Apolipoprotein-E Influences Aspects of Intellectual Ability in Type 1 Diabetes

Stewart C. Ferguson,1 Ian J. Deary,2 Julie C. Evans,3 Sian Ellard,3 Andrew T. Hattersley,3 and Brian M. Frier1

The ε4 allele of the apolipoprotein-E (APOE) gene is associated with poor outcome following various cerebral insults. The relationship between APOE genotype and cognitive function in patients with type 1 diabetes is unknown. In a cross-sectional study of 96 people with type 1 diabetes, subjects were APOE genotyped, previous exposure to severe hypoglycemia was estimated by questionnaire, and cognition was assessed by neuropsychological testing. Cognitive abilities were compared using multivariate general linear modeling (multiple analysis of covariance, MANCOVA) in those with (n = 21) and without (n = 75) the APOE ε4 allele. APOE ε4 selectively influenced cognitive ability in a sex-specific manner (F = 2.3, P = 0.044, η² = 0.15); women with APOE ε4 performed less well on tests of current, non-verbal intellectual ability (Wechsler Adult Intelligence Scale-Revised performance test score, P = 0.001, η² = 0.26) and frontal lobe and executive function (Borkowski verbal fluency, P = 0.016, η² = 0.15). Previous exposure to severe hypoglycemia did not interact with APOE ε4 to produce cognitive disadvantage. The APOE ε4 genotype is associated with specific cognitive disadvantage in young women with type 1 diabetes. APOE ε4 is unlikely to mediate susceptibility to hypoglycemia-induced cognitive disadvantage. Diabetes 52:145–148, 2003

Permanence cognitive impairment is a rare consequence of insulin-induced hypoglycemia. In insulin-treated diabetes, severe hypoglycemia is common, with an annual prevalence of 30% in type 1 diabetes and a higher incidence in people with impaired awareness of hypoglycemia and strict glycemic control (1). Whether recurrent exposure to severe hypoglycemia promotes long-term cognitive sequelae is unresolved. Retrospective cross-sectional studies have indicated that some people with type 1 diabetes suffer a modest cognitive decrement (2), and finding not replicated by prospective observations for up to 10 years (3,4). The cross-sectional studies have reported individual differences in cognitive decrements in those exposed to severe hypoglycemia (2), suggesting that factors other than neuroglycopenia may influence the risk of developing subsequent cognitive impairment. Chronological age, diabetes duration, and coexistent microvascular and macrovascular complications have been proposed as potential mediators of susceptibility to hypoglycemia-induced cognitive impairment (2), whereas genetic susceptibility has not been examined.

Genetic factors influence cognitive aging, and polymorphism of the gene for apolipoprotein-E (APOE) is the most important genetic determinant of late-onset Alzheimer’s disease (5). The APOE gene has three common alleles (ε2, ε3, and ε4) coding for three protein isoforms (designated E2, E3, and E4). Apolipoprotein-E (APOE) mediates central nervous system cholesterol transport in an isoform-specific manner (6). APOE activity is important in neuronal repair and maintenance, and the least active E4-isoform confers a survival disadvantage to injured neurons (6). APOE polymorphism influences cognitive ability in health and disease—apparently healthy middle-aged adults possessing the ε4 allele have, on average, relatively low learning and memory ability (7); the ε4 allele is associated with poorer cognitive and neurological outcome in head injury, demyelinating disease, intracerebral hemorrhage, and cardiopulmonary bypass surgery (8).

The aim of the present study was to examine whether possession of the APOE ε4 allele was associated with cognitive disadvantage in people with type 1 diabetes, and secondly, to determine whether the magnitude of any difference in cognitive ability was modified by preceding exposure to recurrent severe hypoglycemia.

RESEARCH DESIGN AND METHODS

A total of 96 people with type 1 diabetes were recruited and all completed the cross-sectional study protocol. Participants were selected from two preexisting cohorts (63 patients [9], 33 patients [10]), each of which had completed identical neuropsychological assessments. To minimize influences confounding neuropsychological performance, the following exclusion criteria were applied: hypertension (defined as blood pressure >140/90 mmHg), any previous central nervous system pathology, psychiatric disease, alcoholism or drug misuse, or multisystem disease known to affect the central nervous system. Assessment of neuropsychological function. The neuropsychological test battery was chosen to be sensitive to cognitive decrements across diverse cognitive abilities. Assessors blinded to the diabetes characteristics and APOE genotype of participants administered the neuropsychological tests in a fixed order. Incipient hypoglycemia, or hypoglycemia within the preceding 24 h, was excluded before neuropsychological assessment. Evidence of biochemical (blood glucose <3.5 mmol/L) or symptomatic hypoglycemia resulted in rescheduling of the neuropsychological session. The psychometric instruments used were as follows: The Hospital Anxiety and Depression Scale (11) evaluated potential confounding effects of low mood and anxiety.
TABLE 1

Characteristics of participants subdivided by the presence of the APOE ε4 allele

<table>
<thead>
<tr>
<th>Entire cohort (n = 96)</th>
<th>ε4− (n = 75)</th>
<th>ε4+ (n = 21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) 38.9 ± 7.2 (40, 22–54)</td>
<td>38.1 ± 7.4 (39, 22–52)</td>
<td>41.8 ± 5.7 (42, 31–54)</td>
<td>0.033</td>
</tr>
<tr>
<td>Sex (M/F) 53/43</td>
<td>44/31</td>
<td>9/12</td>
<td>0.20</td>
</tr>
<tr>
<td>NART (premorbid IQ score) 32.7 ± 8.6 (3–50)</td>
<td>32.8 ± 8.8 (3–50)</td>
<td>32.1 ± 8.2 (18–45)</td>
<td>0.74</td>
</tr>
<tr>
<td>Age at diagnosis of diabetes (years) 24.3 ± 8.2 (25, 4–45)</td>
<td>24.3 ± 8.4 (25, 4–45)</td>
<td>24.1 ± 7.3 (26, 4–34)</td>
<td>0.93</td>
</tr>
<tr>
<td>Duration of diabetes (years) 14.5 ± 7.3 (12, 5–40)</td>
<td>13.6 ± 6.7 (12, 5–32)</td>
<td>17.7 ± 8.8 (14, 8–40)</td>
<td>0.059</td>
</tr>
<tr>
<td>Severe hypoglycemia Total number of episodes (median) 2.0 (0–100)</td>
<td>3.0 (0–100)</td>
<td>5.0 (0–46)</td>
<td>0.43</td>
</tr>
<tr>
<td>No previous episodes (% patients) 30.2</td>
<td>32.0</td>
<td>23.8</td>
<td>—</td>
</tr>
<tr>
<td>1–10 episodes (% patients) 40.6</td>
<td>42.6</td>
<td>33.3</td>
<td>—</td>
</tr>
<tr>
<td>&gt;10 episodes (% patients) 29.2</td>
<td>25.4</td>
<td>42.9</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are means ± SD with median and range in parentheses.

The Wechsler Adult Intelligence Scale- Revised (WAIS-R) (12) uses performance subtests to measure current intellectual performance (fluid and non-verbal intelligence) and are sensitive to disruption by organic brain disease. Four performance subtests were utilized (picture completion, object assembly, block design, and digit symbol tests).

The National Adult Reading Test (NART) (13) is relatively resistant to the effect of age and some types of organic brain disease. NART performance correlates more closely with premorbid IQ than demographic variables and was used to control for the confounding effects of prior intellectual ability (premorbid IQ, crystallized intelligence) in the present study.

Inspection time (14) was used to assess visual perceptual speed, a component of information processing ability. Participants discriminated between the spatial position (left or right) of the longer of two brieﬂy presented vertical lines. The stimuli were backward-masked; the presentation duration was varied, and the duration of time required to reliably distinguish the stimulus (85% correct) was termed the “inspection time.”

Choice reaction time (15) was used to assess psychomotor speed and completed tests of information processing ability, and 1,2,4- and 8,4,2-choice reaction times were examined.

The Borkowski Verbal Fluency Test (controlled association) (16) is thought to assess frontal lobe and executive function. Participants have 60 s to state as many words as possible, beginning with letters of the alphabet specified by the assessor.

The Paced Auditory Serial Addition Task (17) was used to assess the ability to sustain attention and concentration. Participants listened to a number list, which they were required to add together according to a given rule. After practice, two consecutive 61-number trials were performed with 4 and 2 s between successive digits, respectively.

Assessment of severe hypoglycemia exposure. Severe hypoglycemia was defined as an episode requiring external assistance to facilitate recovery (18), and exposure was assessed retrospectively using a validated and formatted hypoglycemia questionnaire (9). Participants were asked to discuss their history of severe hypoglycemia with family, partners, or friends before completing questionnaires to improve accuracy of estimates. The total number of lifetime episodes, frequency of episodes, and total numbers of hypoglycemic seizures, coma, and episodes requiring medical intervention were recorded.

Determination of APOE genotype. DNA was extracted from venous blood from all subjects using standard methods. APOE genotyping was performed using a PCR-restriction fragment–length polymorphism assay as described by Wrenham et al. (19).

Statistical analysis. SPSS version 10.0 (SPSS, Chicago, IL) was used for statistical analysis. Demographic factors influencing neuropsychological performance (age, sex, and duration of diabetes), premorbid intellectual ability (NART), and the groupings of interest (severe hypoglycemia category, APOE genotype) were evaluated by multivariate general linear modeling (multiple analysis of covariance, MANCOVA). Severe hypoglycemia was dichotomized into two groups (ε4−, ε4+). MANCOVA was used to determine the signiﬁcance (P) and magnitude (Eta^2) of the variables’ effects on cognitive performance.

Subjects. The clinical characteristics of participants are shown in Table 1. Data on glycemic control have not been included as the laboratory reference range, and the methodology for estimating glycated hemoglobin changed across the two time frames during which data from participants were collected. Subdivision of participants by APOE ε4 produced two groups, ε4+ (n = 21) and ε4− (n = 75). The clinical characteristics are shown in Table 1. The subgroups had similar premorbid intellectual ability (NART) and similar exposure to severe hypoglycemia. Those possessing ε4 were slightly older (P = 0.033, t test) and tended to have had diabetes of longer duration (P = 0.059, t test).

RESULTS

Severe hypoglycemia and neuropsychological performance. The range of exposure to severe hypoglycemia was wide, from those with naïve to severe hypoglycemia (30%) to those who had experienced >10 episodes (29%) (Table 1). No signiﬁcant difference in cognitive ability was observed between those previously exposed to severe hypoglycemia and those naïve to severe hypoglycemia (estimated marginal mean differences for those with and without a history of severe hypoglycemia are shown in Table 2).

APOE ε4 and neuropsychological performance. The APOE ε4 allele was associated with a disadvantage in current intellectual performance (WAIS-R performance test score, P = 0.037, Eta^2 = 0.072) and a trend toward poorer frontal lobe and executive functions (Borkowski Verbal Fluency, P = 0.063, Eta^2 = 0.057) after consideration of age, sex, duration of diabetes, preceding severe hypoglycemia, and premorbid intellectual ability (Table 3). The relative disadvantage associated with APOE ε4 appeared to be sex-speciﬁc (APOE X sex interaction: F = 2.28, P = 0.04, Eta^2 = 0.15). Because of the sex specificity, MANCOVA thereafter was performed separately for men and women, using a similar model but excluding sex as a between-subjects factor. APOE genotype signiﬁcantly inﬂuenced cognitive ability in women but not in men (Table 4). Women with APOE ε4 exhibited a signiﬁcant cognitive disadvantage affecting current intellectual performance (WAIS-R performance test score, P = 0.001, Eta^2 = 0.26) and frontal lobe and executive function (Borkowski Verbal Fluency, P = 0.016, Eta^2 = 0.15). The difference in ability associated with possession of APOE ε4 in women was moderate-to-large (Eta^2).

Apolipoprotein-E genotype and severe hypoglycemia. There was no statistical evidence of a detrimental interaction between APOE genotype, previous exposure to severe hypoglycemia, and cognitive ability (APOE X severe hypoglycemia interaction: F = 0.66, P = 0.68, Eta^2 = 0.05). The cognitive ability of subjects with the APOE ε4 allele, subdivided into those exposed previously to severe hypoglycemia (n = 15) and those with naïve to severe hypoglycemia (n = 6), is shown in Table 2. No signiﬁcant difference in cognitive ability was demonstrated between...
TABLE 2
Effect of severe hypoglycemia on cognitive ability and interaction with APOE

<table>
<thead>
<tr>
<th>Neuropsychological test</th>
<th>SH− Estimated marginal mean (SEM)</th>
<th>SH+ Estimated marginal mean (SEM)</th>
<th>Effect of hypoglycemia exposure</th>
<th>APOE by hypoglycemia interaction</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
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<tr>
<td>Performance (IQ WAIS-R raw score)</td>
<td>29</td>
<td>67</td>
<td>96</td>
<td>6</td>
</tr>
<tr>
<td>Frontal and executive function (Borkowski)</td>
<td>126.7 (4.4)</td>
<td>128.1 (2.7)</td>
<td>118.4 (7.6)</td>
<td>124.4 (4.8)</td>
</tr>
<tr>
<td>Early visual processing (inspection time)</td>
<td>37.3 (3.1)</td>
<td>41.3 (1.9)</td>
<td>33.9 (5.4)</td>
<td>38.7 (3.3)</td>
</tr>
<tr>
<td>Psychomotor speed (4-choice reaction time)</td>
<td>77.8 (9.8)</td>
<td>66.8 (6.1)</td>
<td>84.0 (17.2)</td>
<td>55.1 (10.7)</td>
</tr>
<tr>
<td>Attention and concentration (PASAT 2 s)</td>
<td>351.9 (12.9)</td>
<td>352.8 (8.0)</td>
<td>354.3 (22.5)</td>
<td>353.4 (14.1)</td>
</tr>
<tr>
<td></td>
<td>n</td>
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</tr>
<tr>
<td>Performance IQ (WAIS raw score)</td>
<td>38.1 (2.6)</td>
<td>34.8 (1.6)</td>
<td>39.7 (4.6)</td>
<td>34.9 (2.8)</td>
</tr>
</tbody>
</table>

Multivariate linear model [age, sex, NART, severe hypoglycemia category (naïve/previous exposure), and APOE category (ε4+ or ε4−)]. SH−, no previous severe hypoglycemia; SH+, previous severe hypoglycemia; ε4−, no ε4 allele; ε4+, ε4-positivity.

DISCUSSION

The presence of the APOE ε4 allele was associated with a significant disadvantage in current intellectual performance (WAIS-R performance test score) and in frontal lobe and executive functions (Borkowski Verbal Fluency) in women with type 1 diabetes. The deficits did not encompass all aspects of cognitive ability, were present only in women, and were evident at a younger age (39 years) than that which has been reported in people who do not have diabetes (7). Female susceptibility to APOE ε4-associated cognitive disadvantage has been described for WAIS-R performance test ability in healthy elderly women (20), although the pathogenesis of the possible sex difference remains unclear. Age influences the effect of APOE ε4 on cognitive ability; neuropsychological performance in healthy children (21) and healthy young adults (22) is not affected by the ε4 allele, but middle-aged otherwise healthy adults (mean age 46 years) have been observed to have impaired learning and memory ability (7). The younger age (median age 39 years) at which ε4-associated cognitive disadvantage was observed in adults with type 1 diabetes in the present study implies premature susceptibility compared with nondiabetic individuals.

Laboratory and clinical evidence supports the concept of ε4-associated premature cognitive aging in diabetes. Elderly people with type 2 diabetes and the ε4 allele exhibit greater cognitive decrements (23) and an accelerated cognitive decline (24) compared with age-matched healthy control subjects. APOE from people with diabetes is irreversibly glycated and exhibits less in vitro bioactivity (25) compared with nondiabetic control subjects.

TABLE 3
Effect of APOE (ε4− and ε4+), age, and premorbid intellectual ability (NART) on cognitive performance

<table>
<thead>
<tr>
<th>Neuropsychological test</th>
<th>n</th>
<th>Performance IQ (WAIS raw score)</th>
<th>Frontal and executive function (Borkowski)</th>
<th>Early visual processing (inspection time)</th>
<th>Psychomotor speed (4-choice reaction time)</th>
<th>Attention and concentration (PASAT 2 s)</th>
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<td></td>
<td>75</td>
<td>133.4 (2.6)</td>
<td>42.2 (1.8)</td>
<td>75.1 (5.8)</td>
<td>349.9 (7.6)</td>
<td>35.6 (1.5)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>121.4 (4.6)</td>
<td>36.3 (3.2)</td>
<td>69.6 (10.2)</td>
<td>354.9 (13.4)</td>
<td>37.3 (2.7)</td>
</tr>
</tbody>
</table>

Multivariate linear model [age, sex, NART, severe hypoglycemia category (naïve/previous exposure), and APOE category (ε4+ or ε4−)].
Hypoglycemia vs. nagation is required to verify these young women who have type 1 diabetes. Further investigations suggest that APOE/H9280 sectional studies (2) that examined older subjects with short duration, but controlling with retrospective cross-sectional studies (2) that examined older subjects with diabetes of long duration.

In conclusion, the data in this modestly powered study suggest that APOE ε4 confers a cognitive disadvantage in young women who have type 1 diabetes. Further investigation is required to verify these findings and to determine whether the APOE ε4 allele is associated with premature or accelerated cognitive aging in people who have type 1 diabetes, the diabetes-specific factors that may be mediating any such disadvantage, and the possible interaction with sex.

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REFERENCES