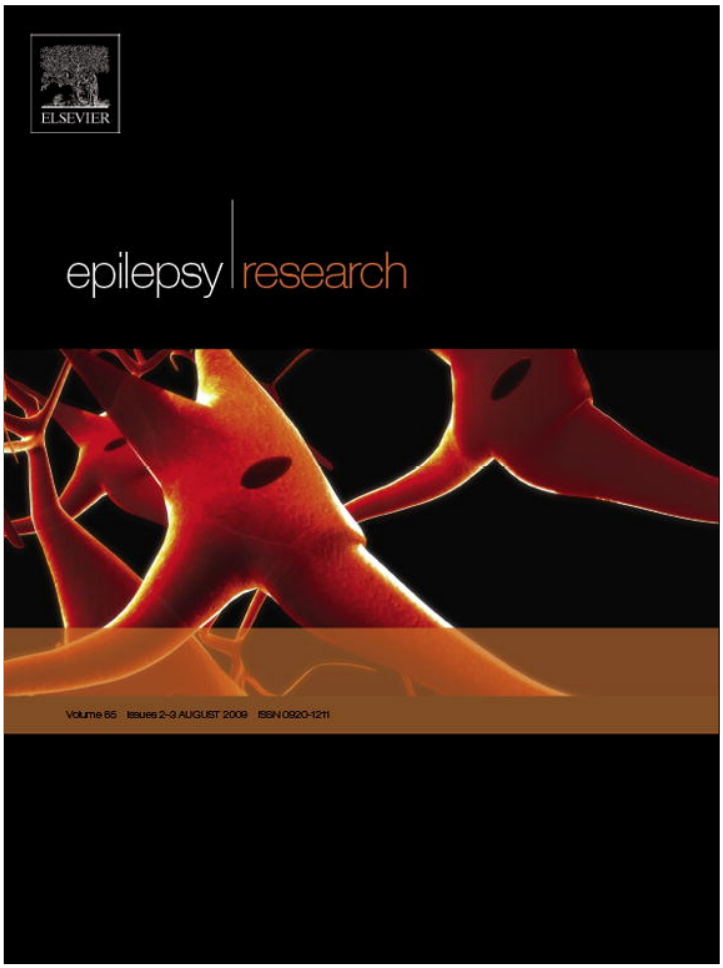

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Serotonin transporter gene variation and refractory mesial temporal epilepsy with hippocampal sclerosis

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Summary We performed a molecular epidemiology study in a population of 105 mesial temporal lobe epilepsy with hippocampal sclerosis (MTE-HS) patients in order to investigate the role of a polymorphism in the serotonin transporter gene (SLC6A4) in the prediction of antiepileptic drug (AED) treatment response. Homozygous carriers of the 12-repeat allele had an almost fourfold increase in risk for a MTE-HS not responding to medical treatment (OR 3.88; CI 95% 1.40—10.7; $p = 0.006$) compared to carriers of the 10-repeat allele. Therefore, a polymorphism of SLC6A4 might be a genetic marker of pharmacoresistance in MTE-HS patients.

Introduction

The majority of patients with epilepsy experience a good response to antiepileptic drug (AED) therapy (Sander, 1993). However, there are particular epileptic syndromes where patients frequently are refractory to medical treatment. Mesial temporal epilepsy with hippocampal sclerosis (MTE-HS) (Wieser, 2004) is such an often paradigmatic cause of refractory epilepsy. Even though there have been a number of studies looking for good prognostic markers in this population of patients, none of the few markers identified so far has been of real clinical usefulness (Briellmann et al., 2007; Hitiris et al., 2007).

A number of pharmacogenetic studies were performed in epilepsy, but the results reported have been mostly inconclusive so far (Szoeke et al., 2006; Shahwan et al., 2007; Tate and Sisodiya, 2007). Nevertheless, genetic factors might influence the phenotypic features of MTE-HS, including the response to AEDs.

Recent evidence supports the role of serotoninergic neurotransmission in epileptogenesis (Bagdy et al., 2007). The serotonin transporter gene (SLC6A4) exhibits a 17 bp variable number of tandem repeats (VNTR) polymorphism in

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intron 2. Since the efficiency of AED treatment might be improved with the use of predictive pharmacogenetic tools, we performed a molecular epidemiology study in a population of MTE-HS patients in order to investigate the role of the VNTR intron 2 polymorphism in the prediction of AED treatment response.

Methods

Patients

Between August 2003 and July 2005, we recruited 105 consecutive MTE-HS patients (55 males, mean age 38.2 years) from the Epilepsy Clinic at the Neurology Division of the Ramos Mejia Hospital in Buenos Aires, Argentina. In supplementary material we present clinical characteristics and procedures performed in their diagnostic work out. All patients were Buenos Aires residents. Previous studies have shown that the genetic contribution of Europeans to the gene pool of Buenos Aires could be estimated at 67.55% (Martinez Marignac et al., 2004). The study was reviewed and approved by the local ethics committee and a written informed consent was obtained from each patient. Additionally, 81 ethnically, age- and sex-matched healthy volunteers were genotyped as control group.

The following clinical features were analyzed: age, sex, response to pharmacological treatment, prior history of status epilepticus, clusters of seizures, and frequency of secondary generalized seizures. Clinical details were assessed retrospectively, blinded to the genotype, by using patient records and personal interviews. We defined non-response to medical treatment according to criteria used previously by Tan et al. (2004). Therefore, a patient was considered refractory to medical treatment when he continued to experience at least four seizures (excluding those associated with missed doses of AED) in a year, in spite of an optimized medical treatment defined by the use of classical AEDs and at least a new AED at maximum tolerated doses. Patient compliance was ascertained by direct questioning the patient about this point. Clustering of seizures was considered positive when three or more seizures occurred within 24 h. A patient was considered to have frequent secondary generalized seizures when he suffered from more than six partial seizures with secondary generalization during the previous year before taking blood sample.

Genotyping

The SLC6A4 repetitive element intron 2 polymorphism was genotyped by PCR-ethidium bromide stained agarose electrophoresis, as previously described (Yeo et al., 2004). For analysis, the few 9-repeat alleles were considered as 10-repeat alleles. The genotyping reactions were performed blinded to clinical features. No other polymorphisms were examined.

Statistical analysis

The Hardy—Weinberg equilibrium was tested using the exact test. We considered the genotype 12/12 as the putative risk factor according to a recessive inheritance model, as previously described (Hranilovic et al., 2004). Therefore, we explored the main effect of this genotype using a multivariate logistic model that considered *treatment response* as the dependent variable. *Sex* and *age of seizure onset* were included as covariates The significance of the model was assessed using likelihood ratio tests. Significance was set at 0.05. All statistical analyses were performed using STATA (Stata Corporation, College Station, TX).

Table 1 Genotypic distribution of SLC6A4 intron 2 polymorphism stratified by response to treatment.

Genotypes	MTE-HS non-responsive, $n = 74$ (%)	MTE-HS responsive, $n = 31$ (%)
12/12	40 (54)	7 (22.6)
12/10	23 (31)	21 (67.7)
10/10	10 (13.5)	3 (9.7)
12/9	1 (1.5)	0 (0)
OR (95% CI)*		3.89 (1.4—10.7)
p-Value**		0.006

MTE-HS, mesial temporal lobe epilepsy with hippocampal sclerosis.
* OR adjusted odds ratio (12/12 vs. 12/10 + 10/10 + 12/9).
** Likelihood ratio test.

Results

The frequency of SLC6A4 genotypes did not deviate from the Hardy—Weinberg equilibrium. Genotype and allele distributions for the SLC6A4 intron 2 polymorphism are summarized in Tables 1 and 2.

We found a significant association between the intron 2 polymorphism and MTE-HS treatment response. Homozygous carriers of the 12-repeat allele had an almost fourfold increase in risk for non-response to medical treatment (OR 3.88; CI 95% 1.40—10.7; LR $X^2 = 7.54$; $p = 0.006$; *Pearson goodness-of-fit*, $p = 0.29$) compared to carriers of the 10-repeat allele. Refractory patients were significantly more likely to be on polytherapy and to have had more AED trials in the past (see supplentary Table 1). The groups were comparable regarding other clinical features, including the antecedent of mood disorders, see supplementary Table 1. Patients have tried similar AEDs irrespective of their genotypes (see supplementary Table 2).

Since SLC6A4 genotyping might be useful as a diagnostic test for the prediction of medical refractory epilepsy, different classical parameters of accuracy and diagnostic ability

Table 2 Genotypic distribution of SLC6A4 intron 2 polymorphism stratified by the presence of frequent secondary generalized complex partial seizures.

Genotypes	MTE-HS non-generalized, $n = 40$ (%)*	MTE-HS generalized, $n = 63$ (%)*
12/12	12 (30)	34 (54)
12/10	20 (50)	23 (36.5)
10/10	8 (20)	5 (7.9)
12/9	0 (0)	1 (1.6)
OR (95% IC)**		2.42 (1.01—6.15)
p-Value***		0.05

MTE-HS, mesial temporal lobe epilepsy with hippocampal sclerosis.
* Reliable information about the frequency of secondary generalization was obtained in 103 patients.
** OR adjusted odds ratio (12/12 vs. 12/10 + 10/10 + 12/9).
*** Likelihood ratio test.

Table 3 Summary of diagnostic ability of SLC6A4 genotyping for prediction of non-response to medical treatment.

Measure	Point estimation	95% Confidence interval
Probability of non-response to medical treatment	70.5%	60.8—79
Sensitivity	54.1%	42.1—65.7
Specificity	77.4%	58.9—90.4
Negative predictive value	41.4%	28.6—55.1
Positive predictive value	85.1%	71.7—93.8
Positive likelihood ratio	2.39	1.21—4.75
Negative likelihood ratio	0.59	0.43—0.81

of this test were estimated. They are summarized in Table 3. The utility of a predictive tool might be expressed in terms of the information gained by its use. Therefore, considering the likelihood ratio of the test and the fact that 70.4% of our MTE-HS patients were refractory to medical treatment, the odds of a given patient experiencing non-responsiveness to medical treatment based on the information obtained by genotyping the serotonin transporter gene polymorphism varies between 41% (for a non 12/12 genotype) and 139% (for the 12/12 genotype).

We also found a significant association between the investigated genetic variation and the presence of frequently secondary generalized complex partial seizures (OR 2.42; CI 95% 1.01—6.15; LR $X^2 = 3.85$; $p = 0.05$). In contrast, we did not find an association with a history of seizures presenting in clusters ($p = 0.92$) or status epilepticus ($p = 0.73$).

We tested the robustness of our findings running analyses with our population stratified according to two other previously published (Siddiqui et al., 2003; Zimprich et al., 2004) definitions of refractoriness. Specifically, Zimprich defined as refractory those patients with a seizure frequency of more than 2 per month, whereas Siddiqui only defined as responder those patients that were seizure free under AED treatment. The gene variant effect was still significant ($p = 0.01$ and $p = 0.04$) irrespective of the definition used, Zimprich and Siddiqui definitions, respectively.

The frequency of SLC6A4 genotypes was not different between MTE-HS patients (12/12 = 47, 12/10 = 44, 10/10 = 13, 12/9 = 1) and controls (12/12 = 31, 12/10 = 35, 10/10 = 15; $p = 0.33$).

Discussion

We found a significant association between MTE-HS refractory to medical treatment and a VNTR in intron 2 of the SLC6A4 gene. The cohort of patients not responding to medical treatment showed higher frequencies of the homozygous 12-repeat genotype. These results suggest that the serotonin transporter gene might be involved in the phenomenon of pharmacoresistance in MTE-HS. Furthermore, SLC6A4 genotyping could be useful as a molecular prognostic tool for refractoriness to medical treatment in this syndrome.

Although well-known non-genetic (biologic and lifestyle-related) factors can account for the variability of response to treatment, genetic differences among patients may also explain an important part of this phenomenon (Goldstein et al., 2007). Accordingly, some genetic markers have been pointed out as potential genetic predictors of pharmacoresistance in epilepsy (Siddiqui et al., 2003; Tate et al., 2005), though none of them turned out as clinically useful yet (Shahwan et al., 2007). Our findings showed that SLC6A4 intron 2 genotyping might be a tool with a moderate predictive ability at the time of a MTE-HS first-consultation. However, our results cannot be considered definitive or ready for clinical application until they are confirmed by other studies.

In contrast to many polymorphisms investigated in the field of complex genetics (Cavalleri et al., 2005), the intron 2 variant in SLC6A4 seems to be functionally active (David et al., 2005). Allele-dependent differential enhancer activity of the polymorphic region in the second intron was demonstrated in gene expression studies, where genotypes homozygous for 12-repeats increased the expression of 5-HTT mRNA around 30%. Moreover, serotonin neurotransmission is increasingly recognized as involved in temporal lobe epileptogenesis. Evidence arising from studies performed in experimental animal models (Merrill et al., 2007), from human functional neuroimaging studies (Theodore et al., 2007) and from genetic studies (Manna et al., 2007) support the relationship between 5-HT brain concentrations and epilepsy, whereas lower levels of serotonin seem to be pro-convulsant. Furthermore, our findings could be relevant for the design of innovative clinical trials that can include selective serotonin reuptake inhibitors as add-on therapies in epilepsy, such as small clinical trials have shown that the selective serotonin reuptake inhibitor fluoxetine might be useful in pharmacoresistant epilepsy therapy (Albano et al., 2006). Finally, considering that subjects homozygous for 12-repeats might express more 5-HTT (Fiskerstrand et al., 1999) leading to a lower synaptic serotonin concentration that would favor a pro-convulsant milieu, our findings seem to be biologically plausible.

Among the caveats of our work, we must mention the retrospective assessment of the seizure frequency that might be subjected to different biases. Therefore, future prospective studies will be needed to better elucidate the role of this polymorphism as a predictor of pharmacoresistance in this and other epileptic syndromes.

In summary, our preliminary results suggest that SLC6A4 gene variability might be involved in the pharmacoresistance in MTE-HS.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.eplepsyres.2009.03.010.

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