No association between ApoE polymorphism and febrile seizures

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Abstract Seizures associated with fever are a common pediatric problem, affecting about 2–7 % of children between 3 months and 5 years of age. Differentiation of febrile seizures from acute symptomatic seizures secondary to central nervous system infections or seizures associated with fever in children with epilepsy is essential to provide appropriate treatment and follow-up care. Here, we tested the hypothesis that children who exhibit simple febrile seizures during early childhood, but do not develop epileptic seizures later in life, might preferentially carry the ApoE2 allele of the gene coding for the apolipoprotein E. We did not find any differences in the distribution of ApoE alleles or genotypes between individuals who exhibited simple febrile seizures ($n = 93$) and age-matched, typically developing subjects ($n = 80$). We found that the observed allele and genotype frequencies did not deviate from Hardy–Weinberg equilibrium, which suggests that the frequencies of ApoE alleles and genotypes are stable in the Swiss population from which our samples were derived. Across both groups of subjects ($n = 173$), we found an ApoE2 allele frequency of 0.064, an ApoE3 frequency of 0.829 and an ApoE4 frequency of 0.107. Our findings are consistent with previous reports of the distribution of ApoE polymorphism for European subjects free of any neurological disorders, and show that the different alleles of the gene coding for the apolipoprotein E are not associated with the occurrence of simple febrile seizures.

Keywords Genetics · Epilepsy · Hippocampus · Fever · Astrocytes · Prostaglandins · Apolipoprotein · Development · Children · Pediatrics

Introduction

Seizures associated with fever are a common pediatric problem, affecting about 2–7 % of children between 3 months and 5 years of age [1]. Although the vast majority of children who present with febrile seizures do not develop epilepsy, the distinction between epilepsy and febrile seizures is important since the first manifestation of the epilepsy syndrome might occur during a fever episode at a young age. If a diagnosis of epilepsy has been established prior to the episode, appropriate therapeutic measures are already in place. If not, however, physicians must wait to see if further seizures develop in order to provide follow-up care. Adequate diagnosis is, therefore, key to adequate treatment and patient education that can alleviate parents' anxiety and allow the family return to normal life. To achieve this goal, a number of researchers and practitioners have called for an increased effort to determine the genetic factors predisposing to febrile seizures and understand the relation between febrile seizures and specific epilepsy syndromes [2–8].

The importance of genetic factors in the incidence of febrile seizures has long been recognized [9, 10], but

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specific genes that affect febrile seizure cases, and which are not part of a broader phenotypic spectrum that includes afebrile seizures, have yet to be conclusively identified [5, 11]. For example, interleukin 1-beta has been linked to the generation of febrile seizures [3], but increased frequency of the interleukin-1-beta-511T allele has also been reported in patients with temporal lobe epilepsy [12]. Moreover, the mechanisms by which variants of these particular genes could contribute specifically to febrile seizures that are not associated with epilepsy have yet to be proposed.

Here, we tested the hypothesis that children who exhibit simple febrile seizures during early childhood, but do not develop epileptic seizures later in life, might preferentially carry the ApoE2 allele of apolipoprotein E. This hypothesis was based on findings showing that a number of genes related to astrocytic processes and functions are differentially expressed in the primate hippocampus across postnatal development [13]. In humans, the highest incidence of seizures is in the first 2 years of life and is most often associated with a febrile illness [14]. Fever begins with the activation of immune response cells that produce interleukin-1, which in turn increases prostaglandin E2 synthesis [15]. Prostaglandins act at the level of the hypothalamus to regulate body temperature and induce fever. Interestingly, prostaglandins also stimulate calcium-dependent glutamate release in astrocytes [16, 17], which can induce abnormal prolonged depolarization with repetitive spiking in CA1 pyramidal neurons leading to seizures [18, 19]. Thus, the relatively high astrocytic coverage of the dense network of CA1 excitatory synapses in the newborn [13] might contribute to a higher incidence of febrile seizures. The decrease in astrocytic coverage of hippocampal excitatory synapses with development might in turn reduce the probability of neuronal depolarization evoked by astrocytic glutamate release, and thus contribute to the decreased susceptibility to febrile seizures with age. The pertinent question, then, is why only 2–7 % of children exhibit febrile seizures [2, 3, 6–8]?

To address this question, we considered the molecular machinery involved in glutamate uptake and release in astrocytes. We focused on ApoE as a candidate gene for three reasons: (1) ApoE is located on chromosome 19q thought to contain susceptibility genes associated with febrile seizures, (2) three different genotypes (ApoE2, ApoE3, ApoE4), linked to single nucleotide polymorphisms, have been identified in humans, and (3) apolipoprotein E found in the brain is synthesized in astrocytes and plays a major role in the maintenance of lipid homeostasis and vesicular trafficking [20, 21]. In Europeans free of any neurological disorders, the distribution of different alleles is stable, with an ApoE2 frequency of 0.068, an ApoE3 frequency of 0.813 and an ApoE4 frequency of 0.119 [22]. ApoE2 is more highly

retained in astrocytes than ApoE3 and ApoE4 (Fan and Fazio, personal communication; see [23] for similar results in macrophages) and might thus facilitate the prostaglandin-induced, calcium-dependent exocytosis of glutamate by astrocytes [16, 17]. In turn, increased glutamate concentration in the synaptic cleft might induce prolonged depolarization with repetitive spiking in neurons leading to seizures. Interestingly, previous studies have considered the potential link between ApoE and seizures, with some studies specifically interested in febrile seizures focusing their analyses on ApoE4 [24], the allele known to be a risk factor for Alzheimer's disease, and other studies considering all three ApoE alleles included other seizure phenotypes [25, 26]. However, none of the previous studies was adequately designed to test the hypothesis that ApoE2 might confer a specific predisposition to febrile seizures.

Materials and methods

We recruited ninety-three children (45 females, 48 males), previously diagnosed with a febrile seizure in two hospitals of the Lemanic Region, Canton of Vaud, Switzerland: the Hôpital du Samaritain, Department of Pediatrics, in Vevey and the Hôpital de l'Enfance, Centre Hospitalier Universitaire Vaudois, in Lausanne. Selection criteria included a documented history of simple febrile seizure (FS), the absence of any other neurological disorders (in particular other seizure disorders), and the absence of a family history of neurological or neuropsychiatric disorders. The characteristics of the febrile seizure and the family history of each patient were evaluated in their personal hospital records, and again by a senior pediatrician (F.C. or M.G.) during the encounter with the family. Patients with unclear history were not enrolled. FS patients experienced a single febrile seizure episode at an average age of 1.8 years (standard deviation 1.0 years, median age 1.5 years) and were enrolled in the study at an average age of 9.5 years (standard deviation 3.1 years, median age 9.7 years). Eighty age-matched typically developing (TD) controls (28 females, 52 males) were also recruited at an average age of 9.5 years (standard deviation 2.5 years, median age 9.5 years), via the same recruitment system. The absence of FS in the patient or his/her family or any other seizure history was also ascertained by a senior pediatrician, at the time of saliva sample collection.

FS and TD subjects were asked to provide a saliva sample to obtain individual samples of genomic DNA. Saliva was collected with the OG-500 Oragene-DNA self-collection kit (DNAgenothek, Kanata, ON, Canada), and returned to our laboratory by the participants via priority mail. Upon receipt, samples were anonymized and heated at 50° for 1 h to ensure that DNA was adequately released

and that nucleases were permanently deactivated, following the manufacturer's protocol (DNAgenothek, Kanata, ON, Canada). DNA extraction and ApoE analyses were performed following the protocols provided with the commercially available ApoE StripAssay[TM] for the identification of apolipoprotein E isoforms E2, E3 and E4 based on polymerase chain reaction (PCR) and reverse hybridization (ViennaLab Labiordiagnostika GmbH, Vienna, Austria). ApoE has three common alleles (E2, E3, and E4), differing by the presence of either the C or T nucleotide at codons 112 and 158 in the fourth exon of the ApoE gene. ApoE2 contains the sequence TGC coding for cysteine at both positions, ApoE3 contains the sequence TGC at codon 112 (coding for cysteine) and the sequence CGC at codon 158 (coding for arginine), and ApoE4 contains the sequence CGC at both codons, therefore, coding for arginine at both positions. The six possible homozygous and heterozygous ApoE genotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, E4/E4) result in a combination of the respective individual isoforms that can easily be visualized with the ApoE StripAssay. The distribution of allele frequencies (ApoE2, ApoE3, ApoE4) was calculated from the observed genotype distributions. Odds ratios were calculated as $(p1 \times (1 - p1))/(p2(1 - p2))$, with p1 corresponding to the probability of the allele in the population of subjects with febrile seizures and p2 corresponding to the probability of the allele in the population of control subjects. Ninety-five percent confidence intervals (95 % CI) of odds ratios were calculated using the following formula $(e(\ln (OR) \pm \text{sum}(1/x)^{0.5})$. Chi square statistical tests were performed to compare the number of individual genotypes and allele distributions in both populations. Statistical significance was set at $p < 0.05$.

This work was approved by the Cantonal Ethics Commission (Vaud) for Human Research (protocol no. 33/10), and conducted in accordance with the Declaration of Helsinki. The parents of all participants gave informed written consent.

Results

Alleles distribution

We did not find any differences in the distribution of ApoE alleles between FS and TD subjects ($\chi^2 = 1.095$, $df = 2$, $p = 0.5783$; Table 1). Across the two groups of subjects recruited in the Lemanic region of Switzerland, we found an ApoE2 allele frequency of 0.064, an ApoE3 frequency of 0.829 and an ApoE4 frequency of 0.107. These findings are consistent with previous findings including 24,262 European subjects free of any neurological disorders, for which the distribution of the different alleles was rather

Table 1 Distribution of ApoE alleles in individuals who exhibited febrile seizures (FS) and age-matched, typically developing subjects (TD)

	E2	E3	E4
Observed numbers			
FS ($n = 93$)	10	154	22
TD ($n = 80$)	12	133	15
Total ($n = 173$)	22	287	37
Chi square	$p = 0.4347$	$p = 0.9747$	$p = 0.4866$
Observed frequencies			
FS	0.0538	0.8280	0.1183
TD	0.0750	0.8313	0.0938
Total	0.0636	0.8295	0.1069
Odds ratio	0.733	1.015	1.227
95 % CI OR	0.420–1.279	0.582–1.771	0.704–2.141

stable, with an ApoE2 allele frequency of 0.068, an ApoE3 frequency of 0.813 and an ApoE4 frequency of 0.119 (computed from data compiled by [22]).

Genotypes distribution

We did not find any differences in the distribution of ApoE genotypes between FS and TD subjects ($\chi^2 = 4.842$, $df = 5$, $p = 0.4354$; Table 2). Across the two groups of subjects recruited in the Lemanic region of Switzerland, we found that the most prevalent genotype was E3–E3 with 70.5 % of subjects, followed by E3–E4 with 16.2 % and E2–E3 with 8.7 %; the other three genotypes (E2–E2, E2–E4, E4–E4) accounted for less than 5 % of the sampled population.

Hardy–Weinberg equilibrium

We compared the observed frequencies of ApoE genotypes (Table 2) with the expected frequencies based on the Hardy–Weinberg principle $((\text{ApoE2} + \text{ApoE3} + \text{ApoE4})^2 = 1$; Table 3) and found that the genotype frequencies did not differ from equilibrium (FS: $\chi^2 = 4.338$, $df = 5$, $p = 0.5018$; TD: $\chi^2 = 2.461$, $df = 5$, $p = 0.7823$; total: $\chi^2 = 6.035$, $df = 5$, $p = 0.3028$). This suggests that the frequencies of ApoE alleles and genotypes are stable in the Swiss population from which our samples were derived.

Discussion

We hypothesized that children who exhibit febrile seizures during early childhood, but do not develop epileptic seizures later in life, might preferentially carry the ApoE2

Table 2 Distribution of ApoE genotypes in individuals who exhibited febrile seizures (FS) and age-matched, typically developing subjects (TD)

	E2–E2	E2–E3	E2–E4	E3–E3	E3–E4	E4–E4
FS (n = 93)	0	7	3	66	15	2
TD (n = 80)	1	8	2	56	13	0
Total (n = 173)	1	15	5	122	28	2
Chi square	$p = 0.3346$	$p = 0.7074$	$p = 0.6449$	$p = 0.2362$	$p = 0.5967$	$p = 0.1713$
Frequencies						
FS	0.0000	0.0753	0.0323	0.7097	0.1613	0.0215
TD	0.0125	0.1000	0.0250	0.7000	0.1625	0.0000
Total	0.0058	0.0867	0.0289	0.7052	0.1618	0.0116

Table 3 Predicted distribution of ApoE genotypes in individuals who exhibited febrile seizures (FS) and age-matched, typically developing subjects (TD), following the Hardy–Weinberg equation for equilibrium

	E2–E2	E2–E3	E2–E4	E3–E3	E3–E4	E4–E4
Numbers						
FS (n = 93)	0	8	1	63	18	1
TD (n = 80)	0	10	1	56	13	1
Total (n = 173)	1	17	2	108	28	2
Frequencies						
FS	0.0029	0.0890	0.0127	0.6855	0.1959	0.0140
TD	0.0056	0.1247	0.0141	0.6910	0.1559	0.0088
Total	0.0040	0.1055	0.0136	0.6880	0.1774	0.0114

The genotype frequencies of ApoE were in Hardy–Weinberg equilibrium for both groups

allele of apolipoprotein E. In contrast, we did not find any differences in the frequencies of ApoE alleles or genotypes between FS and TD individuals. We found that the observed allele and genotype frequencies did not deviate from Hardy–Weinberg equilibrium. Across both groups (n = 173 subjects), we found an ApoE2 allele frequency of 0.064, an ApoE3 frequency of 0.829 and an ApoE4 frequency of 0.107. Our findings are thus consistent with previous reports for European subjects free of any neurological disorders, in particular with previous results for a population of 173 subjects derived from the same Lemanic region of Switzerland (Geneva; [27]): ApoE2 allele frequency of 0.072, ApoE3 frequency of 0.821 and ApoE4 frequency of 0.107. Our findings indicate that the different alleles of the ApoE gene coding for the apolipoprotein E are not associated with a predisposition to exhibit febrile seizures during early childhood. One possible caveat of our study is the fact that we analyzed fewer than 100 subjects per group. However, in order to be clinically relevant, any association between one gene's polymorphism and a medical condition should be significant even with such a small number of individuals. If larger numbers of subjects are required to demonstrate a statistically significant association, such information is unlikely to be useful for everyday practice.

A previous study by Giray et al. [24] also failed to find differences in allele and genotype frequencies between 69 patients with febrile convulsions and 75 control subjects derived from the Turkish population. However, the distribution of ApoE alleles in their control group differed from what was previously reported by Corbo et al. [27] based on the genotyping of 8453 individuals ($\chi^2 = 21.97$, $df = 2$, $p < 0.0001$). The frequency of the ApoE2 allele in their control population also differed from that observed in our control population ($\chi^2 = 4.21$, $df = 1$, $p = 0.0401$), raising some questions regarding the reliability of their sampling or their sequencing methods. The attempt by Giray et al. [24] to further define different genetic predispositions between simple and complex febrile seizures is also difficult to justify given the small number of subjects with complex seizures comprised in their study (n = 17). Other studies have failed to find a causal relationship between ApoE polymorphism and epilepsy: Blumcke et al. [25] failed to demonstrate an association between ApoE polymorphism and early onset temporal lobe epilepsy; Yeni et al. failed to demonstrate an association between ApoE polymorphism and mesial temporal lobe epilepsy with hippocampal sclerosis; Fu et al. [28] reported that ApoE4 does not increase the risk for temporal lobe epilepsy in the Chinese Han population, in the absence of prior trauma. In contrast, Kauffman et al. [29] reported that the ApoE4 allele might be associated with an earlier onset of temporal lobe epilepsy. Thus, in contrast to the well-established association between ApoE polymorphism and the risk of developing Alzheimer's disease [30], there does not seem to be a simple, direct association between ApoE

polymorphism and the susceptibility to exhibit seizures of various etiologies.

In sum, although our hypothesis that ApoE2 might confer a predisposition to simple febrile seizures was not validated, it remains possible that the cellular pathways involved in the regulation of glutamate uptake and release by astrocytes are associated with the susceptibility to exhibit febrile seizures early in life. Since hundreds of genes have the potential to influence these cellular pathways, this hypothesis could be further tested with a genome-wide approach to identify possible genetic markers of febrile seizures. Using this approach, recent studies found that variants of the sodium channel gene SCN1A are associated with general and MMR vaccine-related febrile seizures [4], but also with mesial temporal lobe epilepsy with hippocampal sclerosis, with and without a history of febrile seizures [5]. If such markers can be found, the development and use of cost-effective sequencing technologies available to clinicians should be combined with the development and use of functional analysis tools that can comprehend multiple gene pathways for routine diagnostic. Altogether, such methods might help to better distinguish a benign childhood disorder with an excellent prognosis from other seizure phenotypes associated with an increased risk of developing epilepsy later in life.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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