IJASRM

International Journal of Advanced Scientific Research and Management, Vol. 2 Issue 2, Feb 2017.

www.ijasrm.com

ISSN 2455-6378

# Functional Polymorphisms in the Neuropeptide S Receptor are not associated with Juvenile Myoclonic Epilepsy

**Chiara Marinho[1], Bruna Santos[1], Diego Figueredo[2], Ygor Santos[1], Lívia Gitaí[3], Fernando Gameleira[3], Rodrigo Secolin[4], Marcelo Duzzioni[5], Olagide Castro[6], Tiago Andrade[2] and Daniel Gitaí[1]**

[1] Institute of Biological Sciences and Health, Federal University of Alagoas, Maceió, AL, Brazil

[2] Department of Molecular Biology and Gene Expression, Campus Arapiraca, Federal University of Alagoas, AL, Brazil

[3] Department of Neurology, School of Medicine, Federal University of Alagoas, Maceió, AL, Brazil

[4] Department of Medical Genetics, University of Campinas, Campinas, SP, Brazil

[5] Department of Pharmacology, Institute of Biological Sciences and Health, Federal University of Alagoas, Maceió, AL, Brazil

[6] Department of Physiology, Institute of Biological Sciences and Health, Federal University of Alagoas, Maceió, AL, Brazil

**Abstract**

It is largely accepted that the genetic susceptibility threshold is critical for Juvenile Myoclonic Epilepsy (JME) onset and probably reflects the simultaneous involvement of multiple genes with minor effect interacting with environment factors. The *NPSR1* encoding gene became a high-ranking candidate for epilepsy susceptibility, specifically considering a recent report of the proconvulsive effects of NPS in mice and that gain-of-function *NPSR1* polymorphisms were consistently associated with some epilepsy comorbidities, including sleep and anxiety. This case/control study was designed to investigate whether rs324981, rs2530547 and rs727162 *NPSR1* polymorphisms are associated with JME in the Brazilian population. The polymorphisms were genotyped in 97 JME patients and 193 control subjects by qPCR using TaqMan® SNP Genotyping Assays. Descriptive and statistical analyses were performed using SNPstats software. No significant differences were observed in the genotypic and allelic frequencies of these polymorphisms between cases and controls, even when analyses were restricted to endophenotypes. By Multifactor Dimensionality Reduction (MDR) analysis, we also tested for interactions between polymorphisms, comparing the patients with the control individuals. Even the allele composed by rs2530547C-rs324981A-rs727162C variants that correspond to a highly expressed (-103C) NPSR1 protein, characterized by increased signaling properties (107Asn and 241Ser), did not differ significantly between the groups. These results present no evidence for an association of these polymorphisms with JME. Further studies including other types of epilepsy and/or other functional polymorphisms are

IJASRM
International Journal of Advanced Scientific Research and Management, Vol. 2 Issue 2, Feb 2017.
www.ijasrm.com
ISSN 2455-6378

required to investigate the possible relationship between the *NPSR1* gene and genetic susceptibility to chronic seizures.

***Keywords:*** *SNP; Idiopathic Epilepsy; Juvenile Myoclonic Epilepsy.*

## 1. Introduction

Juvenile myoclonic epilepsy (JME) was recognized as an epileptic syndrome in 1989 [1,2] and has been among the most common types of heritable epilepsies, with an estimated prevalence of 0.1–2 per 100 000 population [3,4]. The age of onset is usually in puberty and the cardinal symptom is myoclonic seizure which often occur on awakening, with or without other seizure types, such as generalized tonic–clonic or absence seizures [5,6]. Typical electroencephalography (EEG) features of JME include interictal generalized spike-wave (GSW) discharges and normal background activity [7,8]. Most patients display a good prognosis under appropriate pharmacological treatment, but frequent seizure recurrence is reported after drug discontinuation [9,10]. It is largely accepted that the genetic susceptibility threshold is critical for JME onset and probably reflects the simultaneous involvement of multiple genes with minor effect interacting with environmental factors [11–13]. Many researchers have used the genetic association approach to investigate these JME susceptibility genes [14–16]. Most of these studies have been directed to candidate genes selected according to their molecular function, such as those coding neuropeptide (or its receptor) that exert modulatory effects on neurotransmission when under conditions of excessively high neuronal firing [17].

The neuropeptide S receptor (NPSR1) is a metabotropic 7-transmembrane G protein-coupled receptor that, when activated by its endogenous ligand, a 20-amino-acid peptide (Neuropeptide S), can activate protein kinases and increase the intracellular cAMP and Ca2+ level [18,19]. This novel peptidergic system is involved in the modulation of important biological functions, which include arousal [20], anxiety [21,22], food intake [23,24], locomotion [25,26], nociception [27], memory [28,29], and drug addiction [30]. Given these central regulatory roles, even a slight change in NPSR function caused by, for example, a functional polymorphism in this gene could contribute to the occurrence of a neurologic disturbance.

The *NPSR1* gene spans 220 kb of DNA sequence on chromosome 7p, containing more than 1000 SNPs and at least four of them with experimental evidence of important functional effects [31]. The rs324981 A>T variation has been widely studied and results in a change of an amino acid (Asn107Ile) in the active center of the receptor binding site. Functional characterization of this variant showed a 10-fold increase in agonist potency in the Ile107 variant (T-allele), leading to higher signal transduction efficiency [32–34]. The more 'efficient' T-allele has been associated with several conditions, including anxiety [35], fear-related traits [36], panic disorder [37], sleep [38], schizophrenia [39] and obsessive-compulsive disorder [40]. Recently, the functional relevance of some other *NPSR1* polymorphisms [31] has been characterized. The rs2530547 C>T variation is located in the promoter region (-103 position) and affects the transcriptional activity, as evidenced by both in vitro luciferase assay and in peripheral blood leukocytes from healthy volunteers. The rs727162 G>C coding polymorphism leads to an arginine to serine exchange at position 241 (Arg241Ser), which is associated with reduced activation of *NPSR1* signaling. Thus, different cis combinations of these three functional SNPs should affect disease risk. Indeed, it was observed that the rs2530547C-rs324981A-rs727162C haplotype has a significant association with reduced risk of Inflammatory Bowel Disease [31].

Recently, Ramos et al. (2012) [41] reported their first experimental evidence regarding the involvement of the NPS-NPSR system on the epileptogenic process. The authors showed that NPS administration in mice (i.v) facilitated the effects of pentylenetetrazole (PTZ) by increasing the duration of seizures. Thus, considering the proconvulsive effects of NPS in mice, we hypothesized that the functional polymorphisms corresponding to a gain-of-function in the NPSR1 protein might influence the risk for epilepsy. Here, we conducted a case/control study in order to evaluate whether rs324981, rs250547 and rs727162 *NPSR1* polymorphisms were associated with Juvenile Myoclonic Epilepsy. In addition, a possible interaction among the three polymorphisms was investigated.

## 2. Materials and Methods

### 2.1. Patients

A total of 97 patients with JME and 193 normal control subjects were included in this study, all recruited from the state of Alagoas in northeastern Brazil. The study was conducted with the approval of the Ethical Committee of the Federal University of Alagoas, Brazil (Nº55395216.0.0000.5013). The cases and controls were matched according to age, sex and geographic location of origin. Individuals with a history of epileptic seizures or

neuropsychiatric disorders were excluded from the control sample. The diagnosis of JME was based on a proposal from the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE, 1989). All patients were submitted to EEG analysis and only those with a GSW were included in this study.

### 2.2 Genotyping

DNA was extracted from peripheral blood leucocytes using the FlexiGene DNA Kit (Qiagen, USA). The SNPs rs2530547 (-103), rs324981 (Asn107lle) and rs727162 (Arg241Ser) were genotyped in an ABI Prism 7500 Fast Sequence Detection System (Applied Biosystems), respectively with TaqMan® SNP Genotyping Assays C___2959938_10, C___2959781_10 and C___2277753_10, according to manufacturer's instructions. Genotyping for 3 SNPs was performed as previously described [31].

### 2.3 Statistical Analysis

In order to investigate the genetic association between case and control groups, SNPstats was used, a web-based tool offered by the Biostatistics and Bioinformatics Web Unit of The Catalan Institute of Oncology [42]. The following parameters were analyzed: Allele and genotype frequencies; Hardy-Weinberg Equilibrium; SNP association with a response variable and odds ratio (OR) together with a 95% confidence interval (CI).

The Fisher's exact test was used to compare categoric variable (sex) between case and controls groups, calculated by Graph Pad Prism 5 software.

The statistical power for the present study was calculated by G*Power v3.1.3 software [43,44], using the following parameters: a logistic regression test; two-tailed analysis; a size sample for each SNP (rs2530547, n=282; rs727162, n=290; rs324981, n=289); a minimum OR = 1.5; and a statistical significance level $\alpha = 0.05$.

The gene-gene interactions analysis was performed using MDR software [45]. Hypothesis testing and multiple comparisons adjustment were performed using a permutation approach by MDR-Permutation Testing Software (beta 0.4.5), which generates empirical null distribution of prediction accuracy and compares it with the value of the final best model. We used 10,000 permutations for hypothesis testing. Significance was considered at both the 5% and 10% level for multivariate and MDR analysis.

## 3. Results

Some clinical features of the unrelated patients with JME are illustrated in Table 1.

**Table 1**. Phenotypic features of 97 patients with JME and 193 healthy controls.

| Parameters | Patients (n=97) | Controls (n = 193) | p-value** |
|---|---|---|---|
| Mean age at seizure onset | 13.43±4.24 | - | - |
| M:F | 33:64 | 71:122 | 0.6978 |
| MS | 97 | - | - |
| GTCS | 93 | - | - |
| Absence | 70 | - | - |
| Monotherapy | 64 | - | - |
| MS* | 2 | - | - |
| MS + GTCS | 24 | - | - |
| MS + Absence | 2 | - | - |
| MS + GTCS + Absence | 68 | - | - |

JME (juvenile myoclonic epilepsy); M (male); F (female); MS (myoclonic seizures); GTCS (generalized tonic-clonic seizures); *only MJs; **p-values for differences between patients and controls.

The data for genotype distribution and allele frequencies of *NPSR1* rs2530547, rs727162, and rs324981 polymorphisms for the patients and the controls are summarized in Table 2.

Genotype proportions and allele frequencies for the three variants did not differ significantly between the groups (Table 2). The distribution of these polymorphisms did not deviate significantly from that expected by the Hardy-Weinberg equilibrium, as estimated by the Chi-square test (Supplementary Table 1). In order to investigate any possible association with specific JME characteristics, we split the sample and performed the analysis using three subgroups of patients with: 1) diurnal preferential seizure occurrence (n=73) (Supplementary Table 2), 2) the triad of myoclonus, absences and generalized tonic-clonic seizures (n=68) (Supplementary Table 3) and 4) the combination of myoclonus and generalized tonic-clonic seizures (n=24) (Supplementary Table 3). No differences were found comparing each one versus the control individuals. By MDR analysis, we also tested for interactions between polymorphisms, comparing the patients with the control individuals. The best model consisted of the interaction among SNPs rs2530547, rs727162 and rs324981, showing a testing accuracy of 0.5452. The cross-validation consistency of this model was 10/10. Permutation testing did not differ significantly (p=0.7123).

IJASRM

International Journal of Advanced Scientific Research and Management, Vol. 2 Issue 2, Feb 2017.

www.ijasrm.com

ISSN 2455-6378

**Table 2.** Comparative analysis between genotypic frequencies obtained in groups of patients with epilepsy and controls (without adjustment for gender).

| Model | Genotype | Controls | Patients | OR (95% IC) | p-value* |
|---|---|---|---|---|---|
| **rs2530547 (n=282)** | | | | | |
| **Codominant** | T/T | 55 (29.3%) | 30 (31.9%) | 1.00 | 0.72 |
| | T/C | 85 (45.2%) | 44 (46.8%) | 0.95 (0.53-1.69) | |
| | C/C | 48 (25.5%) | 20 (21.3%) | 0.76 (0.38-1.52) | |
| **Dominant** | T/T | 55 (29.3%) | 30 (31.9%) | 1.00 | 0.65 |
| | T/C-C/C | 133 (70.7%) | 64 (68.1%) | 0.88 (0.52-1.51) | |
| **Recessive** | T/T-T/C | 140 (74.5%) | 74 (78.7%) | 1.00 | |
| | C/C | 48 (25.5%) | 20 (21.3%) | 0.79 (0.44-1.43) | 0.43 |
| **Overdominant** | T/T-C/C | 103 (54.8%) | 50 (53.2 %) | 1.00 | |
| | T/C | 85 (45.2%) | 44 (46.8%) | 1.07 (0.65-1.75) | 0.8 |
| **Log-additive** | --- | --- | --- | 0.88 (0.63-1.23) | 0.46 |
| **rs727162 (n=290)** | | | | | |
| **Codominant** | C/C | 92 (47.7%) | 54 (55.7%) | 1.00 | |
| | C/G | 87 (45.1%) | 36 (37.1%) | 0.70 (0.42-1.18) | 0.41 |
| | G/G | 14 ( 7.2%) | 7 ( 7.2%) | 0.85 (0.32-2.24) | |
| **Dominant** | C/C | 92 (47.7%) | 54 (55.7%) | 1.00 | |
| | C/G-G/G | 101 (52.3%) | 43 (44.3%) | 0.73 (0.44-1.18) | 0.2 |
| **Recessive** | C/C-C/G | 179 (92.8%) | 90 (92.8%) | 1.00 | 0.99 |
| | G/G | 14 ( 7.2%) | 7 (7.2%) | 0.99 (0.39-2.55) | |
| **Overdominant** | C/C-G/G | 106 (54.9%) | 61 (62.9%) | 1.00 | 0.19 |
| | C/G | 87 (45.1%) | 36 (37.1%) | 0.72 (0.44-1.19) | |
| **Log-additive** | --- | --- | --- | 0.81 (0.54-1.21) | 0.3 |
| **rs324981 (n=289)** | | | | | |
| **Codominant** | A/A | 66 (34.2%) | 31 (32.3%) | 1.00 | |
| | A/T | 89 (46.1%) | 43 (44.8%) | 1.03 (0.59-1.80) | 0.81 |
| | T/T | 38 (19.7%) | 22 (22.9%) | 1.21 (0.63-2.42) | |
| **Dominant** | A/A | 66 (34.2%) | 31 (32.3%) | 1.00 | |
| | A/T-T/T | 127 (65.8%) | 65 (67.7%) | 1.09 (0.65-1.83) | 0.75 |
| **Recessive** | A/A-A/T | 155 (80.3%) | 74 (77.1%) | 1.00 | |
| | T/T | 38 (19.7%) | 22(22.9%) | 1.21 (0.67-2.20) | 0.53 |
| **Overdominant** | A/A-T/T | 148 (69.8%) | 53 (55.2 %) | 1.00 | |
| | A/T | 58 (30.2%) | 43 (44.8%) | 0.95 (0.58-1.55) | 0.83 |
| **Log-additive** | --- | --- | --- | 1.10 (0.79-1.54) | 0.57 |

OR (*Odds Ratio*); *Logistic Regression.

**Table 3.** MDR analysis for polymorphisms.

| Model | *Training balance accuracy* | *Testing balance accuracy* | CVC | p-value* |
|---|---|---|---|---|
| **P1-P3** | 0.5739 | 0.5319 | 9/10 | 0.8403 |
| **P1-P2-P3** | 0.6235 | 0.5452 | 10/10 | 0.7123 |

CVC= *cross-validation consistency*; *p-value obtained value of 10,000 permutation tests. Abbreviations: P1 (rs2530547), P2 (rs727162) and P3 (rs324981) of gene *NPSR1*.

## 4. Discussion

To our knowledge, this is the first association study between the functional *NPSR1* polymorphisms and epilepsy. The investigation was conducted on JME, which presents a strong influence of the genetic component [46,47]. The *NPSR1* encoding gene became a high-ranking candidate for epilepsy susceptibility, specifically because of recent evidence showing that: i) NPS-NPSR1 forms a novel neurotransmitter system that regulates many brain functions [19]; ii) NPS administration evoked a proconvulsive effect in mice [41]; iii) *NPSR1* contains well-characterized gain-of-function polymorphisms [31]; and iv) *NPSR1* gene variants have been associated with some epilepsy comorbidity pathologies [35,38]. However, our data did not show a significant difference in the genotype and allele frequencies of these polymorphisms between the JME patients and the controls, even when OR was adjusted for sex and/or ethnicity. This suggests that there is no association of rs324981, rs2530547 and rs727162 with JME in this Brazilian sample.

However, we have to take into account that epilepsies result from an interaction among genetic variants present in the same or in different genes [12]. Thus, each *NPSR1* polymorphism may be only a weak contributor (and may not be sufficiently informative) and the effects on disease risk likely depend on their respective combinations in cis. Indeed, recently, [31] the rs2530547C-rs324981A-rs727162C haplotype was shown to correspond to allele with increased *NPSR1* mRNA expression and weaker intracellular signaling. The authors also observed that this "functional haplotype" is associated with reduced risk of inflammatory bowel disease. We also performed a genetic interaction analysis, shown in Table 3. Although we detected all possible combinations of alleles, which confirm that these polymorphisms are not in strong linkage disequilibrium (Supplementary Table 5), none of them differed significantly between the groups. Our data suggest that even the allele composed by rs2530547C-rs324981A-rs727162C variants which correspond to a highly expressed (2103C) NPSR1 protein, characterized by increased signaling properties (107Asn and 241Ser), is not related to JME susceptibility.

Another important confounder in genetic association studies is the heterogeneity in phenotype definition [48]. Collecting phenotypes narrowly and consistently is a relevant strategy for investigating susceptibility genes. Therefore, since JME displays a number of differentiable subclinical categories that may reflect differing underlying genetic influences, we performed the genetic association analysis using JME patients grouped according to specific endophenotypes, including the predominant time of seizures (Supplementary Table 2), and the types of seizures (Supplementary Table 3 and 4). Also, the allelic and genotypic frequencies for the three polymorphisms were not different between the groups. However, proof that genetic association between *NPSR1* variants and JME does not exist would need replication of our findings in independent samples. Moreover, further studies including other types of epilepsy and/or other functional polymorphisms are required in order to investigate the relationship between *NPSR1* genes and genetic susceptibility to chronic seizures.

The statistical test showed that this study has a power of >76% to detect association with JME. Although the sample size used was small the investigation included only JME patients rather than analyzing a clinically broader population (e.g. Idiopathic generalized epilepsy patients) with several epilepsy syndromes. This approach might have minimized possible bias from the limited sample size.

IJASRM

International Journal of Advanced Scientific Research and Management, Vol. 2 Issue 2, Feb 2017.

www.ijasrm.com

ISSN 2455-6378

## 5. Conclusion

Our results present no evidence for an association of these polymorphisms with JME. Further studies including other types of epilepsy and/or other functional polymorphisms are required to investigate the possible relationship between the NPSR1 gene and genetic susceptibility to chronic seizures.

## Appendix

**Supplementary Table 1.** Hardy-Weinberg equilibrium for polymorphisms rs2530547, rs727162, rs324981.

| | | | | | | |
|---|---|---|---|---|---|---|
| **rs2530547 (n=282)** | | | | | | |
| | **T/T** | **T/C** | **C/C** | **T** | **C** | **p-value*** |
| **All subjects** | 85 | 129 | 68 | 299 | 265 | 0.19 |
| **Controls** | 55 | 85 | 48 | 195 | 181 | 0.19 |
| **Patients** | 30 | 44 | 20 | 104 | 84 | 0.68 |
| **rs727162 (n=290)** | | | | | | |
| | **C/C** | **C/G** | **G/G** | **C** | **G** | **p-value*** |
| **All subjects** | 146 | 123 | 21 | 415 | 165 | 0.56 |
| **Controls** | 92 | 87 | 14 | 271 | 115 | 0.39 |
| **Patients** | 54 | 36 | 7 | 144 | 50 | 0.79 |
| **rs324981 (n=289)** | | | | | | |
| | **A/A** | **A/T** | **T/T** | **A** | **T** | **p-value*** |
| **All subjects** | 97 | 132 | 60 | 326 | 252 | 0.23 |
| **Controls** | 66 | 89 | 38 | 221 | 165 | 0.46 |
| **Patients** | 31 | 43 | 22 | 105 | 87 | 0.41 |

*Exact test by SNPStats.

**Supplementary Table 2.** Comparative analysis of genotypic frequencies obtained in groups of patients with predominant time of seizures and control group (without adjustment for gender).

| | | | | | |
|---|---|---|---|---|---|
| **rs2530547 (n=258)** | | | | | |
| **Model** | **Genotype** | **Controls** | **Patients** | **OR (95% IC)** | **p-value*** |
| **Codominant** | T/T | 55 (29.3%) | 20 (28.6%) | 1.00 | |
| | T/C | 85 (45.2%) | 34 (48.6%) | 1.10 (0.58-2.10) | 0.87 |
| | C/C | 48 (25.5%) | 16 (22.9 %) | 0.92 (0.43-1.97) | |
| **Dominant** | T/T | 55 (29.3%) | 20 (28.6%) | 1.00 | 0.91 |
| | T/C-C/C | 133 (70.7%) | 50 (71.4%) | 1.03 (0.56-1.90) | |
| **Recessive** | T/T-T/C | 140 (74.5%) | 54 (77.1%) | 1.00 | 0.66 |
| | C/C | 48 ( 25.5%) | 16 (22.9%) | 0.86 (0.45-1.65) | |
| **Overdominant** | T/T-C/C | 103 (54.8%) | 36 (51.4%) | 1.00 | 0.63 |
| | T/C | 85 (45.2%) | 34 (48.6%) | 1.14 (0.66-1.98) | |
| **Log-additive** | --- | --- | --- | 0.96 (0.66-1.40) | 0.85 |
| **rs727162 (n=266)** | | | | | |
| **Codominant** | C/C | 92 (47.7%) | 39 (53.4%) | 1.00 | |
| | C/G | 87 (45.1%) | 28 (38.4%) | 0.76 (0.43-1.34) | 0.61 |
| | G/G | 14 (7.2%) | 6 (8.2%) | 1.01 (0.36-2.82) | |
| **Dominant** | C/C | 92 (47.7%) | 39 (53.4%) | 1.00 | |
| | C/G-G/G | 101(52.3%) | 34 (46.6%) | 0.79 (0.46-1.36) | 0.4 |
| **Recessive** | C/C-C/G | 179 (92.8%) | 67 (91.8%) | 1.00 | |
| | G/G | 14 (21.3%) | 6 (8.2%) | 1.14 (0.42-3.10) | 0.79 |
| **Overdominant** | C/C-G/G | 106 (54.9%) | 45 (61.6%) | 1.00 | |
| | C/G | 87 (45.1%) | 28 (38.4%) | 0.76 (0.44-1.31) | 0.32 |
| **Log-additive** | --- | --- | --- | 0.88 (0.57-1.37) | 0.58 |
| **rs324981 (n=265)** | | | | | |
| **Codominant** | A/A | 66 (34.2%) | 19 (26.4%) | 1.00 | |
| | A/T | 89 (46.1%) | 33 (45.8%) | 1.29 (0.67-2.46) | 0.28 |
| | T/T | 38 (19.7%) | 20 (27.8%) | 1.83 (0.87-3.85) | |
| **Dominant** | A/A | 66 (32.8%) | 19 (26.4%) | 1.00 | |
| | A/T-T/T | 127 (65.8%) | 53 (73.6%) | 1.45 (0.79-2.65) | 0.22 |
| **Recessive** | A/A-A/T | 155 (80.3%) | 52 (72.2%) | 1.00 | |
| | T/T | 38 (19.7%) | 20 (27.8%) | 1.57 (0.84-2.93) | 0.16 |
| **Overdominant** | A/A-T/T | 104 (53.9%) | 39 (54.2%) | 1.00 | |
| | A/T | 89 (46.1%) | 33 (45.8%) | 0.99 (0.57-1.70) | 0.97 |
| **Log-additive** | --- | --- | --- | 1.35 (0.93-1.96) | 0.11 |

OR (*Odds Ratio*); AIC (Akaike Information Criterion); BIC (Bayesian Information Criterion); *Logistic Regression.

**Supplementary Table 3.** Comparative analysis of genotypic frequencies obtained of polymorphisms in *NPSR1* gene in groups of patients with types of seizures* and control group (analysis without adjustment for gender).

| | | | | | |
|---|---|---|---|---|---|
| **rs2530547 (n=254)** | | | | | |
| **Model** | **Genotype** | **Controls** | **Patients** | **OR (95% IC)** | **p-value**** |
| **Codominant** | T/T | 55 (29.3%) | 21 (31.8%) | 1.00 | |
| | T/C | 85 (45.2%) | 31 (47%) | 0.96 (0.50-1.83) | 0.77 |
| | C/C | 48(25.5%) | 14 (21.2%) | 0.76 (0.35-1.67) | |
| **Dominant** | T/T | 55 (29.3%) | 21 (31.8%) | 1.00 | 0.7 |
| | T/C-C/C | 133 (70.7%) | 45 (68.2%) | 0.89 (0.48-1.62) | |
| **Recessive** | T/T-T/C | 140 (74.5%) | 52 (78.8%) | 1.00 | 0.48 |
| | C/C | 48 ( 25.5%) | 14 (21.2%) | 0.79 (0.40-1.54) | |
| **Overdominant** | T/T-C/C | 103 (54.8%) | 35 (53%) | 1.00 | 0.81 |
| | T/C | 85 (45.2%) | 31 (47%) | 1.07 (0.61-1.88) | |
| **Log-additive** | --- | --- | --- | 0.88 (0.60-1.29) | 0.51 |
| **rs727162 (n=261)** | | | | | |
| **Codominant** | C/C | 92 (47.7%) | 38 (55.9%) | 1.00 | |
| | C/G | 87 (45.1%) | 25 (36.8%) | 0.70 (0.39-1.25) | 0.47 |
| | G/G | 14 (7.2%) | 5 (7.3%) | 0.86 (0.29-2.57) | |
| **Dominant** | C/C | 92 (47.7%) | 38 (55.9%) | 1.00 | |
| | C/G-G/G | 101(52.3%) | 30 (44.1%) | 0.72 (0.41-1.25) | 0.24 |
| **Recessive** | C/C-C/G | 179 (92.8%) | 63 (92.7%) | 1.00 | |
| | G/G | 14 (21.3%) | 5 (7.3%) | 1.01 (0.35-2.93) | 0.98 |
| **Overdominant** | C/C-G/G | 106 (54.9%) | 43 (63.2%) | 1.00 | |
| | C/G | 87 (45.1%) | 25 (36.8%) | 0.71 (0.40-1.25) | 0.23 |
| **Log-additive** | --- | --- | --- | 0.81 (0.51-1.27) | 0.35 |
| **rs324981 (n=260)** | | | | | |
| **Codominant** | A/A | 66 (34.2%) | 19 (28.4%) | 1.00 | |
| | A/T | 89 (46.1%) | 31 (46.3%) | 1.21 (0.63-2.33) | 0.53 |
| | T/T | 38 (19.7%) | 17 (25.4%) | 1.55 (0.72-3.34) | |
| **Dominant** | A/A | 66 (32.8%) | 19 (28.4%) | 1.00 | |
| | A/T-T/T | 127 (65.8%) | 48 (71.6%) | 1.31 (0.71-2.41) | 0.38 |
| **Recessive** | A/A-A/T | 155 (80.3%) | 50 (74.6%) | 1.00 | |
| | T/T | 38 (19.7%) | 17 (25.4%) | 1.39 (0.72-2.67) | 0.33 |
| **Overdominant** | A/A-T/T | 104 (53.9%) | 36 (53.7%) | 1.00 | |
| | A/T | 89 (46.1%) | 31 (46.3%) | 1.01 (0.58-1.76) | 0.98 |
| **Log-additive** | --- | --- | --- | 1.24 (0.85-1.83) | 0.26 |

OR (*Odds Ratio*); AIC (Akaike Information Criterion); BIC (Bayesian Information Criterion); *types of seizures (myoclonic seizures + generalized tonic-clonic seizures + absence); **Logistic Regression.

**Supplementary Table 4.** Comparative analysis of genotypic frequencies obtained of polymorphisms in *NPSR1* gene in groups of patients with types of seizures* and control group (analysis without adjustment for gender).

| | | | | | |
|---|---|---|---|---|---|
| **rs2530547 (n=211)** | | | | | |
| **Model** | **Genotype** | **Controls** | **Patients** | **OR (95% IC)** | **p-value**** |
| **Codominant** | T/T | 55 (29.3%) | 8 (34.8%) | 1.00 | |
| | T/C | 85 (45.2%) | 10 (43.5%) | 0.81 (0.30-2.18) | 0.85 |
| | C/C | 48(25.5%) | 5 (21.7%) | 0.72 (0.22-2.34) | |
| **Dominant** | T/T | 55 (29.3%) | 8 (34.8%) | 1.00 | 0.59 |
| | T/C-C/C | 133 (70.7%) | 15 (65.2%) | 0.78 (0.31-1.93) | |
| **Recessive** | T/T-T/C | 140 (74.5%) | 18 (78.3%) | 1.00 | 0.69 |
| | C/C | 48 ( 25.5%) | 5 (21.7%) | 0.81 (0.29-2.30) | |
| **Overdominant** | T/T-C/C | 103 (54.8%) | 13 (56.5%) | 1.00 | 0.87 |
| | T/C | 85 (45.2%) | 10 (43.5%) | 0.93 (0.39-2.23) | |
| **Log-additive** | --- | --- | --- | 0.84 (0.47-1.52) | 0.57 |
| **rs727162 (n=217)** | | | | | |
| **Codominant** | C/C | 92 (47.7%) | 14 (58.3%) | 1.00 | |
| | C/G | 87 (45.1%) | 8 (33.3%) | 0.60 (0.24-1.51) | 0.54 |
| | G/G | 14 (7.2%) | 2 (8.3%) | 0.94 (0.19-4.58) | |
| **Dominant** | C/C | 92 (47.7%) | 14 (58.3%) | 1.00 | |
| | C/G-G/G | 101(52.3%) | 10 (41.7%) | 0.65 (0.28-1.54) | 0.32 |
| **Recessive** | C/C-C/G | 179 (92.8%) | 22 (91.7%) | 1.00 | |
| | G/G | 14 (21.3%) | 2 (8.3%) | 1.16 (0.25-5.46) | 0.85 |
| **Overdominant** | C/C-G/G | 106 (54.9%) | 16 (66.7%) | 1.00 | |
| | C/G | 87 (45.1%) | 8 (33.3%) | 0.61 (0.25-1.49) | 0.27 |
| **Log-additive** | --- | --- | --- | 0.77 (0.38-1.57) | 0.47 |
| **rs324981 (n=217)** | | | | | |
| **Codominant** | A/A | 66 (34.2%) | 10 (41.7%) | 1.00 | |
| | A/T | 89 (46.1%) | 10 (41.7%) | 0.74 (0.29-1.88) | 0.77 |
| | T/T | 38 (19.7%) | 4 (16.7%) | 0.69 (0.20-2.37) | |
| **Dominant** | A/A | 66 (32.8%) | 10 (41.7%) | 1.00 | |
| | A/T-T/T | 127 (65.8%) | 14 (58.3%) | 0.73 (0.31-1.73) | 0.47 |
| **Recessive** | A/A-A/T | 155 (80.3%) | 20 (83.3%) | 1.00 | |
| | T/T | 38 (19.7%) | 4 (16.7%) | 0.82 (0.26-2.53) | 0.72 |
| **Overdominant** | A/A-T/T | 104 (53.9%) | 14 (58.3%) | 1.00 | |
| | A/T | 89 (46.1%) | 10 (41.7%) | 0.83 (0.35-1.97) | 0.68 |
| **Log-additive** | --- | --- | --- | 0.81 (0.45-1.48) | 0.5 |

OR (*Odds Ratio*); AIC (Akaike Information Criterion); BIC (Bayesian Information Criterion); *types of seizures (myoclonic seizures + generalized tonic-clonic seizures); **Logistic Regression.

**Supplementary table 5.** Linkage Disequilibrium* between polymorphisms genotyped in the study.

| **SNP** | **rs2530547** | **rs727162** | **rs324981** |
|---|---|---|---|
| rs2530547 | -- | 0.0941 | 0.018 |
| rs727162 | -0.0559 | -- | 0.0016 |
| rs324981 | 0.0168 | -9e04 | -- |

D′ is above the diagonal; $r^2$ is below the diagonal. *Analysis performed by SNPStats.

**Acknowledgments**

We thank all the participants in the research and the Neurology care unit team of Professor Alberto Antunes Hospital at the Federal University of Alagoas. This work was supported by CAPES, CNPq and FAPEAL. Language assistance, editing and proofreading was performed by Robert Edward Dower.

## References

[1] Genton P, Gelisse P. The history of juvenile myoclonic epilepsy. Epilepsy Behav 2013;28 Suppl 1:S2–7. doi:10.1016/j.yebeh.2013.01.002.

[2] Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 30:389–99. doi: 10.1111/j.1528-1157.1989.tb05316.x.

[3] Jallon P, Latour P. Epidemiology of idiopathic generalized epilepsies. Epilepsia 2005;46 Suppl 9:10–4. doi:10.1111/j.1528-1167.2005.00309.x.

[4] Camfield CS, Striano P, Camfield PR. Epidemiology of juvenile myoclonic epilepsy. Epilepsy Behav 2013;28 Suppl 1:S15–7. doi:10.1016/j.yebeh.2012.06.024.

[5] Janz D, Christian W. Impulsiv-Petit mal. Dtsch Zeitschrift Für Nervenheilkd 1957;176:346–86. doi:10.1007/BF00242439.

[6] Genton P, Thomas P, Kasteleijn-Nolst Trenité DGA, Medina MT, Salas-Puig J. Clinical aspects of juvenile myoclonic epilepsy. Epilepsy Behav 2013;28 Suppl 1:S8–14. doi:10.1016/j.yebeh.2012.10.034.

[7] Usui N, Kotagal P, Matsumoto R, Kellinghaus C, Lüders HO. Focal semiologic and electroencephalographic features in patients with juvenile myoclonic epilepsy. Epilepsia 2005;46:1668–76. doi:10.1111/j.1528-1167.2005.00262.x.

[8] Zifkin B, Andermann E, Andermann F. Mechanisms, genetics, and pathogenesis of juvenile myoclonic epilepsy. Curr Opin Neurol 2005;18:147–53.

[9] Pavlović M, Jović N, Pekmezović T. Antiepileptic drugs withdrawal in patients with idiopathic generalized epilepsy. Seizure 2011;20:520–5. doi:10.1016/j.seizure.2011.03.007.

[10] Martínez-Juárez IE, Alonso ME, Medina MT, Durón RM, Bailey JN, López-Ruiz M, et al. Juvenile myoclonic epilepsy subsyndromes: family studies and long-term follow-up. Brain 2006;129:1269–80. doi:10.1093/brain/awl048.

[11] Steinlein OK. Gene polymorphisms and their role in epilepsy treatment and prognosis. Naunyn Schmiedebergs Arch Pharmacol 2010;382:109–18. doi:10.1007/s00210-010-0531-8.

[12] Greenberg DA, Pal DK. The state of the art in the genetic analysis of the epilepsies. Curr Neurol Neurosci Rep 2007;7:320–8.

[13] Gitaí DLG, Romcy-Pereira RN, Gitaí LLG, Leite JP, Garcia-Cairasco N, Paço-Larson ML. Genes e epilepsia I: epilepsia e alterações genéticas. Rev Assoc Med Bras 2008;54:272–8. doi:10.1590/S0104-42302008000300023.

[14] Gitaí LLG, de Almeida DH, Born JPL, Gameleira FT, de Andrade TG, Machado LCH, et al. Lack of association between rs211037 of the GABRG2 gene and juvenile myoclonic epilepsy in Brazilian population. Neurol India 2013;60:585–8. doi:10.4103/0028-3886.105191.

[15] Born JPL, Santos BP Dos, Secolin R, Gameleira FT, Andrade TG de, Machado LCH, et al. Lack of association between the prothrombin rs1799963 polymorphism and juvenile myoclonic epilepsy. Arq Neuropsiquiatr 2015;73:289–92. doi:10.1590/0004-282X20150010.

[16] Santos B, Marques T, Gameleira F, Secolin R, Andrade T, Gitaí L, et al. Epilepsy & Behavior polymorphisms are not associated with juvenile myoclonic epilepsy. Epilepsy Behav 2014;36:82–5. doi:10.1016/j.yebeh.2014.04.024.

[17] Hökfelt T, Broberger C, Xu ZQ, Sergeyev V, Ubink R, Diez M. Neuropeptides--an overview. Neuropharmacology 2000;39:1337–56. doi: 10.1016/S0028-3908(00)00010-1.

[18] Reinscheid RK, Xu Y-L. Neuropeptide S and its receptor: a newly deorphanized G protein-coupled receptor system. Neuroscientist 2005;11:532–8. doi:10.1177/1073858405276405.

[19] Pitti T, Manoj N. Molecular evolution of the

neuropeptide S receptor. PLoS One 2012;7:e34046. doi:10.1371/journal.pone.0034046.

[20] Xu Y-L, Reinscheid RK, Huitron-Resendiz S, Clark SD, Wang Z, Lin SH, et al. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 2004;43:487–97. doi:10.1016/j.neuron.2004.08.005.

[21] Lukas M, Neumann ID. Nasal application of neuropeptide S reduces anxiety and prolongs memory in rats: social versus non-social effects. Neuropharmacology 2012;62:398–405. doi:10.1016/j.neuropharm.2011.08.016.

[22] Jüngling K, Seidenbecher T, Sosulina L, Lesting J, Sangha S, Clark SD, et al. Neuropeptide S-mediated control of fear expression and extinction: role of intercalated GABAergic neurons in the amygdala. Neuron 2008;59:298–310. doi:10.1016/j.neuron.2008.07.002.

[23] Beck B, Fernette B, Stricker-Krongrad A. Peptide S is a novel potent inhibitor of voluntary and fast-induced food intake in rats. Biochem Biophys Res Commun 2005;332:859–65. doi:10.1016/j.bbrc.2005.05.029.

[24] Fedeli A, Braconi S, Economidou D, Cannella N, Kallupi M, Guerrini R, et al. The paraventricular nucleus of the hypothalamus is a neuroanatomical substrate for the inhibition of palatable food intake by neuropeptide S. Eur J Neurosci 2009;30:1594–602. doi:10.1111/j.1460-9568.2009.06948.x.

[25] Smith KL, Patterson M, Dhillo WS, Patel SR, Semjonous NM, Gardiner J V, et al. Neuropeptide S stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake. Endocrinology 2006;147:3510–8. doi:10.1210/en.2005-1280.

[26] Rizzi A, Vergura R, Marzola G, Ruzza C, Guerrini R, Salvadori S, et al. Neuropeptide S is a stimulatory anxiolytic agent: a behavioural study in mice. Br J Pharmacol 2008;154:471–9. doi:10.1038/bjp.2008.96.

[27] Li W, Chang M, Peng Y-L, Gao Y-H, Zhang J-N, Han R-W, et al. Neuropeptide S produces antinociceptive effects at the supraspinal level in mice. Regul Pept 2009;156:90–5. doi:10.1016/j.regpep.2009.03.013.

[28] Okamura N, Garau C, Duangdao DM, Clark SD, Jüngling K, Pape H-C, et al. Neuropeptide S Enhances Memory During the Consolidation Phase and Interacts with Noradrenergic Systems in the Brain. Neuropsychopharmacology 2010;36:744–52. doi:10.1038/npp.2010.207.

[29] Han R-W, Yin X-Q, Chang M, Peng Y-L, Li W, Wang R. Neuropeptide S facilitates spatial memory and mitigates spatial memory impairment induced by N-methyl-d-aspartate receptor antagonist in mice. Neurosci Lett 2009;455:74–7. doi:10.1016/j.neulet.2009.03.023.

[30] Cao J, de Lecea L, Ikemoto S. Intraventricular administration of neuropeptide S has reward-like effects. Eur J Pharmacol 2011;658:16–21. doi:10.1016/j.ejphar.2011.02.009.

[31] Anedda F, Zucchelli M, Schepis D, Hellquist A, Corrado L, D'Alfonso S, et al. Multiple polymorphisms affect expression and function of the neuropeptide S receptor (NPSR1). PLoS One 2011;6:e29523. doi:10.1371/journal.pone.0029523.

[32] Reinscheid RK, Xu Y-L, Okamura N, Zeng J, Chung S, Pai R, et al. Pharmacological characterization of human and murine neuropeptide s receptor variants. J Pharmacol Exp Ther 2005;315:1338–45. doi:10.1124/jpet.105.093427.

[33] Bernier V, Stocco R, Bogusky MJ, Joyce JG, Parachoniak C, Grenier K, et al. Structure-function relationships in the neuropeptide S receptor: molecular consequences of the asthma-associated mutation N107I. J Biol Chem 2006;281:24704–12. doi:10.1074/jbc.M603691200.

[34] Nepomuceno D, Sutton S, Yu J, Zhu J, Liu C, Lovenberg T, et al. Mutagenesis studies of neuropeptide S identify a suitable peptide tracer for neuropeptide S receptor binding studies and peptides selectively activating the I(107) variant of human neuropeptide S receptor. Eur J Pharmacol 2010;635:27–33. doi:10.1016/j.ejphar.2010.03.008.

[35] Laas K, Reif A, Akkermann K, Kiive E, Domschke K, Lesch K-P, et al. Interaction of the neuropeptide S receptor gene $Asn^{107}Ile$ variant and environment: contribution to affective and anxiety disorders, and suicidal behaviour. Int J Neuropsychopharmacol 2014;17:541–52. doi:10.1017/S1461145713001478.

[36] Glotzbach-Schoon E, Andreatta M, Reif A,

Ewald H, Tröger C, Baumann C, et al. Contextual fear conditioning in virtual reality is affected by 5HTTLPR and NPSR1 polymorphisms: effects on fear-potentiated startle. Front Behav Neurosci 2013;7:31. doi:10.3389/fnbeh.2013.00031.

[37] Domschke K, Reif A, Weber H, Richter J, Hohoff C, Ohrmann P, et al. Neuropeptide S receptor gene -- converging evidence for a role in panic disorder. Mol Psychiatry 2011;16:938–48. doi:10.1038/mp.2010.81.

[38] Spada J, Sander C, Burkhardt R, Häntzsch M, Mergl R, Scholz M, et al. Genetic association of objective sleep phenotypes with a functional polymorphism in the neuropeptide S receptor gene. PLoS One 2014;9:e98789. doi:10.1371/journal.pone.0098789.

[39] Lennertz L, Quednow BB, Schuhmacher A, Petrovsky N, Frommann I, Schulze-Rauschenbach S, et al. The functional coding variant Asn107Ile of the neuropeptide S receptor gene (NPSR1) is associated with schizophrenia and modulates verbal memory and the acoustic startle response. Int J Neuropsychopharmacol 2011;15:1205–15. doi:10.1017/S1461145711001623.

[40] Lennertz L, Franke PE, Grabe HJ, Rampacher F, Schulze-Rauschenbach S, Guttenthaler V, et al. The functional coding variant Asn107Ile of the neuropeptide S receptor gene (NPSR1) influences age at onset of obsessive-compulsive disorder. Int J Neuropsychopharmacol 2013;16:1951–8. doi:10.1017/S1461145713000382.

[41] Ramos SF, Mendonça BP, Leffa DD, Pacheco R, Damiani AP, Hainzenreder G, et al. Effects of neuropeptide S on seizures and oxidative damage induced by pentylenetetrazole in mice. Pharmacol Biochem Behav 2012;103:197–203. doi:10.1016/j.pbb.2012.09.001.

[42] Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics 2006;22:1928–9. doi:10.1093/bioinformatics/btl268.

[43] Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods 2009;41:1149–60. doi:10.3758/BRM.41.4.1149.

[44] Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175–91.

[45] Moore JH, Gilbert JC, Tsai C-T, Chiang F-T, Holden T, Barney N, et al. A flexible computational framework for detecting, characterizing, and interpreting statistical patterns of epistasis in genetic studies of human disease susceptibility. J Theor Biol 2006;241:252–61. doi:10.1016/j.jtbi.2005.11.036.

[46] Delgado-Escueta A V, Koeleman BPC, Bailey JN, Medina MT, Durón RM. The quest for juvenile myoclonic epilepsy genes. Epilepsy Behav 2013;28 Suppl 1:S52–7. doi:10.1016/j.yebeh.2012.06.033.

[47] Kjeldsen MJ, Corey LA, Solaas MH, Friis ML, Harris JR, Kyvik KO, et al. Genetic factors in seizures: a population-based study of 47,626 US, Norwegian and Danish twin pairs. Twin Res Hum Genet 2005;8:138–47. doi:10.1375/1832427053738836.

[48] Greenberg DA, Subaran R. Blinders, phenotype, and fashionable genetic analysis: a critical examination of the current state of epilepsy genetic studies. Epilepsia 2011;52:1–9. doi:10.1111/j.1528-1167.2010.02734.x.