J. Cell. Mol. Med. Vol 9, No 3, 2005 pp. 698-703

Association of refractory complex partial seizures with a polymorphism of ApoE genotype

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Received: May 26, 2005; Accepted: August 29, 2005

Abstract

Apolipoprotein E (ApoE) is a constituent of many types of lipoproteins that play a role in metabolism of cholesterol and lipids in the body as well as in the brain. ApoE is synthesised in astrocytes and microglia and enter to neurons through LDL, LRP and VLDL receptors. Recently it was shown that ApoE is also produced in neurons. ApoE has a role in modulating learning and memory, structural plasticity, mobilization of cholesterol in repair, growth and maintenance of myelin and neuronal membranes during development and aging, and cell death after ischemic, convulsive, or other type of brain injury. The aim of this research was to investigate the possible association of ApoE gene polymorphism with the development of resistance to pharmacological therapy in patients with partial complex seizures with or without secondary generalization. In this prospective matched-pair controlled study, 60 patients with cryptogenic epilepsy with complex partial seizures, with or without secondary generalization, who have been suffering for five or more years, were studied. The first group comprised 30 patients refractory to the current therapy, while the second group consisted of patients with well-controlled seizures. The refractory and non-refractory groups of patients differed significantly in their phenotypes. Phenotype E3/4 was six times more frequent in refractory group than among non-refractory group. The lack of response was shown to be significantly associated with the presence of ε4 allele. This study provided evidence that the presence of ε4 allele is more often associated with a lack of response to current antiepileptic drugs as compared to ε2 and ε3 alleles.

Keywords: ApoE polymorphism • refractory epilepsy • complex partial seizures • ischaemia/reperfusion • non-responders • pharmacotherapy

Introduction

Epilepsy is a common disorder affecting up to 1% of the population. Complex partial seizures (CPS) occur in about 35% of patients with epilepsy. Twenty percent of patients with CPS have uncontrolled seizures, refractory to anticonvulsant therapy. The etiology of epilepsy is often multifactorial, with 60–70% of all cases without clear cause, although much is known about the physiological bases of abnormal discharges accompanying seizure phenomena. It seems likely that there is a primary defect in the neuronal membrane that results in the instability of the resting membrane potential [1]. There is no clear biological

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or neurophysiological marker that will predict resistance to therapy.

Apolipoprotein E (ApoE) is a constituent of many types of lipoproteins that play a role in metabolism of cholesterol and lipids in the body as well as in the brain. ApoE is a major determinant of the recognition and the uptake of the lipoproteins *via* low density lipoproteins (LDL) receptor, the LDL receptor related protein (LRP), the apoE receptor 2 and very low density lipoprotein (VLDL) receptor and megalin. ApoE is synthesised in astrocytes and microglia and enter to neurons through LDL, LRP and VLDL receptors. Recently it was shown that ApoE is also produced in neurons [2]. ApoE has a role in modulating learning and memory, structural plasticity, mobilization of cholesterol in repair, growth and maintenance of myelin and neuronal membranes during development and aging, and cell death after ischemic, convulsive brain injury or other brain injury [3]. The human ApoE gene shows polymorphism, with the three different alleles, termed ε2, ε3, ε4 that give rise to six different phenotypes (E2/2, E2/3, E2/4, E3/3, E3/4, E4/4). ApoE 3 is the most common isoform (77–78%) in general population, while ApoE 2 is found in 7–8% and ApoE 4 in 14–16% of individuals. ApoE ε3 and ApoE ε2 bind preferentially high-density lipoproteins (HDL) [4]. ApoE ε2 binds defectlively to the LDL receptors and carriers have high risk to develop type III hyperlipoproteinemia. Apo ε4 carriers have a higher risk of cardiovascular diseases, several neurodegenerative disorders including Alzheimers disease, Lewy Body Dementia and frontal lobe dementia. [5].

Apolipoprotein E first came to the attention of neurologists as a result of the genetic association between ApoE ε4 and a late onset of Alzheimer's disease [6]. Furthermore, ApoE ε4 is associated with an increased risk of ischemic cerebral infarction [7], predicts poor outcome after intracerebral haemorrage [8] and severe traumatic brain injury [9] and is associated with faster progression of disability in multiple sclerosis [10]. The continuous occurrence of epileptic seizures despite the use of several antiepileptic drugs, even as polytherapy at maximal tolerated doses, is the major health problem in epileptology. The role of Apo E polymorphism in epilepsy, especially intractable epilepsy has been investigated in several studies with various results. ApoE levels were significantly increased in rats hippocampi several days after status epilepticus and that was confirmed *in vitro* on organotypic cultures with kainate treatment. These observations indicate that lipid metabolism is modified in the lesioned structure that follows severe seizures [11].

The aim of this research is to investigate the possible association of ApoE gene polymorphism with the development of resistance to pharmacological therapy in patients with CPS with or without secondary generalization.

Patients and methods

Patients

In this prospective matched-pair controlled study, 60 adult patients with cryptogenic epilepsy with complex partial seizures, with or without secondary generalization, who have been suffering for five or more years, were included (Table 1). The study was performed during 3 years (2001–2004), in Univerity Hospital Zagreb, Croatia. The first group comprised 30 patients refractory to the current therapy (non-responders), while the second group consisted of patients with well-controlled seizures (responders). Refractory epilepsy was defined as four or more fits in a 4-week period during the last 12 weeks with two or more antiepileptic drugs in therapy. Well-controlled seizures were defined without any fit in the same period. Patients who were between this two groups are excluded from the study. Responders took significantly lower number of medications ($Z=-3.576$; $p=0.0003$). Median value of taken medications was 2 in responders and 3 in non-responders (Table 2). Compliance was determined by measuring concentration of anticonvulsants in the serum [12, 13]. Patients with a history of the central nervous system infection, head trauma, brain tumor, pseudo-attacks, neurodegenerative and psychiatric diseases were excluded from the study.

The study was approved by local ethical committee, and all patients have signed informed consent to take part in the study.

Methods

Genotyping

A blood sample for the estimation of ApoE genotype was taken from all patients. The sample was cen-

Table 1 Basic characteristics of study groups

	Non-refractory	Refractory
N (% from total)	30 (50)	30 (50)
Positive genetic loading (%)	4 (13.3)	3 (10.0)
Age at the onset of disease*	19.5 (11.9)	12.9 (6.5)*
Duration of illness - mean (±S.D)	21.3 (12.2)	24.1 (15.7)
Febrile convulsions (%)	3 (10.0)	5 (16.7)

The disease was of a significantly earlier onset in the refractory group of patients (F=7.2; p=0.001). Still, the overall duration of the disease did not differ between groups (F=0.58; p=0.449)
* significantly earlier onset in the group of refractory patients

trifuged, the cells were separated from the liquid blood phase, and both fractions were deeply frozen. Leukocyte DNA was extracted as it is described by Hixson J *et al.* [14]. Restriction isotyping (restriction enzyme isoform genotyping) was used for rapid typing of common apolipoproteine E isoforms. ApoE restriction isotyping used oligonucleotides to amplify apolipoprotein E gene sequences containing amino acid positions 112 and 158. Each amplification reaction contained 1µg of leukocyte DNA, 1pmol/µL of each primer, 10% dimethyl sulfoxide and 0.025 units/µL of Tag polymerase in a final volume of 30µL. Each reaction mixture was heated at 95°C for 5 minutes for denaturation and subjected to 30 cycles of amplification by primer annealing, extension and denaturation. Amplification of apoE products that were suitable for Hhal. After PCR (polymerase chain reaction) 5 units of Hhal were added directly to each reaction mixture for digestion of apoE sequences (>3h at 37°C). Each reaction mixture was loaded onto an 8% polyacrylamide

Table 2 Medication

	Responders N (%)	Non-responders N (%)
carbamazepine	28 (93.3)	28 (93.3)
methylphenobarbitone	12 (40.0)	9 (30.0)
phenytoin	3 (10.0)	10 (33.3)
sodium valproate	2 (6.7)	5 (16.7)
lamotrigine	8 (26.7)	17 (56.7)
topiramate	1 (3.3)	3 (10.0)
gabapentin	1 (3.3)	2 (6.7)
primidone	1 (3.3)	4 (13.3)

Two medications were more frequently used in non-responders group: phenytoin (p=0.028) and lamotrigine (p=0.018). Carbamazepine, methylphenobarbitone, sodium valproate, topiramate, gabapentin and primidone did not differ significantly.

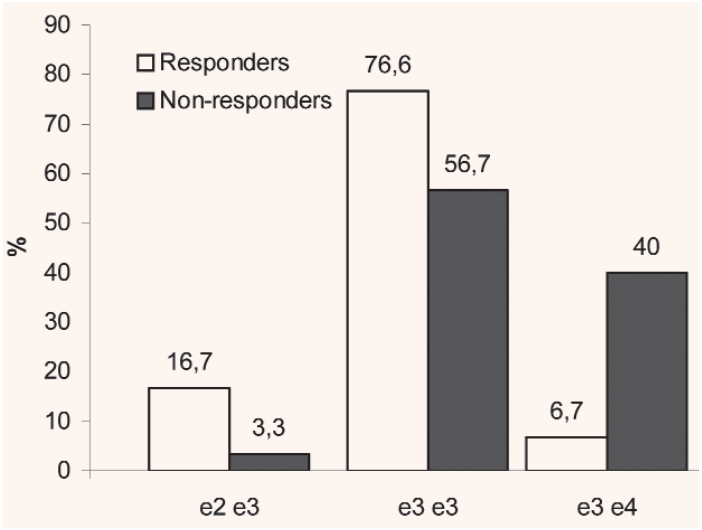

Fig. 2 There is a significant genotype difference between responders and non-responders. The most frequent phenotype in both groups was E3/3. Phenotype E3/4 was six times more frequent in refractory group than among non-refractory group. Five subjects (16.7%) in the non-refractory group, and 1 (3.3%) in the refractory group had present ε2 allele, a difference below the level of significance (the Fisher exact one-sided p=0.097). The lack of response was shown to be significantly associated with the presence of ε4 allele: ε4 allele was present in only 2 (6.7%) subjects who responded to the treatment, and in 12 (40.0%) who did not respond (the Fisher exact one-sided p=0.002). 95%CI of the observed difference in proportions are assessed between -0.53 and -0.14 (CI 95% = -0.53-0.14).

nondenaturing gel (1.5mm thick x 25cm long) and electrophoresed for 3h under constant current (45mA). After electrophoresis, the gel was treated with ethidium bromide (0.2mg/L) for 10 minutes and then DNA fragments were visualized. Each of the isoforms was distinguished by a unique combination of Hhal fragment size that enabled unambiguous typing of all homozygotic and heterozygotic combinations [15].

Statistics

As this was a pilot study, no formal sample size calculation was undertaken.

Continuous variables were analyzed with 1-way ANOVA and categorical variables with χ^2 test. The association of treatment response was analyzed by Fisher's exact test. All tests were applied at α=0.05.

Results

There is a significant genotype difference between responders and non-responders. The most frequent phenotype in both groups was E3/3. Phenotype E3/4 was six times more frequent in refractory group than among non-refractory group. Five subjects (16.7%) in the non-refractory group, and 1 (3.3%) in the refractory group had present ε2 allele, a difference below the level of significance (the Fisher exact one-sided p=0.097). The lack of response was shown to be significantly associated with the presence of ε4 allele: ε4 allele was present in only 2 (6.7%) subjects who responded to the treatment, and in 12 (40.0%) who did not respond (the Fisher exact one-sided p=0.002). 95% CI of the observed difference in proportions are assessed between -0.53 and -0.14 (CI 95% = -0.53-0.14) (Fig. 1).

The disease was of a significantly earlier onset in the refractory group of patients (F=7.2; p=0.001). Still, the overall duration of the disease did not differ between groups (F=0.58; p=0.449) (Table 1).

Among medications used to treat epilepsy in the study patients two drugs were significantly more frequent in non-responders: phenytoin (p=0.028) and lamotrigine (p=0.018). Carbamazepine, methylphenobarbitone, sodium valproate, topiramate, gabapentin and primidone did not differ significantly (Table 2).

Discussion

Our study indicates that the lack of response to anticonvulsants in patient with CPS might be associated with the presence of ε4 allele. In addition, the presence of ε2 genotype was five times more frequent among the responders (non-refractory group of patients). This data is particularly interesting since *in vivo* studies using transgenic mice models that express human ApoE 3, ApoE 4 demonstrated that ApoE 3/3 mice are more protected than E3/4 mice against excitotoxin induced degeneration, while E4/4 mice shows no protection. This data may sug-

gest that ApoE 4 is not only less protective than ApoE 3, but act as dominant negative factor that interferes with the beneficial function of ApoE 3 [16]. Thus we may suggest that in the epilepsy presence of ApoE 4 weaken the recovery/plasticity of the cell membrane due to the destabilization of lipid binding, distribution of lipids and cholesterol homeostasis (this is opposite for ApoE 2) what may lead to the instability in the neuronal membrane, more frequent uncontrolled discharging and more often refractory events in the epileptic patients. Lipid redistribution and cholesterol homeosthasis have been shown to participate in processes of growth, remodeling and regeneration of synapses. In general, anticonvulsants are believed to work either by blocking action potentials or by interfering with synaptic transmission. The mechanisms by which they accomplish this include limitation of sustained repetitive firing (inhibiting Na channels and stabilizing neuronal membranes), enhancement of GABA-mediated inhibition, attenuation of activity of voltage sensitive Ca channels and decrements in glutamate-mediated excitation [17, 18]. Even a medication with anticonvulsants needs to have stabile membranes as a target and that might not be case in refractory epilepsy. In majority of medications used to treat epilepsy in our patients two medications were more frequently used in non-responders group: phenytoin ($p=0.028$) and lamotrigine ($p=0.018$). The significance of this finding is still unclear and rather indicate that this anticonvulsants of second and fourth generation are the most often prescribed as add on therapy in patients with partial attacks. This two antiepileptics, together with carbamazepine, act through inhibition of Na^+ channels and stabilization of neuronal membranes, in apparent opposition to our suggestion regarding the function of Apo E4. If the presence of ε4 allele of ApoE in epileptic patients leads to worsening of the patients outcome by refractory attacks, than ε4 allele can be considered a factor predisposing the epileptic population to be more prone to attacks (despite therapy) and less prone to post attack recovery.

The continuous occurrence of epileptic seizures despite the use of a polytherapeutic approach with several antiepileptic drugs is the major health problem in epileptology. Early onset, remote symptomatic causes, multiple seizure types, and a high frequency of seizures before initiation of treatment are some factors that may predict intractability (19–21). Further on, the polymorphism in the drug transporter gene ABCB1 (P glycoprotein 170) has biological plausibility in patients with multidrug resistant epilepsy [22]. The biological basis of resistance is likely to be multifactorial and variable. Some possible contributing factors are: ion channelopathies, altered neurotransmitter receptors, reactive autoimmunity (against glutamic acid decarboxylase), causative neuropathology, hyperexcitable and disinhibited neuronal network reorganisation and impaired antiepileptic drug penetration. Our results also suggest that the disease has a significantly earlier onset among non-responders (the group of refractory patients).

The role of Apo E polymorphism in epilepsy has been investigated in few studies. It has been reported that apolipoprotein E polymorphism is not associated with the development of major seizure disorders including temporal lobe epilepsy [23–25]. Moreover, the inheritance of the ApoE ε4 allele is associated with increased risk of late posttraumatic seizures [26] and with earlier onset of temporal lobe epilepsy [27]. Intractable temporal lobe epilepsy with senile plaques in epileptogenic focus had a 70% ApoE ε4 carrier frequency as compared to 27% among age-matched controls without senile plaques [28]. ApoE ε4 allele and disease duration affect verbal learning in mild temporal lobe epilepsy [29].

In conclusion, results of our preliminary study suggest a possible association of ApoE gene polymorphism with the development of resistance to pharmacological therapy in patients with CPS with or without secondary generalization. Thus, ApoE genotype might be an early susceptibility factor which can indicate the prospective clinical picture and detect responders and non-responders among patients with refractory complex partial seizures.

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