The APOE ε4 Allele Affects Cognitive Functions Differently in Carriers of APP Mutations Compared to Carriers of PSEN1 Mutations in Autosomal-Dominant Alzheimer’s Disease

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Abstract: Mounting evidence shows that the APOE ε4 allele interferes with cognition in sporadic Alzheimer’s disease. Less is known about APOE in autosomal-dominant Alzheimer’s disease (adAD). The present study explored the effects on cognition associated with the gene–gene interactions between the APOE gene and the APP and PSEN1 genes in adAD. This study includes mutation carriers (MC) and non-carriers (NC) from adAD families with mutations in APP (n=28 and n=25; MC and NC, respectively) and PSEN1 (n=12 and n=15; MC and NC, respectively) that represent the complete spectrum of disease: AD dementia (n=8) and mild cognitive impairment (MCI, n=15 and presymptomatic AD, n=17). NC represented unimpaired normal aging. There was no significant difference in the distribution of APOE ε4 (absence vs. presence) between the APP vs. PSEN1 adAD genes and mutation status (MC vs. NC). However, episodic memory was significantly affected by the interaction between APOE and the APP vs. PSEN1 genes in MC. This was explained by favorable performance in the absence of APOE ε4 in PSEN1 compared to APP MC. Similar trends were seen in other cognitive functions. No significant associations between APOE ε4 and cognitive performance were obtained in NC. In conclusion, cognitive effects of APOE–adAD gene interaction were differentiated between the PSEN1 and APP mutation carriers, indicating epistasis.

Keywords: APOE; autosomal-dominant Alzheimer’s disease; APP; PSEN1; cognition; epistasis

1. Introduction

Alzheimer’s disease (AD) is defined at autopsy by extracellular deposits of β-amyloid accumulated in plaques and neurofibrillary tangles of intracellular phosphorylated tau [1]. According to empirical evidence, the APOE ε4 allele has a deleterious effect on disease onset in sporadic Alzheimer’s disease (sAD) that is related to the number of APOE ε4 alleles [2]. APOE ε4 influences lipid metabolism in the brain, β-amyloid and tau processing, synapticogenesis, glucose metabolism, mitochondrial function, vascular integrity, and neuroimmune modulation [3,4]. In this way, sAD as well as normal aging is affected by various APOE-related functions. However, the precise mechanisms for these effects are far from known.

Less is known about the relationship between APOE and possible effects in autosomal-dominant AD (adAD). In recent studies of adAD, the APP, PSEN1 and PSEN2 genes have been combined into one group of mutation carriers. The impact of APOE ε4 on β-amyloid burden has not been clear, making a conclusion impossible in adAD [5,6]. Years to estimated clinical onset (YECO) was driving changes across time. The YECO–APOE ε4 interaction did not add any power to the time-related cognitive changes in adAD. In a cohort of the
specific Colombian PSEN1 mutation, no significant effect was found in association with APOE ε4 [7].

The combination of mutation carriers from various genes into one combined group may be problematic, because adAD genes and mutations may be associated with different phenotypes of cognition in APP and PSEN1 genes [7–11].

The aim of the present study was to investigate the possible interaction between the APOE gene and the APP and PSEN1 genes in adAD as observed on various cognitive functions.

2. Methods

2.1. Participants

Adult mutation carriers (MC, n = 40) and non-carriers (NC, n = 40) from six adAD families harboring a mutation in the APP or PSEN1 genes were invited to the Memory Clinic, Karolinska University Hospital Huddinge, Sweden, to participate in research. Three families carried an APP mutation (Swedish, p.KM670/671NL; Arctic, p.E693G and London, p.V717I) and three families carried a PSEN1 mutation (p.I143T, p.M146V, and p.H163Y). The demographic characteristics (age, gender, years of education, years to expected clinical onset of disease and APOE ε4 percentage) of MC and NC are presented in Table 1.

Table 1. Demographic characteristics (age, gender, YECO = years to estimated clinical onset, years of education, % APOE ε4) of adAD mutation carriers (MC) and non-carriers (NC) from APP and PSEN1 families. There were 12 MC with APP Swedish, 15 with APP Arctic, 1 with APP London, 3 with PSEN1 I143T or M146V and 9 with PSEN1 H163Y mutation. In addition, p-values are reported using two-way (Mutation and Gene) ANOVAs with each background factor as dependent variable.

| APP                  | PSEN1               | p   |
|----------------------|---------------------|-----|
|                      | MC      | NC    |      | MC      | NC    | M   | G   | MxG  |
| N (% females)        | 28 (36) | 25 (44) | 12 (33) | 15 (47) | ns    | ns  | ns  | ns   |
| Age, years (Mean ± SD)| 47.9 ± 11.2 | 47.4 ± 9.9 | 36.2 ± 9.3 | 33.8 ± 11.1 | ns    | ns  | ***| ns   |
| YECO (Mean ± SD)     | −7.1 ± 11.0       | −7.5 ± 9.7       | −11.1 ± 10.9       | −8.2 ± 11.8       | ns    | ns  | ns  | ns   |
| Education, years (Mean ± SD) | 10.9 ± 2.5 | 10.8 ± 2.9 | 11.8 ± 3.0 | 10.6 ± 1.5 | ns    | ns  | ns  | ns   |
| APOE ε4, %           | 46      | 28    | 50    | 40    | ns    | ns  | ns  | ns   |

Note. M = (MC/NC), G = (APP/PSEN1), MxG = interaction, ns = not significant, and *** = p < 0.001.

Analyses of the comparability of MC and NC groups from APP and PSEN1 families showed that APP individuals were significantly older than PSEN1 individuals (F = 25.52, df 1/76, p < 0.001, η² = 0.25), while advancement of disease as years to estimated clinical onset (YECO; see below) was not significant as well as the effects of gender, education and the APOE ε4 allele (present vs. absent). There were no significant differences between MC and NC in background characteristics or significant interaction effects, except for years of education (F = 9.34, df 1/53, p = 0.004, η² = 0.15). These results support that the groups of MC and NC in APP and PSEN1 families are roughly comparable in background characteristics.

2.2. Examination and Diagnosis

The standardized clinical examination included somatic, neurological, and psychiatric status, often an interview with a close informant, cognitive screening (MMSE) [12], cognitive assessment, Magnetic imaging, electric encephalography, and biochemical assessments of urine, blood, Cerebrospinal fluid markers (Abeta, total tau and phosphorylated tau), and fibroblast. Studies on non-cognitive outcomes have been reported elsewhere [13,14].

The clinical diagnosis was decided by a consensus meeting of medical professionals and based on all available reports, excluding information on the mutation status. At baseline, eight carriers (6 APP and 2 PSEN1) were diagnosed with dementia [15] and as having AD [16], 15 were diagnosed with mild cognitive impairment (MCI, 8 APP and 7 PSEN1) [17] and 17 (14 APP and 3 PSEN1) were asymptomatic. No NC was diagnosed with AD, MCI, or any other disease affecting the brain, neither at baseline nor at any follow-up examination.
2.3. APOE Genotyping

APOE genotyping was performed for SNPs rs7412 and rs429358 using TaqMan® SNP Genotyping Assays (ABI, Foster City, CA, USA) according to manufacturer’s protocol. The amplified products were run on the 7500 fast Real-Time PCR Systems (ABI, Foster City, CA, USA).

2.4. Years to Estimated Clinical Onset (YECO)

The years to estimated clinical onset of disease was calculated as the participant’s present age minus the family-specific age of clinical onset in correspondence with previous research [18,19]. This measure is invariant within families, variable between families and significantly associated with the observed age of onset and parental age of onset [18,19].

2.5. Cognitive Assessment

The cognitive assessment focused on tests varying in sensitivity for the development of AD [17,20]. The earliest change typically occurs in episodic memory, followed by executive function and visuospatial ability, and later by attention/processing speed and verbal ability. In the present study, the tests of Rey Auditory Verbal Learning (RAVL, episodic memory), Digit Symbol (executive function), Block Design (visuospatial ability), the Trailmaking A test (TMT A, attention) and Similarities (verbal ability) were used as outcome measures [18]. Raw test scores were standardized into z-scores based on data from normal individuals [21].

2.6. Ethical Approval

All participants were given written and oral information about the risk to inherit AD before this study began. All participants provided written informed consent to participate in this study. This study was approved by the Regional Ethics Committee in Stockholm (2006/901-31/3). This study was performed according to the declaration of Helsinki and subsequent revisions.

2.7. Statistical Analyses

A two-way ANOVA analyzed the distribution of APOE ε4 (present/absent) and demographic characteristics as related to mutation status (MC vs. NC) and adAD genes (APP vs. PSEN1). Five two-way ANOVAs, one for each cognitive test, analyzed the effect of the APOE gene, the adAD genes (APP vs. PSEN1) and their interaction separately in mutation carriers and non-carriers. Two-way ANCOVAs were used to analyze the effect on tests related to mutation (MC/NC) and gene (APP/PSEN1) with YECO and education as covariates.

3. Results

The cognitive test results across the five domains for APP and PSEN1 MC and NC are presented in Table 2. In NC, the results scatter around the mean performance for cognitively unimpaired individuals as expected. In contrast, the test results for MC vary from normal to clearly impaired as seen in episodic memory in APP MC, although the majority are in the preclinical stage of disease. The cognitive trajectory for episodic memory of these cross-sectional test results across time (YECO) is presented in Figure 1. The APP MC seem to start the decline decades before the clinical diagnosis is possible to verify. The PSEN1 MC seem to keep a normal performance level until approximately 10 years ahead of the clinical diagnosis; then the decline is steep.
Table 2. Cognitive test results (Mean ± SD) in z-score based on normal individuals in mutation carriers (MC) and non-carriers (NC) from APP and PSEN1 families. ANCOVA p-values are reported as well as related to mutation status, gene, mutation–gene interaction and the effects of covariates (YECO and years of education).

| Domain/Test                     | APP             | PSEN1           | p     |
|---------------------------------|-----------------|-----------------|-------|
|                                 | MC              | NC              |       |
| scholarship                    | MC              | NC              |       |
|                                               |                 |                 |       |
| Visuospatial/Block Design       | −1.18 ± 1.56    | −0.54 ± 0.92    |       |
|                                 | −0.28 ± 1.67    | −0.88 ± 0.98    | ns    |
|                                 | ns              | ns              | *     |
|                                 | ns              | ns              | **    |
| Episodic memory/RAVL            | −0.71 ± 1.79    | +0.05 ± 1.54    |       |
|                                 | +0.26 ± 2.33    | +0.73 ± 1.03    |       |
|                                 | *               | ns              | ***   |
|                                 | ns              | ns              | ***   |
| Executive/Digit Symbol          | −1.29 ± 1.37    | −0.12 ± 0.83    |       |
|                                 | −0.41 ± 1.55    | +0.03 ± 1.13    |       |
|                                 | ***             | ns              |       |
|                                 | ns              | ns              | ***   |
| Attention/TMT A                 | −0.73 ± 1.58    | +0.28 ± 0.99    |       |
|                                 | +0.35 ± 1.70    | +0.63 ± 1.08    |       |
|                                 | **              | *               | ***   |
|                                 | *               | ns              |       |
|                                 | ns              | **              |       |
|                                 | +0.05 ± 2.25    | +0.65 ± 0.78    |       |
|                                 | *               | ns              |       |
|                                 | ns              | ns              |       |

Note: Note. M = (MC/NC), G = (APP/PSEN1), MxG = interaction, Y = YECO, E = education, * = p < 0.05, ** = p < 0.01, *** = p < 0.001, and ns = not significant.

Figure 1. The trajectories for APP and PSEN1 MC as well as all NC across YECO using cross-sectional data.

The possible effect of mutation (MC/NC) and gene (APP/PSEN1) together with covariates (YECO and education) on test results are presented in Table 2. Mutation status was significant in four tests, gene in one test and the interaction was not significant in any test. YECO was significantly influencing test results in all tests and years of education was significant in four tests.

In MC, there was a significant effect of the APOE–adAD gene interaction on episodic memory (the RAVL learning test) (F = 6.66, df = 1/33, p = 0.014, η² = 0.17), see Figure 2. The graph shows a typical crossed pattern seen in statistical interaction: The APP MC performed poorer in episodic memory (p > 0.1), when APOE ε4 was absent (M ± SD: −1.62 ± 1.33) compared to present (M ± SD: −0.93 ± 1.38), while the opposite was seen in PSEN1 MC, namely significantly (p = 0.04) better performance when APOE ε4 was absent (M ± SD: 0.47 ± 0.71) compared to present (M ± SD: −1.25 ± 1.71).

Among the other four cognitive tests, there was a trend of significant interaction effect in visuospatial ability (Block Design) (F = 3.46, df = 1/36, p = 0.071, η² = 0.09), and in executive function (Digit Symbol) (F = 3.03, df = 1/20, p = 0.10, η² = 0.13), while the APOE–adAD gene interaction was not significant in verbal ability (Similarities) and attention/processing speed (p’s > 0.1). The pattern of results across the four cognitive tests demonstrated the same pattern as seen in episodic memory, i.e., a favorable performance...
for PSEN1 MC when the APOE ε4 was absent compared to present. For APP MC, the results were better when the ε4 allele was present compared to absent. In executive function (Digit Symbol), this effect was close to significant (p = 0.053), better in ε4 carriers (M ± SD: −0.11 ± 1.34) than in ε4 non-carriers (M ± SD: −1.26 ± 1.34). There were no significant interaction effects in NC. There were no significant main effects of APOE or adAD gene in any cognitive test (all p’s > 0.1).

**Figure 2.** A graph showing mean (95% CI) episodic memory performance (RAVL learning) between APOE ε4 positive and ε4 negative mutation carriers of APP and PSEN1 adAD genes.

**4. Discussion**

This study investigated the possible interaction effect on cognitive functions between APOE and the APP and PSEN1 genes in adAD in a sample of MC and NC from six families harboring an adAD gene. This study covered the whole range of cognitive impairment from the presymptomatic stage across mild cognitive impairment and finally AD dementia, i.e., more than 40 years (30 years before and 10 years after the expected onset). The APP and PSEN1 MC were comparable in background factors.

The main finding was that the APOE–adAD gene interaction was significant in episodic memory (the RAVL learning test) that accounted for 17% of the total test variance. Furthermore, there was a trend to significant interaction effect in visuospatial ability that accounted for 9% of the variance (the Block Design test) and in executive function that accounted for 13% of the variance (the Digit Symbol test). The statical interaction was observed as a favorable and significant effect in PSEN1 MC when APOE ε4 was absent compared to a favorable effect on cognition in APP MC when APOE ε4 was present (although not significant), i.e., opposite interaction effects.

In addition, a similar pattern of segregated ε4 effects (absence vs. presence) for PSEN1 vs. APP MC were obtained in the other cognitive tests. The APOE–adAD gene interaction effect was significant and favorable in APP MC on executive function (the Digit Symbol test), when APOE ε4 was present, while the interaction effect was positive (although not significant) in PSEN1 MC, when APOE ε4 was absent, i.e., opposite.

The interaction between the APOE and adAD genes have not been reported previously to our knowledge. The finding was based on a relatively small convenient sample of participants carrying a mutation in the APP or PSEN1 genes in adAD. The PSEN2 gene was not represented and several mutations in the APP and PSEN1 adAD genes were not investigated in the present study. These limitations make it necessary to await larger studies until a more reliable conclusion can be reached.
That said, the gene–gene interactions observed in the present study point to the possible complexity of function involved in the APOE gene. This complexity has recently been dealt with in recent publications. It seems clear that APOE is a significant factor in sAD [5,6] and that the APOE ε4 allele has gain of function effect in the earliest ontogenesis [22], in aging [23], in environments differing in infectious burden in Amazonas versus Western societies [24], and in demanding cognitive tasks [23] compared overlearned cognitive tasks [25,26]. Assuming that the findings are valid, they illustrate that the APOE gene is involved in both gene–gene and gene-environment interactions seen both within individuals across tasks and between individuals. This effect has been suggested to be a response to varying degree of neural stress [25].

The lack of knowledge about APOE functions may depend on the fact that many mechanisms are involved in APOE functioning, not only those related to lipid metabolism, β-amyloid and tau processes, but also synaptogenesis, glucose metabolism, mitochondrial function, vascular integrity, and neuroimmune modulation [3,4]. These various functions of APOE have led to contrasting outcomes like resistance to toxicity and loss of neuroprotection, summarized as the phenomena of pleiotropi.

Furthermore, we have previously shown that APP processing varies between APP and PSEN1 mutations as well as between different mutations in the same gene [13]. Thus, it is possible that the APOE-adAD gene interaction in parts reflects differences in the interaction caused by the different APP-processing products generated via the amyloidogenic as well as non-amyloidogenic pathways.

In conclusion, an interaction was observed between the APOE ε4 allele and the APP and PSEN1 genes in adAD. The effect of APOE ε4 on cognition was favorable in PSEN1 MC without an APOE ε4 allele and favorable in APP MC in the presence of an APOE ε4 allele. These results are an indication of epistasis.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved in 2006, September 25, by the Regional Ethics Committee in Stockholm (code 2006/901-31/3).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available upon reasonable request to the corresponding author (Caroline Graff).

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References
1. Lane, C.A.; Hardy, J.; Schott, J.M. Alzheimer’s disease. Eur. J. Neurol. 2018, 25, 59–70. [CrossRef] [PubMed]
2. Corder, E.H.; Saunders, A.M.; Strittmatter, W.J.; Schmechel, D.E.; Gaskell, P.C.; Small, G.W.; Roses, A.D.; Haines, J.L.; Pericak-Vance, M.A. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. Science 1993, 261, 921–923. [CrossRef] [PubMed]
3. Suidan, G.L.; Ramaswamy, G. Targeting Apolipoprotein E for Alzheimer’s disease: An Industry perspective. Int. J. Mol. Sci. 2019, 20, 2161. [CrossRef]
