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Lack of association between *COMT* Val158met polymorphism and Juvenile Myoclonic Epilepsy

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Abstract

Juvenile myoclonic epilepsy (JME) is the most common genetic generalized epilepsy, representing 5% to 10% of all epilepsies. The genetic component is an important factor in the etiology of JME, which may show Mendelian or genetically complex inheritance. Therefore, the identification of susceptibility genes for JME has aroused the interest of researchers. In this context, the gene encoding for the enzyme Catecholamine O-methyl Transferase (*COMT*), whose function is the deactivation of catecholamines in the synaptic cleft, is a strong candidate. The dysregulation of these neurotransmitters may contribute to the generation and modulation of seizures. Thus, the objective of this case/control study is to investigate whether the *COMT* val158met polymorphism (rs4680) is associated with JME. Genotyping of 96 patients and 200 controls was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the statistical analyzes were done through the SNPStats platform. No significant differences were observed in the genotype and allele frequencies of this polymorphism between cases and controls. The results present no evidence for an association of *COMT* val158met with JME. Further studies including other functional polymorphisms are required to investigate the involvement of *COMT* gene in the genetic susceptibility to JME.

Keywords: *COMT, polymorphism, juvenile myoclonic epilepsy.*

1. Introduction

Juvenile myoclonic epilepsy (JME) is the most common form of genetic generalized epilepsy. The frequency of JME is 5-10% of all people with epilepsy] (Jallon et al., 2005). JME typically manifests during adolescence and is characterized by arrhythmic myoclonic seizures, also experiencing generalized tonic-clonic seizures (GTCS) and, less often, absence seizures (ILAE., 1989).

JME have a complex genetic inheritance, making it difficult to identify susceptibility genes. In complex disorders such as these, interactions between different susceptibility genes may predispose to disease. A specific set of alleles in each determines the genetic threshold of susceptibility to a particular condition. In general, individuals with a high susceptibility threshold to epilepsy do not present epileptic seizures even when exposed to epileptogenic disorders or precipitating factors of seizures. On the other hand, individuals with low susceptibility threshold present chronic or reactive seizures when exposed, respectively, to epileptogenic disorders or precipitating factors of seizures. In order to obtain a synthetic view of the process of epileptogenesis, based on the above discussion, we can infer that epileptogenic disorders and precipitating factors, in a facilitating genetic environment, induce alterations in specific molecular pathways, which interfere, directly or indirectly, in the cerebral circuits, and may lead to the onset of epileptic seizures (Gitaí et al., 2008).

Thus, the identification of susceptibility genes helps to understand the genetic mechanisms involved in epilepsy. *COMT* is an essential gene controlling neural activity. This enzyme catalyzes the transfer of a methyl grouping of S-adenosylmethionine to catecholamines, which include dopamine, epinephrine, and norepinephrine. This mechanism interrupts the synaptic transmission exerted by these neurotransmitters. As epilepsy is related to

uncontrolled synaptic discharges, the genes responsible for this control may be altered in epileptic patients. The most studied functional genetic alteration in this gene is the Val158met polymorphism (rs4680) in which a single G/A base-pair substitution leads to a valine (Val) to methionine (Met) substitution at codon 158, producing an enzyme with reduced activity (Singh et al., 2012). Therefore, the *COMT* gene is a strong candidate.
This gene has been associated with genetically complex neuropsychiatric conditions, such as panic disorder and depression. As previously mentioned, these conditions are among the most frequent comorbidities associated with epilepsy. In fact, in a recent study, Woo et al. (2002) investigated the association of the *COMT* gene Val158Met SNP with panic disorder in the city of Seoul, Korea. In the control group, individuals homozygous for the polymorphism represented 2.2%, whereas, in the group with panic disorder, this percentage was 19.6%. The results suggested that this genotype may be related to the development and consequent treatment of patients with panic disorder. In another study, Âberg et al. (2011) investigated the association of this polymorphism with depression in a Swedish population, homozygous genotypes for polymorphism were more common in depressive men. In women, there was no association.

2. Materials and Methods

2.1 Patients and controls

This study included 296 individuals composed of 96 unrelated patients with JME (63 females, mean of age 25.0) and 200 healthy control subjects (133 females, mean of age 30.5), all of whom were recruited from the state of Alagoas in northeastern Brazil. The study was approved by the Ethics Committee of the Federal University of Alagoas, Brazil and all subjects gave written informed consent before blood collection was performed (CAEE: 17265313.6.0000.5013). Cases were matched with controls 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. Diagnostic criteria of the Commission on Classification and Terminology of the International League against Epilepsy were used to diagnose probands with JME. All patients were submitted to electroencephalography analysis, and only those with generalized spike-wave pattern were included in this study.

2.2 Genetic analysis

DNA of patients and controls was extracted from leukocytes of peripheral blood by FlexiGene DNA Kit (Qiagen,USA). Initially, PCR was performed using the protocol described by Taq polymerase (Fermentas Life Science) in DNA Thermal Cycler (MJ96G BioCycler). Then, 10 µL of the amplicon was submitted to restriction reaction with 5U (1uL) Nla III Restriction Enzyme (New England Biolabs, UK), at 37°C for 18h. Digestion products were separated by electrophoresis on 8% Acrylamide gels and then were stained with ethidium bromide and visualized by ultraviolet light. Here, the bands containing the fragments in sizes 114pb, 83bp and 20bp are G/G genotypes, while the bands containing the 96bp, 83bp, 20bp, and 18bp sizes are A/A genotypes. Bands containing all fragment sizes are G/A genotypes.

2.3 Statistical analysis

The statistical analysis of the data were performed using SNPstats, a web-based tool offered by the Biostatistics and Bioinformatics Unit Web of The Catalan Institute Oncology (Solé, 2006). Allele and genotype frequencies were calculated by counting and to verify if the populations studied were in Hardy-Weinberg equilibrium the chi-square statistical test was performed, using different inheritance models. To assess the association between a *COMT* polymorphism and juvenile myoclonic epilepsy, logistic regression analysis was performed, and the odds ratio (OR) with the 95% confidence interval (95% CI) was calculated for association with the respective genotypes.

3. Results

The mean age at onset of the JME probands' was 13.4 (range 4-27) years, and 65.6% of them were females. The triad of myoclonus, absences, and generalized tonic-clonic seizures was observed in 69.8% of the patients, the combination of myoclonus and generalized tonic-clonic seizures in 25%, the combination of myoclonus and absence was observed in 2% and the myoclonus alone in 2% of patients. Of the 96 patients, 66.7% of patients were on monotherapy and the remaining patients were on polytherapy.
The genotype distribution did not deviate significantly from that expected by Hardy–Weinberg equilibrium ($p = 0,28$), as estimated by chi-square test. Duplicated genotyping of 20% of samples revealed 100% of genotyping concordance. The Proportions of G homozygotes, G/A heterozygotes

and A/A were 34%, 47% and 19% in JME patients, respectively, whereas in control group were 32%, 55%, and 14%, respectively. The allele frequencies of G and A were, respectively, 58% and 42% in JME patients and 41% and 59% in the control group. Genotype proportions and allele frequencies for the *COMT* val158met polymorphism in both groups were not significantly different (Table 1). No significant differences were observed in the genotype and allele frequencies of this polymorphism between cases and controls.

Table 1. Genotype frequencies of COMT val158met polymorphism in the controls and JME patients.

Model	Genotype	Patients	Controls	OR (95% CI)	P-value
Codominant	G/G	33 (34.4%)	63 (31.5%)	1.00	0.4
	G/A	45 (46.9%)	109 (54.5%)	0.79 (0.46-1.36)	
	A/A	18 (18.8%)	28 (14%)	1.23 (0.59-2.54)	
Dominant	G/G	33 (34.4%)	63 (31.5%)	1.00	0.62
	G/A-A/A	63 (65.6%)	137 (68.5%)	0.88 (0.52-1.47)	
Recessive	G/G-G/A	78 (81.2%)	172 (86%)	1.00	0.3
	A/A	18 (18.8%)	28 (14%)	1.42 (0.74-2.71)	
Overdom inant	G/G-A/A	51 (53.1%)	91 (45.5%)	1.00	0.22
	G/A	45 (46.9%)	109 (54.5%)	0.74 (0.45-1.20)	
Log-additive	---	---	---	1.04 (0.73-1.50)	0.82

4. Discussion

Juvenile myoclonic epilepsy is a common genetic generalized epilepsy that is characterized by arrhythmic myoclonic seizures. Since the genetic component of JME is strong, the identification of susceptibility genes is important to unravel molecular mechanisms and guide therapeutics.

In this study, we show that the genotype distribution and allele frequencies of *COMT* val158met polymorphism in patients with JME was not different when compared to healthy subjects. JME can show a genetically complex inheritance pattern, which can difficult the identification of susceptibility genes. Indeed, 22 chromosomal loci and seven genes have been associated with the disease, six of which encode for ion channel subunits (Santos et al., 2017; Striano and Nobile, 2018).

The catecholamines are abundant in CNS, and acts in the modulation of seizure susceptibility (Weinshenker and Szot, 2002). Dopamine signaling, for example, has a substantial role in epileptogenesis (Bozzi and Borrelli 2013). Studies have already shown that the manifestations of JME as a short-term memory loss are related to the areas of dopaminergic innervation and that striatal dopaminergic transmission is involved in the interruption of seizures. In addition, myoclonus are related to deficiencies in the dopaminergic pathways (Ciumas et al. 2008). In adrenergic pathways, it is known that damage in adrenergic circuitry increased susceptibility for epileptic seizures and that the reduction in adrenergic transmission is associated with refractory epilepsy (Giogi et al. 2004).

Genetic associations studies can suffer from low statistical power due to the small sample size, which leads to increased rate of both false-positive and false-negative results (Hirschhorn and Altshuler, 2002). Furthermore, it is known that genotype distributions can differ across different populations because differences in genetic background (Colhoun et al. 2003).

5. Conclusions

In summary, our study showed a lack of association between COMT val158met polymorphism and JME. However, it is necessary to conduct more studies using larger number of patients with epilepsy and well-matched controls. This can clarify and increase our understanding of the association between Val158Met polymorphism and JME.

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References

[1] Âberg, E., Fandinô-Losada, A., Sjoholm, L. k., Forsell, Y and Laverbratt, C. The functional Val158Met polymorphism in catechol-O-methyltransferase (COMT) is associated with depression and motivation in men from a Swedish population-based study.J Affect disord, Vol 129 (1-3): 158-66, (2011).

[2] Bozzi, Y., Borrelli and E. The role of dopamine signaling in epileptogenesis. Front Cell Neurosci Vol 7:157, (2013).
[3] Ciumas, C., Wahlin, T. B., Jucaite, A., Lindstrom, P., Halldin, C and Savic, I. Reduced dopamine transporter binding in patients with juvenile myoclonic epilepsy. Neurology, Vol 71 (11): 788-94, (2008).
[4] Colhoun, H. M., McKeigue, P. M and Smith, G. D. Problems of reporting genetic associations with complex outcomes. The Lancet, Vol 361 (9360): 865-872, (2003).
[5] Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia, Vol 36 (65): 8–61, (1989).
[6] Giogi, F. S., Pizzanelli C., Biagioni F., Murri L and Fornai, F. The role of norepinephrine in epilepsy: from the bench to the bedside. Neurosci. Biobehav. Rev, Vol 28 (5): 507-24, (2004).
[7] Gitai, D.L.G., Romcy-pereira, R. N., Gitai, L. L. G., Leite, J. P. P., Garcia-Cairasco, N and Paço-Larson, M. L. Genes e Epilepsia I: Epilepsia e Alterações Genéticas. Rev Assoc Med Bras, Vol 54 (3): 272-8, (2008).
[8] Hirschhorn, J. N and Altshuler, D. Once and Again—Issues Surrounding Replication in Genetic Association Studies. The Journal of Clinical Endocrinology & Metabolism, Vol 87 (10): 4438-4441, (2002).
[9] Jallon, P and Latour, P. Epidemiology of idiopathic generalized epilepsies. Epilepsia, Vol 46 (9): 10-4, (2005).
[10] Singh, J. P., Volavka, J., Czobor, P and Dorn, R. A. V. A Meta-Analysis of the Val158Met COMT Polymorphism and Violent Behavior in Schizophrenia. PLoS ONE, Vol 7 (8): 43423. doi:10.1371/journal.pone.0043423 (2012).
[11] Santos, B. P., Marinho, C. R. M., Maruqes, T. E. B. S., Angelo, L. K. G., Malta, M. V. S., Duzzioni, M., Castro, O. W., Leite, J. P., Barbosa, F. T and Gitaí, D. L. G. Genetic susceptibility in Juvenile Myoclonic Epilepsy: Systematic review of genetic association studies. PlosOne, Vol 12 (16): : e0179629, (2017)
[12] Solé, X., Guino, E., Valls, J., Iniesta, R and Moreno, V. SNPStats: a web tool for the analysis of association studies. Bioinformatics, Vol 22 (15): 1928-1929, (2006).
[13] Striano, P and Nobile, E. The genetic basis of juvenile myoclonic epilepsy. The Lancet, Vol 17 (6): 493-495, (2018).
[14] Weinshenker, D and Szot P. The role of catecholamines in seizure susceptibility: new results using genetically engineered mice. Pharmacol. Ther. Vol 94 (3): 213-233, (2002).
[15] Woo, J. M., Yoon, K. S and Yu, B. H. Cathechol O-Methiltransferase Genetic Polymorphism in Panic Desorder. Am J Psychiatry, Vol 159 (10):1783-1785, (2002).