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ApoE Epsilon4 Allele and Disease Duration Affect Verbal Learning in Mild Temporal Lobe Epilepsy

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Summary: *Purpose:* To clarify the possible role of other factors including the ApoE ε4 allele for memory decline in temporal lobe epilepsy (TLE).

Methods: We conducted a neuropsychological and molecular study in 138 consecutive patients (78 female patients; mean age, 50.2 years, SD ± 17.9; range, 14 to 87 years) with mild nonlesional TLE, who rarely or never had seizures at long-term follow-up. The mean age at seizure onset was 33.0 years (SD, ±21.7), and the mean duration of epilepsy was 17.1 years (SD, ±15.7).

Results: Thirty-four (25%) of 138 patients had test scores indicating verbal learning deficit (VLD). The presence of an ApoE ε4 allele was associated with an increased risk of VLD (OR, 4.18; 95% CI, 1.66–10.55). The effect of the ApoE genotype was independent of both the age at epilepsy onset and disease duration as well as of a low educational level, which were separately associated with VLD (p values = 0.045, 0.001, and 0.001, respectively). A significant linear trend (p = 0.005) was seen in the relation between disease duration and cognitive impairment, with the highest risk being in patients with an epilepsy duration ≥25.5 years (OR, 7.06; 95% CI, 1.67–29.85), especially if they carried the ε4 allele (OR, 32.29; 95% CI, 5.23–195.72).

Conclusions: These results provide evidence for an alteration in cognitive performance as a function of the presence of the ApoE ε4 allele and point to the critical role of disease duration itself for cognitive impairment in TLE. **Key Words:** Verbal memory—Temporal lobe epilepsy—Genetic predisposition—ApoE.

In patients with temporal lobe epilepsy (TLE), significant cognitive deficits frequently develop, especially in the domains of memory and word finding, and impaired verbal memory in left TLE is the most consistent finding (1). Verbal memory is the prime basis of day-to-day learning and memory, so its impairment may significantly disrupt the patient's life (2). Several factors may adversely affect cognition, including the underlying pathology, the effects of the epilepsy itself, and the impact of recurrent seizures (3). In addition, antiepileptic drugs (AEDs) can affect memory by reducing the efficiency of coding and retrieval processes via changes in concentration and cognitive processing (4).

This issue of cognitive impairment in TLE has been addressed mainly in patients with severe, pharmacoresistant epilepsy (5,6), and it would appear to be basically a direct consequence of continued seizures. Nonetheless, the possibility also exists that other factors including a genetic susceptibility may contribute to the memory decline in TLE (7). A highly plausible genetic risk factor for memory impairment in TLE is the apolipoprotein E (ApoE) 4 allele, owing to the deposition of amyloid plaques in the temporal lobe (8), especially in patients with TLE (9–11). Besides, substantial evidence from animal models supports the negative influence of the ApoE polymorphism on the brain's ability to repair damage and form new synapses (12).

To clarify the possible role of other factors including the ApoE 4 allele for memory decline in TLE, we conducted a detailed neuropsychological and molecular study in a large group of patients with mild nonlesional TLE, who rarely or never had seizures at long-term follow-up (13).

METHODS

Patients

Data and evaluation procedures on our TLE patients have been reported in greater detail elsewhere (13–15).

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The study group consisted of 138 consecutive unrelated patients (78 women) who received a diagnosis of TLE on the basis of a constellation of clinical, EEG, and magnetic resonance imaging (MRI) criteria, which are considered to be reliable interictal indicators of TLE. The diagnosis of TLE was based mainly on typical temporal auras and/or interictal EEG discharges with a maximum over the temporal lobes (16). Lateralization was based on lateralized EEG discharges, with or without lateralized seizure features. Epileptiform discharges were diagnosed in the presence of focal spikes or sharp waves followed by slow waves. Waking and sleep EEG recordings were obtained in all patients and always included T_1 and T_2 electrodes. In all patients, brain MRIs were obtained by using sequences and slices to optimize visual detection of mesial temporal structures. Based on the MRI study, TLE was classified as nonlesional if no focal mass lesion such as cerebral tumor, cortical dysgenesis, vascular lesion or malformation, or posttraumatic scars were detected. TLE patients with neuroimaging evidence of mesial temporal sclerosis were included. Neurologic examination was unremarkable in all patients. None of our patients had mental retardation. No patient had a history of head injury or neurologic illness other than epilepsy. At the time of the study, the age of the patients ranged from 14 to 87 years (mean, 50.2 years; SD, ±17.9). Forty-six (33.3%) of 138 patients had a family history of seizures or febrile convulsions (FCs) in one or more first- to third-degree relatives. Sixteen (11.6%) of 138 patients had a personal history of FCs, which were simple in all of them but one. The mean age at seizure onset was 33.0 years (SD, ±21.7) with a range of 1–83 years. The mean duration of epilepsy was 17.1 years (SD, ±15.7). The follow-up time at our epilepsy clinic was always >12 months (range, 12–103 months). Of 138 patients, 42 (31%) had right-sided TLE, and 58 (42%) had left-sided TLE, whereas 17 (12%) of 138 had bilateral independent temporal paroxysmal activity. Interictal EEG did not show any epileptiform activity in the remaining 21 (15%) patients. Twenty-four (17%) patients had MRI abnormalities indicative of mesial temporal sclerosis. Patients with pharmacoresistant TLE were deliberately not included. All patients had only mild TLE, as they were seizure free, or had either occasional auras or not more than two disabling (complex partial or secondarily generalized) seizures per year, with or without appropriate antiepileptic medication (AED). Most patients (122; 88.4%) were receiving monotherapy, and the most-used drug was carbamazepine (CBZ), usually at subtherapeutic dosage. Of the 138 patients, three were untreated because of the very mild ictal symptoms, whereas the remaining 13 patients were taking a combination of two AEDs. The patients gave their informed consent to participate in this study.

Neuropsychological testing

The measures of cognitive performance were selected to sample from several broad domains of functioning, including general cognitive ability, verbal/figural memory as representative of temporal lobe functions, and tests of attention, verbal fluency, and abstract and conceptual reasoning skills. Summary measures of cognitive functioning with relative cutoff scores are reported in Table 1. General cognitive ability was indexed with the Mini-Mental State Examination (MMSE) (17). Verbal memory was assessed by Rey's 15-words Immediate and Delayed Recall (IR and DR) test (18). The IR score is the sum of the words recalled in the five trials; the DR score is the number of words recalled after 15 min. Visuospatial memory was assessed by the Immediate Visual Memory test (the number of correct recognitions) (19) and the Corsi Block-Tapping Task (20). The abstract and conceptual reasoning skills were explored by using (a) the Verbal Fluency test (21), with a score determined by the sum of correct words produced in 1 min for three letters (i.e., G, I, S); (b) phrase construction (22), with a score determined by the sum of the responses judged to be correct for each of five phrases; (c) the Raven's Progressive Matrices '38, with a score determined by number of correct identifications of picture completions (23); and (d) the modified version of Wisconsin Card Sorting Test persevering errors (24). Language was assessed by the Token test (25). Attention was indexed with the Stroop test (26).

All tests were administered by following standardized procedures (19), by examiners that were blind to the patients' electroclinical and genetic characteristics. The time required for the test administration was ~90 min. Normative data were already available for all the tests but the Stroop test, as they were obtained by means of testing a sample of normal Italian subjects matched for sex, age, and educational level (27–29). For each test, a subject was defined impaired when he or she was beyond the cut-off value

TABLE 1. *Neuropsychological brief-test battery performed in each patient*

Cognitive measures	Cutoff normal value
Mini-Mental State Examination (MMSE)[a]	
Attention	
Stroop test (color words)	<358.7
Verbal memory	
Rey's 15-words Immediate Recall (IR)	>28.53
Rey's 15-words Delayed Recall (DR)	>4.69
Visuospatial memory	
Visual Memory Test	>13.85
Corsi block-tapping task	>3.75
Abstract and conceptual reasoning	
Verbal fluency test	>17.35
Phase construction	>8.72
Raven Progressive Matrices	>18.96
Wisconsin Card-Sorting Test	<3
Language comprehension	
Token test	>26.5
Mood	
Hamilton Psychiatric Rating Scale	<8

[a]The MMSE was scored with reference to large population-based data from Italy (Grigoletto et al., 1999).

of that test. The procedure for obtaining these normative data, the correction factors, and the cut-off scores are reported elsewhere (27,28). The MMSE was scored with reference to large population-based data from Italy (29). Forty-six normal subjects matched for age, sex, and education served as controls for the Stroop test. For data analysis,we used the third Stroop card showing color words printed inink of different colors, and abnormality was defined as at least two SD below the mean value of the controls. Before the cognitive testing, each patient also was evaluated for depression with the Hamilton Psychiatric Rating Scale for Depression, with a score >8 indicating depressed mood (30).

Molecular methods

Venous blood was drawn from each individual after obtaining informed consent, and DNA was extracted from peripheral blood leukocytes by proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation, and the ApoE genotyping was performed by using previously described primers and scoring methods (31). Each reaction mixture was heated at 94°C for 5 min and put through 35 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, followed by extension at 70°C for 1 min and 30 s, with a final extension at 70°C for 10 min. After polymerase chain reaction (PCR) amplification, 10 units of Cfo I (Boehringer Mannheim) were added directly to each reaction mixture for digestion of ApoE sequences for ≥3 h at 37°C. Each reaction mixture was loaded onto a 20% polyacrylamide nondenaturing gel and electrophoresed at 100 V for 16 h and then viewed and photographed under ultraviolet light after staining with ethidium bromide.

The molecular study also was performed in 220 normal individuals matched for age, gender, and ethnicity. We did not perform stratification studies because all our patients as well as the controls are white and were born in southern Italy. An increasing belief is found that the problem of population admixture is not serious if association studies avoid gross levels of population structure [32].

Statistical analysis

The Hardy–Weinberg equilibrium was tested using the random-permutation procedure (33) and implemented in the Arlequin package, because the asymptotic methods were unreliable, given the very low expected frequencies for genotypes $\varepsilon2/\varepsilon2$, $\varepsilon2/\varepsilon4$ and $\varepsilon4/\varepsilon4$. Categoric variables were presented as counts and percentages, at univariate analysis, and were analyzed with the χ^2 test. Distributions of continuous variables were expressed as means and standard deviations. Differences in quantitative variables were assessed for statistical significance with Mann–Whitney *U* test because these data were not normally distributed. The frequencies of the ApoE genotypes were compared among cognitively impaired and cognitively preserved patients by using a standard χ^2 test. We also performed comparison when patients were grouped as carriers of at least one $\varepsilon4$ allele ($\varepsilon4+$ group, coded as "1") and in noncarriers of the $\varepsilon4$ allele ($\varepsilon4$-group, coded as "0").

The association of duration of epilepsy and cognitive impairment also was evaluated. We treated disease duration both as a continuous variable and as a categoric variable. We categorized it by using quartiles of the combined distribution of preserved and impaired subjects (quartiles were 4, 12, and 25.5). Odds ratios with 95% confidence intervals (95% CIs) were estimated by univariate logistic-regression analysis.

To control for potential confounding, we performed a multivariate logistic-regression analysis. Variables with a p value (at univariate test) <0.15 were considered as candidates for the multivariate model. Because age at onset and duration of epilepsy were co-linear, only duration was included in the multivariate analysis. A Likelihood Ratio test (LRT) was carried out to select the most important variables at multivariate level, comparing models with and without a given variable. To test whether a linear trend in the relation existed between disease duration and cognitive impairment (in the logit scale) orthogonal polynomials were used. LRT also was used to test the interactions between the variables in the final model with the only main effect. For each test, Bonferroni's adjustment was applied. Statistical analysis was performed with Statistical Package for Social Sciences software (SPSS version 11.0.1; Chicago, IL, U.S.A.) for Windows.

RESULTS

General cognitive ability as indicated by the MMSE was preserved in all patients, as were basic languages and perceptual functions. The mean education level was 7.38 years (SD, ±4.0), and the mean MMSE score was 25.9 (SD, ±4.1). Almost all patients performed within theaverage range on measures of visuoperceptual/visuospatial ability, attention, and executive functioning. The percentage of subjects impaired for visuospatial memory was very low [13 (9.4%) of 138 patients].

Importantly, 34 (25%) patients had test scoresindicating Verbal Learning Deficit (VLD), reflected by significantly low IR or DR scores or both. Examination of demographic characteristics did not show any statistically significant differences between the unimpaired and impaired patients for chronologic age (Table 2). Conversely, significant main effects on VLD were noted for patients with different ages at seizure onset ($p = 0.045$) and duration of epilepsy ($p < 0.001$; Tables 2 and 3). Moreover, on average, patients with VLD had significantly lower ($p < 0.001$) levels of education (Tables 2 and 3). At multivariate logistic-regression analysis, we found a significant linear trend ($p < 0.005$) in the relation between

TABLE 2. *Relation between demographic variables and verbal memory performance in patients with mild temporal lobe epilepsy*

Variables	Total (n = 138)	Impaired (n = 34)	Preserved (n = 104)	p Value
Antecedent FCs, no. (%)	16 (11.6)	4 (11.8)	12 (11.5)	0.971[a]
Female sex, no. (%)	78 (56.5)	25 (73.5)	53 (51.0)	0.021[a]
Education (yr), mean (SD)	7.38 (4.0)	5.35 (3.63)	8.05 (3.91)	<0.001[b]
Age (yr): mean (SD)	50.2 (17.9)	53.6 (15.7)	49.1 (18.5)	0.195[b]
Age at onset (yr): mean (SD)	33.0 (21.7)	26.2 (17.3)	35.3 (22.6)	0.045[b]
Duration of epilepsy (yr): mean (SD)	17.1 (15.7)	27.4 (19.7)	13.7 (12.5)	<0.001[b]
Depressed mood, no. (%)	17 (12.7)	6 (17.7)	11 (10.6)	0.359[c]
ε4+ no. (%)	24 (17.4)	12 (35.3)	12 (11.5)	0.004[d]
ε4– no. (%)	114 (82.6)	22 (64.7)	92 (88.5)	

[a]p Values are based on asymptotic χ^2distributions with one degree of freedom.
[b]Asymptotic p values for the Mann–Whitney test.
[c]Empirical p value is based on 10,000 assignments.
[d]p Value is based on asymptotic χ^2 distributions with one degree of freedom and corrected by using the Bonferroni method (uncorrected p = 0.002).

disease duration and cognitive impairment, with the highest risk in the latter quartile (Table 4; i.e., epilepsy duration ≥25.5 years (OR, 7.06; 95% CI, 1.67–29.85)]. Although a significantly different distribution was seen between impaired and preserved patients at univariate analysis for variable females (p value = 0.021, χ^2test; Table 2), this was not significant at the multivariate level. Evidence of a low mood was found in 17 (12.4%) patients, but depression did not have a significant impact on VLD (Table 2).

The relations between lateralization of EEG temporal focus and verbal memory are presented in Table 5. Epileptiform abnormalities were identified in 33 of 34 patients who had impaired verbal memory. No significant differences were noted between the two groups in terms of age, years of education, age at epilepsy onset, or disease duration. The two groups were thus comparable with respect to these variables. We found that bitemporal independent spiking mainly accounted for VLD (p = 0.001) in these TLE patients. Moreover, when comparing strictly unilateral left- and right-temporalpatients only, we found that left temporal epileptiform abnormalities also were associated with VLD (p = 0.025).

Finally, the relatively small number [24 (17%)] of patients who had MRI abnormalities indicative of mesial temporal sclerosis did not allow any conclusions to be drawn about its relation with verbal memory.

TABLE 3. *Odds ratios and 95% confidence interval in patients affected by mild temporal lobe epilepsy with verbal memory impairment*

	Odds ratio (95% CI)	
Variable	Univariate analysis	Multivariate analysis[a]
ε4+ vs. ε4–	4.18 (1.66–10.55)	4.94 (1.71–14.30)
Duration of TLE (yr)	1.31 (1.15–1.50)[b]	1.26 (1.09–1.45)[b]
Education (yr)	0.37 (0.21–0.67)[b]	0.42 (0.21–0.81)[b]

[a]Multivariate logistic regression model including ApoE status, education and duration of TLE.
[b]Odds ratios and 95% CI for interval of 5 years.

Apolipoprotein E allele and cognitive function

Patients were then divided into two groups according to the presence or absence of the ApoE ε4 allele: ε4 (n = 24; two, 2/4; 21, 3/4; one, 4/4) and non-ε4 (n = 114; 13, 2/3; 101, 3/3). None of the observed genotype counts deviated from those expected according to the Hardy–Weinberg equilibrium (exact p value: all patients, p = 0.905; patients with memory impairment, p = 0.657; patients without memory impairment, p = 0.474). We found that the presence of an ApoE ε4 allele was associated with an increased risk of VLD (OR, 4.18; 95% CI, 1.66–10.55; Table 3). Indeed, 12 (50%) of 24 patients carrying one ε4 allele had VLD, compared with only 22 (19%) of 114 in the noncarrier group (p = 0.004; Table 2).

We also found that patients with the ε4 allele had an onset of habitual seizures on average 7 years earlier than patients not carrying the ε4 allele (with ε4, 26.2 ± 20.1 years; without ε4, 33.9 ± 20.7 years; p = 0.08). So we examined whether the effect of the ApoE genotype on verbal memory was influenced by both age at onset of epilepsy and disease duration, as well as by a low educational level. The multivariate analysis revealed that taking these variables into account did not alter these findings (OR, 4.94; 95% CI, 1.71–14.30; Table 3). Moreover, patients carrying the ε4 allele who also had long disease durations (≥25.5 years) had the highest risk (OR, 32.29; 95% CI, 5.23–195.72) of developing verbal memory impairment in comparison with those not carrying the ε4 allele with a shorter (>4 years) duration of epilepsy and similar educational level. Furthermore, analyses examining the distribution of the ApoE genotype by age group did not reveal any significant differences, indicating that presence or absence of an ε4 allele was evenly distributed. The average education was comparable between carriers and noncarriers of the ε4 allele.

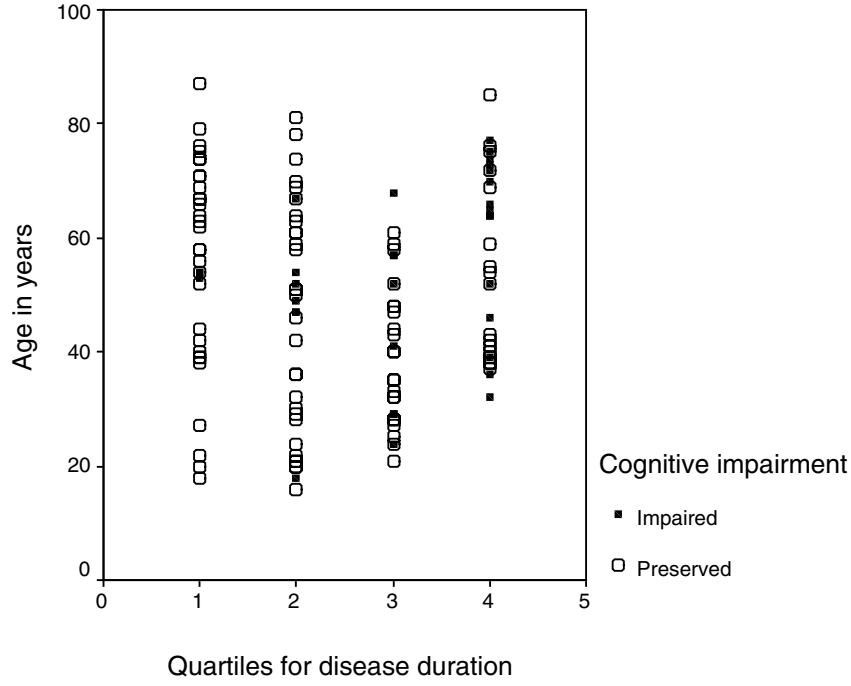
Finally, consistent with our previous findings (14), the proportion of each ApoE genotype or allele in our patients was very similar to that found in the controls (Table 6), as well as in the general population in southern European countries (34).

TABLE 4. *Relation between duration of epilepsy and risk of memory decline*

A. Distribution of preserved and impaired subjects per quartiles, with relative odds ratios

Duration of epilepsy	Impaired (n = 34)	Preserved (n = 103)	p Value[a]	Odds ratio[b] (95% CI)
Duration <4 yr	3 (8.6)	32 (91.4)	0.005	1.0
4 < duration < 12 yr	7 (18.9)	30 (81.1)		2.28 (0.50–10.39)
12 < duration < 25.5 yr	8 (25.8)	23 (74.2)		4.48 (0.96–2 1.00)
Duration > 25.5 yr	16 (47.1)	18 (52.9)		7.06 (1.67–29.85)

B. Plots of verbal memory performance for each patient



[a]p Value is based on orthogonal polynomials method to test linear trend in logistic regression.
[b]ORs and 95% CIs were estimated by using multivariate logistic-regression model including the variables: ApoE and education.

DISCUSSION

Our results indicate strong evidence for an alteration in verbal learning performance as a function of the presence of at least one $\varepsilon 4$ allele in a patient with TLE. Patients with ApoE $\varepsilon 4$ were about fourfold as likely as those without ApoE $\varepsilon 4$ to perform worse on measures of verbal memory functioning. The effect of the ApoE genotype on verbal learning was not influenced by either the patient's educational level, age at onset of epilepsy, or disease duration, which independently increased the risk of verbal learning impairment. Moreover, the combined effect of long disease duration and the ApoE $\varepsilon 4$ allele greatly increased the risk of cognitive dysfunction.

These results are consistent with recent evidence indicating that even in TLE, the ApoE $\varepsilon 4$ allele promotes the intracerebral accumulation of β-amyloid (11), which plays a central role in the pathogenesis of memory dysfunction, probably by increasing neuronal susceptibility to damage (35). In this way, the present study adds to the evidence that ApoE may play an active role in modulating some clinical features, such as age at disease onset and prognosis, not only in Alzheimer disease (36) but in other neurologic conditions as well (36,37), without being a significant risk factor for their eventual occurrence.

TABLE 5. *Relation between interictal EEG abnormalities and verbal memory function in temporal lobe epilepsy (A) with relative odds ratios (B)*

A

Temporal epileptiform activity	Impaired (n = 33)	Preserved (n = 84)	p Value[a]
Bilateral	10 (30.3)	7 (8.3)	0.001
Right	5 (15.2)	37 (44.0)	
Left	18 (54.5)	40 (47.6)	

B

Temporal epileptiform activity	OR (95% CI)	p Value
Bilateral vs. unilateral (R or L)	4.783 (1.637–13.976)	0.002
Left vs. right	3.330 (1.123–9.875)	0.025

[a]Empiric p value is based on 10,000 assignments.

TABLE 6. *Allelic and genotypic distributions of the apolipoprotein E polymorphisms in patients with temporal lobe epilepsy and in controls.*

ApoE polymorphism	Patients (n = 138)	Controls (n = 297)	p Value
Genotype (%)			
ε2-2	0	1 (0.3%)	
ε2-3	13 (9.5%)	38 (12.8%)	
ε2-4	2 (1.4%)	3 (1.0%)	
ε3-3	101 (73.2%)	227 (76.4%)	
ε3-4	21 (15.2%)	27 (9.2%)	
ε4-4	1 (0.7%)	1 (0.3%)	NS
Allele (%)	(n = 276)	(n = 594)	
ε2	15 (5.5%)	43 (7.2%)	
ε3	236 (85.5%)	519 (87.4%)	
ε4	25 (9.0%)	32 (5.4%)	NS

Moreover, our results remain consistent with a recent study that found the ApoE ε4 to be related to a significantly accelerated memory decline with age in healthy subjects (38). In another study (39), some evidence indicated that patients with severe partial epilepsy carrying the ε4 allele had significantly lower IQs. Moreover, a recent association study in patients with severe TLE illustrated that the ApoE ε4 allele was associated with an earlier onset of habitual seizures by shortening the silent interval before the onset of TLE (40). Similarly, in our patients, we observed that the age at onset of TLE tended to be lower in those carrying the ApoE ε4 allele, but this trend was not significant.

Another, no less important, finding of the present study was that, in parallel with the ApoE deleterious effect, the disease duration–related decrement in verbal learning performance was seen. An earlier age at seizure onset also had a significant but lesser impact on verbal memory, whereas the patient's chronologic age had no effect. A plausible interpretation of these findings is that, because epilepsy in our patients was very mild with almost no seizures at long-term follow-up, the underlying epileptic process itself may cause additional damage to temporal structures with consequent remodeling of synaptic organization leading to related memory decline. In accordance with this hypothesis, good evidence from experimental studies indicates that long-term alteration in neuronal plasticity and memory function may be the consequence of even single or brief repetitive seizures (41,42). Our view is also supported by a recent longitudinal imaging study that illustrated that secondary hippocampal volume loss may occur even in milder forms of TLE (43). Moreover, a larger study of a community-based population found evidence of significant focal or diffuse cortical volume losses in a significantly higher proportion of patients with newly diagnosed epilepsy, compared with age-matched controls (44). The fact that most of our patients had normal MRIs did not allow us to make a comparison between the degree of temporal atrophy and several variables including epilepsy duration, memory deficit, and the ApoE genotype. Only computerized quantitative MRI studies are sensitive enough techniques to reveal subtle focal temporal or generalized atrophy (43,44).

We cannot rule out, however, the possibility that a longer exposure to AEDs might also contribute to verbal learning impairment in our patients. Nonetheless, because most of our patients were taking low dosages of CBZ, which has only a modest, dose-related, adverse cognitive effect (45), it is conceivable that the long-term AED therapy had almost no contribution to verbal memory impairment in our patients. The impact of AEDs on memory, indeed, is strongly related to high dosages and polytherapy (4). Accordingly, most of our patients performed well on neuropsychological measures, such as visuoperceptual/visuospatial ability and attention, which mainly are affected by AEDs (46).

To our knowledge, this the first systematic neuropsychological study carried out in consecutive patients with TLE, who had mild courses and typically entered remission with or without AEDs. With the exception of an old report (47), mild TLE has received little attention in the past, probably because most studies on TLE have originated from groups with special interests in surgical treatment. However, as also supported by epidemiologic studies (48,49), in the last few years, evidence has emerged that mild TLE is a rather common epileptic syndrome with typical onset in adulthood (13,43), in which genetic factors seem to play a major etiopathogenetic role (15,50). The relation between mild TLE and severe pharmacoresistant TLE seems to be far more complex, and genetic studies give some suggestion that these conditions might lie on a biologic continuum (51). The results of the present study illustrate that, similar to severe TLE (5,6), even patients with mild TLE may develop some cognitive dysfunction, which is strictly focused on verbal learning difficulty. The patients were defined as impaired by using the cut-off at a rather low level, so persons with mild impairment might be in the nonimpaired group. Nonetheless, we preferred to be conservative because we used only two tests for defining VLD and also because each test was scored with reference to large population–based data.

Interesting, the verbal learning impairment in our patients correlated with the occurrence of bitemporal spiking activity, further supporting the theory that worst memory performance is associated with bitemporal lobe dysfunction in patients with TLE (52). It is not surprising that a protective effect on verbal memory is induced by a higher educational level, as demonstrated in some of our patients. Evidence exists that persons with higher educational levels are more resistant to the effects of cognitive decline, probably as a result of having greater cognitive reserve and increased complexity of neuronal synapses (53).

Finally, the results of recent studies (15,54–56), including ours, reinforce the belief that association studies seem to be a powerful approach to identify genes implicated in complex multietiologic diseases such as TLE (57). TLE, indeed, is typically a complex disorder in which more than one gene, with or without the influence of acquired factors, acts in a multifactorial fashion and results in the specific clinical problems that clinicians attempt to unravel and treat in any given patient. We are, however, aware of the limits and subtle biases of any association study (57). Moreover, because of the lack of longitudinal data on the participants, we also are unable to describe any ApoE-related changes in cognitive functioning. In addition, the scarcity of individuals homozygous for the $\varepsilon 4$ allele ($n = 1$) makes an exploration of dose–response relations not feasible. We await further studies in independent population samples to confirm our results. It should be noted, however, that our study fulfils the fundamental requisites for performing genetic investigations of complex multietiologic diseases (57). Particularly important is the fact that we collected patients in whom confounding influences for developing cognitive impairment in TLE (3), such as recurrent uncontrolled seizures, previous head trauma or severe cerebral insults, structural lesion, and polytherapy with AEDs, were all absent.

In conclusion, the results of the present study provide good evidence for an alteration in cognitive performance as a function of the presence of the ApoE $\varepsilon 4$ allele and point to the critical role of disease duration itself for cognitive impairment in TLE. These findings on the factors influencing cognitive performance in TLE will prompt new directions in researching better and more appropriate strategies in the treatment of epilepsy.

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