk of AD was significantly reduced for people with genotypes epsilon2/epsilon2 (Table 2). The same significant result was noticed where the results were recalculated in order that the presence of at least one epsilon2 allele was taken into account, without any epsilon 4 allele, in each person. (Table 3).

Table 2   Occurrence of ApoE in the population of Lublin region, Poland. Odds ratios, confidential intervals and strength of the test.

ApoE genotype	OR, 95%CI; AD/Control	Power, > 0,08 alpha 0,005;
Epsilon2/epsilon2	0.1743, 0.0463 to 0.6564*	*0,93
Epsilon2/epsilon3	0.6899, 0.2783 to 1.7101	0.118
Epsilon2/epsilon4	3.6556, 0.7207 to 18.5423	0.374
Epsilon3/epsilon3	1.4583, 0.6401 to 3.3224	0.136
Epsilon3/epsilon4	2.8485, 0.7101 to 11.4268	0.315
Epsilon4/epsilon4	3, 0.3015 to 29.8506	0.153

Table 3   ApoE allele epsilon 2 and epsilon 4 as a risk or protective factor for AD in the population of Poland, Lublin region.

Patient with Allele	OR, 95%CI	Power, > 0,08; alpha 0. 005	AD	Control
Epsilon 2 non 4	0.2668, 0.1162 to 0.6126*	*0.97	14	29
Epsilon 4 non 2	3.0854, 0.9115 to 10.4442	0.45	11	4

Not shown was an increased risk with epsilone 4 allele occurrence. An increased risk in epsilon 4 carriers was observed, but the test did not reach the required level of power, probably because of analyzed sample being too small (Table 4).

Table 4   ApoE allele epsilon 2 and epsilon 4 as a risk or protective factor for AD in the population of Lublin region.

Patient with Allele	OR, 95%CI	Power, > 0,08; alpha 0,005	AD	Control
Epsilon 4	3.6667, 1.3175 to 10.2049	0,78	18	6
Epsilon 2	0.4152, 0.1874 to 0.92	0,58	21	31

DISCUSSION

Pinpointing risk factors for Alzheimer's disease may have a widespread social impact. From a population perspective, it is possible to calculate the attributable risk of a factor if one knows the frequency of the risk factor in the matched, non-affected population, and the relative risk or odds ratio.

There is much data concerning ApoE in the pathology of Alzheimer's disease. In fact, apolipoprotein E epsilon 4 allele is the only well confirmed genetic risk factor for sporadic AD. As it contributes to 40-70% of AD cases, a large proportion of genetic variance may be determined by additional loci. Many studies have confirmed that the epsilon4 allele is a dose-response risk factor for AD, and the epsilon4/epsilon4 genotype is associated with a significantly earlier age of onset. Moreover, it has been proved that the epsilon2 allele was a dose-response protective factor for AD, and the epsilon3 allele exerts a weaker dose-response protective effect for risk of AD, compared with epsilon2 [10-12, 20].

ApoE epsilon4 is associated with small vessel arteriolo-sclerosis, microinfarcts of the deep nuclei, neuritic senile plaque density, and amyloid angiopathy in patients with autopsy-proved Alzheimer disease. These results suggest a role for epsilon4 in some of the microvascular changes commonly found in AD, and are consistent with a potential amyloidogenic role for epsilon4 [27].

In the population examined, recruited from the BERCAL study, however, ApoE4 seemed to be the least prevalent, both among AD and control subjects.

On the other hand, multi-centre studies undertaken by Farrer et al. [28] confirmed the high occurrence of ApoE4 in people with AD.

The low percentage of ApoE4 allele in the population examined in the presented study may suggest that factors other than ApoE4 factors may contribute to the occurrence of Alzheimer's disease in the Lublin region of Poland.

Farrer et al. [28] examined more closely the association between apolipoprotein E genotype and Alzheimer disease by age and sex in populations of various ethnic and racial denominations. Forty research teams contributed data on ApoE genotype, sex, age at disease onset, and ethnic background for 5,930 patients who met criteria for probable or definite AD, and 8,607 controls without dementia who were recruited from clinical, community, and brain bank sources.

Similar to the presented study, Farrer et al. [27] studied odds ratios and 95% confidence intervals for AD, adjusted for age, and stratified by major ethnic group (Caucasian, African American, Hispanic, and Japanese, for ApoE genotypes epsilon2/epsilon2, epsilon2/epsilon3, epsilon2/epsilon4, epsilon3/epsilon4, and epsilon4/epsilon4 relative to the epsilon3/epsilon3 group. For the purpose of this discussion, only the results from Caucasian subjects have been taken into consideration, among whom the risk of AD was significantly increased for people with genotypes epsilon2/epsilon4 (OR=2.6, 95% CI=1.6-4.0), epsilon3/epsilon4 (OR=3.2, 95% CI=2.8-3.8), and epsilon4/epsilon4 (OR=14.9, 95% CI= 10.8-20.6).

In the population under study, increased risk in epsilon 4 carriers was observed, but the test did not reach the required level of test power, probably because the sample analyzed was too small. Even if had been powerful enough, the test would not, however, have the same influence. In the population of the Lublin region, the carrier state of epsilon 4 would not increase the risk by even 4 times and the occurrence of E4/E4

by even 3 times, as opposed to the 15 times reported by Farrer's metha–analysis [27].

In Farrer's study, ORs were decreased for people with genotypes epsilon2/epsilon2 (OR=0.6, 95% CI=0.2-2.0) and epsilon2/epsilon3 (OR=0.6, 95% CI=0.5-0.8).

In the presented study, the same effect was observed in the AD group for epsilon2/epsilon2. In the case of epsilon2/epsilon3 genotype, however, the data were not significant. Despite the fact that the results of OR seemed to grant protection, the test was insufficiently powerful. This effect may be explained simply by the small sample size.

Farrer et al. [28] concluded that the ApoE epsilon4 allele represents a major risk factor for AD in all ethnic groups studied, across all ages from 40-90 years, and in both genders.

In the presented study, however, ApoE epsilon 4 alleles did not fulfill the criteria for a major AD risk factor, and population analysis should be continued to recognize more factors contributing to a risk. If these risk factors are simply additive at the population level, family history/ApoE4 would account for over 30% of the attributable risk for AD. For example, Mortimer [29] calculated the attributable risk due to family history as 26%. A similar calculation for ApoE4 suggested that over 30% of cases between the ages of 65-80 may be attributed to the E4 allele.

Verification of these risk factors for Alzheimer's disease obviously has important sociological implications. This study suggested that the well known genetic factor, mainly ApoE, even if of noticeable importance, may not be a main risk factor in the population of the Lublin region in Poland.

Alzheimer's disease needs analysis other then the genetic risk factors in its epidemiological characteristic.

REFERENCES

1. Murayama S, and Saito Y: Neuropathological diagnostic criteria for Alzheimer's disease. *Neuropathology* 2004, **24**, 254-260.
2. McKeel DW Jr, Price JL, Miller JP, Grant EA, Xiong C, Berg L, Morris JC: Neuropathologic criteria for diagnosing Alzheimer disease in persons with pure dementia of Alzheimer type. *J Neuropathol Exp Neurol* 2004, **63**, 1028-1037.
3. Gandy S: The role of cerebral amyloid beta accumulation in common forms of Alzheimer disease. *J Clin Invest* 2005 May, 115(5).
4. Hardy J: Has the amyloid cascade hypothesis for Alzheimer's disease been proved? *Curr Alzheimer Res* 2006 Feb, **3(1)**, 71-73.
5. Patterson C, Feightner JW, Garcia A, Hsiung GY, MacKnight C, Sadovnick AD: Diagnosis and treatment of dementia: 1. Risk assessment and primary prevention of Alzheimer disease. CMAJ 2008 Feb 26, **178(5)**, 548-556.
6. Müller T, Meyer HE, Egensperger R, Marcus K: The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. *Prog Neurobiol* 2008 Aug, **85(4)**, 393-406.
7. Resende R, Ferreiro E, Pereira C, Resende de Oliveira C: Neurotoxic effect of oligomeric and fibrillar species of amyloid-beta peptide1-42: involvement of endoplasmic reticulum calcium release in oligomer-induced cell death. *Neuroscience* 2008 Aug 26, **155(3)**, 725-737.
8. Delacourte A: Tau pathology and neurodegeneration: an obvious but misunderstood link. *J Alzheimers Dis* 2008 Aug, **14(4)**, 437-440.
9. Tang BL, Liou YC: Novel modulators of amyloid-beta precursor protein processing. *J Neurochem* 2007 Jan, **100(2)**, 314-323.
10. Pericak-Vance MA, Bebout JL, Gaskell PC Jr, Yamaoka LH, Hung WY, Alberts MJ, Walker AP, Bartlett RJ, Haynes CA, Welsh KA, et al.: Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. *Am J Hum Genet* 1991 Jun, **48(6)**, 1034-1050.
11. Schellenberg GD, Pericak-Vance MA, Wijsman EM, Moore DK, Gaskell PC Jr, Yamaoka LA, Bebout JL, Anderson L, Welsh KA, Clark CM, et al.: Linkage analysis of familial Alzheimer disease, using chromosome 21 markers. *Am J Hum Genet* 1991 Mar, **48(3)**, 563-583.

12. Saunders AM, Strittmatter WJ, Schmechel p, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ, Helette C, Crain B, Goldgaber D, Roses AD: Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993, **43**, 1467-1472.

13. Roses AD: On the discovery of the genetic association of Apolipoprotein E genotypes and common late-onset Alzheimer disease. *J Alzheimers Dis* 2006, **9(3 Suppl),** 361-366.

14. Pericak-Vance MA, Grubber J, Bailey LR, Hedges D, West S, Santoro L, Kemmerer B, Hall JL, Saunders AM, Roses AD, Small GW, Scott WK, Conneally PM, Vance JM, Haines JL: Identification of novel genes in late-onset Alzheimer's disease. *Exp Gerontol* 2000 Dec, **35(9-10),** 1343-1352.

15. Riley KP, Snowdon DA, Saunders AM, Roses AD, Mortimer JA, Nanayakkara N: Cognitive function and apolipoprotein E in very old adults: findings from the Nun Study. *J Gerontol B Psychol Sci Soc Sci* 2000 Mar, **55(2),** 69-75.

16. Sanan DA, Weisgraber KH, Russell SJ, Mahley RW, Huang D, Saunders A, Schmechel D, Wisniewski T, Frangione B, Roses AD, et al.: Apolipoprotein E associates with beta amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform apoE4 associates more efficiently than apoE3. *J Clin Invest* 1994 Aug, **94(2),** 860-869.

17. Carter DB: The interaction of amyloid-beta with ApoE. *Subcell Biochem* 2005, **38**, 255-272.

18. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J,Salvesen GS, Roses AD: Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA* 1993 Mar 1, **90(5),** 1977-1981.

19. Burke JR, Roses AD: Genetics of Alzheimer's disease. *Int J Neurol* 1991-1992, **25-26,** 41-51.

20. Mayeux R, Stem Y, Ottman R, Tatemichi TK, Tang MX, Maestre G, Ngai C, Tycko B, Ginsberg H. The apolipoprotein 04 allele in patients with Alzheimer's disease. *Ann Neurol* 1993, **34**, 752- 754.

21. Carmo Martins M, Lima Faleiro L, Rodrigues MO, Albergaria I, Fonseca A: Influence of the APOE genotypes in some atherosclerotic risk factors. *Acta Med Port* 2008 Sep-Oct, **21(5),** 433-440.

22. Gustaw K, Woźnica I, Bylina J: ROZPOWSZECHNIENIE ZESPOLOW OTEPIENNYCH W TYM CHOROBY ALZHEIMERA W POPULACJI MIESZKANCÓW WOJEWÓDZTWA LUBELSKIEGO. *Medycyna Ogólna* 2008, **14 (XLIII), 4,** 381-394.

23. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (4th ed). American Psychiatric Association 2000. Washington, DC, USA. Text Revision (DSM-IV-TR).

24. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: 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* 1984, **34**, 939-944.

25. Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *HhaI.J Lipid Res* 1990 Mar, **31(3),** 545-548.

26. Hixson JE, Vernier DT, Powers PK: Detection of SstI restriction site polymorphism in human APOC3 by the polymerase chain reaction. *Nucleic Acids Res* 1991 Jan 11, **19 (1),** 196.

27. Yip AG, McKee AC, Green RC, Wells J, Young H, Cupples LA, Farrer LA: APOE, vascular pathology, and the AD brain. *Neurology* 2005 Jul 26, **65(2),** 259-265.

28. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM: Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* 1997 Oct 22-29, **278(16),** 1349-1356.

29. Mortimer JA: Epidemiology of dementia: cross-cultural comparisons. *Adv Neurol* 1990, **51**, 27-33.