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SLC6A4 gene variants and temporal lobe epilepsy susceptibility: a meta-analysis

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Abstract There seems to be a role for serotoninergic neuro-transmission in the pathophysiology of the epilepsies. Different groups have studied the role of regulatory variants in the SLC6A4 gene, which code for the central serotonin transporter, in the complex genetics of temporal lobe epilepsy (TLE) obtaining contradictory findings. Therefore, a systematic review and critical analysis of this topic seem to be timely. Published studies up to October 2011 of TLE and the SLC6A4 promoter and intron 2 variant number repeat polymorphisms (VNTR) were identified by searches of Medline, Scopus and ISI-Web of Sciences databases. Meta-analysis of TLE case-control data were performed to assess the association of SLC6A4 VNTRs with TLE susceptibility. Pooled odds ratios were estimated by means of a genetic-model-free approach. The quality of the included studies was assessed by a score. The studies included compared a total of 991 TLE cases and 1,202 controls. We did not find synthetic evidence of association between SLC6A4 promoter and intron 2 variants and the risk of TLE. However, the intron 2 VNTR seems to have opposite effects in different populations. In this meta-analysis our findings were inconclusive in order to associate any of the 5-HT receptor gene variants with the risk of TLE.

Keywords Epilepsy · Hippocampal sclerosis · Genetics · SLC6A4 · Serotonin

Introduction

There seems to be a role for serotoninergic neuro-transmission in the pathophysiology of the epilepsies. A reduced serotoninergic tone could be epileptogenic [1]. Moreover, genetic epilepsy-prone rats have reduced hippocampal 5-HT1a receptor expression. In mesial and neocortical temporal lobe epilepsy (TLE), human studies have demonstrated decreased 5-HT1a receptor binding in ictal spreading regions, including the temporal lobes, the midbrain raphe and the thalamus [2].

Transcriptional activity of the SLC6A4 gene, which code for the serotonin central transporter, could be under the influence of two polymorphisms [3]. Different groups have studied the role of these variants in the complex genetics of TLE. These studies showed heterogeneity in their design with contradictory findings. Therefore, a systematic review and critical analysis of this topic seem to be timely [4, 5].

We therefore carried out a systematic review of the evidence relating SLC6A4 gene polymorphisms with TLE risk, aiming to estimate allele frequencies, the presence and

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magnitude of association between variants and the disorder and to assess methodological bias that could account for the reported differences.

Methods

Search strategy

The MEDLINE, SCOPUS, ISI-Web of Sciences and Huge Navigator databases were searched with no language restrictions from their inception to October, 2011. We used a combination of keywords as free text in the following search strategy: ["serotonin" OR "serotonin transporter" OR "SLC6A4 gene"] AND ["epilepsy" OR "temporal lobe epilepsy" OR "seizures"] for studies examining an association between the SLC6A4 gene variants and TLE. All references cited in these studies and published reviews were reviewed to identify additional works not indexed by the databases selected. Where there were multiple publications from the same study group, the most complete and recent results were used.

Inclusion criteria

Eligible studies had to meet all of the following criteria: (a) published in a peer-reviewed journal and independent studies using original data; (b) provided sufficient data to calculate the odds ratio (OR) with confidence interval (CI) and *P* value; (c) investigated SLC6A4 polymorphisms; (d) described the genotyping method or provided reference to it; (e) included patients with a diagnosis of TLE; (f) used healthy individuals as controls. Authors were contacted in cases in which there were queries regarding their studies.

Data extraction

Two investigators (M.K. and M.C.) independently extracted the following data from each publication: author; country of origin; selection and characteristics of cases and controls; demographic information; racial descent of the study population; numbers of eligible and genotyped cases and controls; and numbers of cases and controls for each of SLC6A4 genotypes (5HTTLPR and 5HTTVNTR variants dichotomized into high and low efficient transcriptional polymorphisms). Disagreements were resolved by consensus.

Quality score assessment

Methodologic quality was independently assessed by two reviewers (M.K. and M.C.), according to a set of predefined criteria (Supplementary Table 1), based on the scale of Thakkinstian et al. [6]. Disagreements were resolved by consensus. Scores ranged from 0 (lowest) to 7 (highest).

Statistical analyses

Data analyses were performed as follows. First, the pooled prevalence of the putative risk allele in controls was estimated by the inverse variance method (Appendix of Ref. [7]). A Q test for heterogeneity was done for the total control cohort. Under the null hypothesis of no difference in effect across studies, the Q statistic is X^2-distributed with degrees of freedom (df) equal to the number of studies minus one. Second, for the controls in each study, Hardy–Weinberg equilibrium (HWE) was assessed using the exact test. Third, we estimated the overall gene effect by use of logistic regression with fixed and random-effects model as described by Bagos et al. [8]. In case of finding a significant overall gene effect, the most appropriate genetic model would be used to collapse the three genotypes into two groups; the pooled estimate of risk would be obtained using the fixed effect inverse variance method. Fourth, we also applied other genetic model-free approach estimating generalized odds ratio (OR_G) for each study. The individual studies' OR_Gs were also synthesized using a random effect model [9]. Publication bias was assessed using Egger's and Begg-Matzumdar tests. Statistical analysis was done with Stata, version 9 (Stata Corporation, College Station, TX). $P < 0.05$ was considered statistically significant, except for heterogeneity, Egger's and Begg's tests, where a level of 0.10 was used. The OR_G (individual and pooled) was estimated using ORGGASMA [9].

Results

Study inclusion and characteristics

The combined search yielded 15 references. After overlapping references and those that did not meet inclusion criteria were discarded, six references were retained. These references were then filtered to ensure conformity to inclusion criteria. Populations included in the studies of Hecimovic et al. [10]. and Stefulj et al. [11]. were the same, thus they were counted once. The only published genome wide association study done on an epilepsy population [12] did not include these serotonin variants. Finally, five references met our criteria for inclusion (see Tables 1, 2, 3). Four of them analyzed the association between both SLC6A4 promoter and intron 2 polymorphisms with TLE [4, 11, 13, 14], whereas one only investigated the intron 2 variant [5]. Therefore, the studies compared a total of 991 TLE cases and 1,202 controls. Pooled 5HTTLPR variants frequencies were 0.52; 0.44–0.61 and 0.48; 0.38–0.55 for alleles L and S, respectively. 5HTTVNTR variants pooled frequencies were 0.67; 0.50–0.83 and 0.33; 0.16–0.49 for alleles 12 and 10, respectively. There were marked

Table 1 Genotype frequencies in cases and controls from the 4 studies included in the analysis of 5HTTLPR polymorphism

First author, year of publication	Country	*n*	Controls				Patients					
			LL	LS	SS	L carriers	LL	LS	SS	L carriers	Quality score	HS (%)
Manna, 2007	Italy	585	90	142	77	232	77	146	53	223	7	17
Stefulj, 2010	Croatia	271	60	93	17	153	42	45	14	87	7	44
Schenkel, 2011	Brazil	330	54	64	37	118	48	91	36	139	6	19
Li, 2011	China	797	36	167	276	203	24	130	164	154	6	23

HS Hippocampal sclerosis

Table 2 Genotype frequencies in cases and controls from the 5 studies included in the analysis of 5HTTVNTR polymorphism

First author, year of publication	Country	*n*	Controls				Patients					
			12/12	12/10	10/10	12 carriers	12/12	12/10	10/10	12 carriers	Quality score	HS (%)
Manna, 2007	Italy	585	115	136	58	251	126	112	38	238	7	17
Kauffman, 2009	Argentina	186	31	35	15	66	47	44	14	91	7	100
Stefulj, 2010	Croatia	259	64	74	24	138	30	46	21	76	7	44
Schenkel, 2011	Brazil	330	67	67	21	134	62	81	32	143	6	19
Li, 2011	China	821	429	57	1	486	276	55	3	331	6	23

HS Hippocampal sclerosis

Table 3 Strengths and weaknesses of each included study

Author, year	Strengths	Weaknesses
Manna, 2007	Controls adequately matched for ethnicity	Potential confounding population variables not completely considered, such as age, TLE-subtype and response to treatment
Kauffman, 2009	Homogeneous TLE population, including only TLE-HS patients	Possible bias with the retrospective assessment of the seizure frequency and the definition of drug response
	Analysis limited to one variable (e.g., treatment response)	Relatively small sample
Stefulj, 2010	More comprehensive analysis of serotonergic genes	Potential confounding population variables not completely considered, like age, TLE-subtype and response to treatment
Schenkel, 2011	Potential confounding population variables were considered and analyzed	Potential population ethnic admixture bias
		Not homogeneity of TLE sample
Li, 2011	Relatively big sample	Potential confounding population variables not completely considered, such as age, TLE-subtype and response to treatment

heterogeneity across the studies ($P < 0.001$). TLE populations differed in proportion of hippocampal sclerosis (HS) cases and epilepsy severity (see Tables 1, 2). Manna et al. included mostly benign TLE cases whereas Kauffman et al. included mostly HS drug-resistant subjects. The other populations studied were in an intermediate situation. The quality of studies ranged from 6 to 7, out of a possible score of 7. Two studies were conducted in Europe [4, 11], two in South American [5, 13] and one in Asia [14]. All studies reported HWE analysis results. All control populations were in HWE. Research groups used three different genotypic methods previously described in [15, 16], whereas Manna et al. [4] used a fluorescence-based previously un-described method. A summary of strengths and weaknesses of each study is presented in Table 3.

Association results

None of the individual studies investigating the association between *5HTTLPR* and the risk of TLE showed significance for the allele contrast and OR_G (see Table 4) comparisons.

Two individual studies showed association between the *5HTTVNTR* and the risk of TLE for the allele contrast and OR_G (see Table 5) comparisons. However, genetic effects

Table 4 Individual association analyses from the 4 studies included in the analysis of 5HTTLPR polymorphism

Author, year	OR_G (95 CI)	Allele contrast (S as risk factor)
Manna, 2007	0.90 (0.68–1.19)	0.91 (0.72–1.15)
Stefulj, 2010	0.89 (0.57–1.39)	0.95 (0.66–1.36)
Schenkel, 2011	1.11 (0.77–1.60)	1.08 (0.80–1.47)
Li, 2011	0.81 (0.62–1.05)	0.85 (0.68–1.07)

OR_G Generalized odds ratio. Pooled OR_G: 0.90; CI 95, 0.77–1.06

Table 5 Individual association analyses from the 5 studies included in the analysis of 5HTTVNTR polymorphism

Author, year	OR_G (95 CI)	Allele contrast (10 as risk factor)
Manna, 2007	0.72 (0.54–0.95)	0.75 (0.59–0.95)
Kauffman, 2009	0.76 (0.46–1.25)	0.78 (0.50–1.18)
Stefulj, 2010	1.44 (0.94–2.22)	1.37 (0.95–1.97)
Schenkel, 2011	1.35 (0.93–1.37)	1.30 (0.95–1.78)
Li, 2011	1.56 (1.05–2.3)	1.55 (1.07–2.25)

OR_G Generalized odds ratio. Pooled OR_G: 1.10; CI 95, 0.78–1.57

were in opposite directions. Manna et al., found the 10 allele to be a protective factor for epilepsy whereas Li et al., found it as a risk factor. Noteworthy, Stefulj [11] and Schenkel [13] found a trend in the same direction of Li [14], whereas Kauffman [5] found a trend in the same direction of Manna.

The overall analysis between both *5HTTLPR* and *5HTTV -NTR* variants and the risk of TLE showed no statistically significant association for the allele contrast and OR_G (see Tables 4, 5). We did not find evidence of heterogeneity between studies investigating the *5HTTLPR* variant. On the other hand, there was heterogeneity between studies investigating the *5HTTVNTR* variant. An influence analysis that excluded data from Kauffman et al. study that only included patients with HS showed similar OR_G estimates (1.20; CI 95, 0.80–1.78). There was no evidence of publication bias ($P > 0.5$ for Begg and Egger tests) in studies investigating both variants.

Discussion

In this study we did not find synthetic evidence of association between SLC6A4 *promoter* and *intron 2* variants and the risk of TLE. Noteworthy, intron 2 VNTR seems to have opposite effects in different populations. Whereas Manna and Kauffman found the 10 allele a protective factor for epilepsy [4, 5], the other researchers found it as a risk factor [11, 13, 14]. These differences could be due to linkage disequilibrium of the putative and unidentified disease causing allele with different alleles at the marker locus in different populations [17].

The dataset analyzed is of a relatively small magnitude limiting the robustness of our findings. The quality of the studies is homogeneus. However, there is an heterogeneus structure of the populations investigated. In the future, this should be controlled during study design or during the analysis by quantifying and controlling for substructures. Furthermore, there were variables not completely analyzed such as age, ethnicity, and gender that could bias results. Unfortunately, this small dataset impeded us to perform stratified sub-analyses in order to better assess these potential biases. So future studies and meta-analyses with a greater number of cases and designs of better quality are needed to provide a better estimate of the effect of this polymorphism in the development of epilepsy.

Therefore, there is insufficient evidence to identify absolutely any gene variant as a putative risk factor for the development of epilepsy [12]. In this meta-analysis our findings were inconclusive in order to associate any of the 5-HT receptor gene variants with the risk of TLE. More evidence is needed from epidemiologic studies to provide a better characterization of the role of this gene and its common variant, if any, in the genetic susceptibility to develop TLE and other epileptic disorders. Therefore, it is not possible to recommend systematic analysis of the SLC6A4 gene in the routine management of patients with epilepsy, but the understanding of the 5-HT receptor gene in epilepsy will certainly have potential research implications for a better understanding of epileptogenicity.

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