Polymorphism of the SLC6A4 gene of the SERT transporter in individuals with completed suicide

Original Article

Guillén-Rangel, Guadalupe C1; Contreras-Pérez, Carlos Manuel2; Barrientos-Salcedo, Carolina3

SUMMARY

Introduction. Suicide has been related to diverse affective pathologies, including depression; this entity has been linked to polymorphisms of the genes that code for the serotonin transporters and other neurotransmitters, which could occur in individuals with completed suicides.

Material and Methods: Blood samples were taken from 9 individuals who died due to suicide attempt in the Medical Forensic Service of the Jaliscience Institute of Forensic Sciences; DNA was extracted by means of the Promega Wizard Genomic DNA Purification Kit. For the detection of the polymorphism the endpoint PCR technique was performed with specific primers for SCT6A4 of SERT, for detection of the s / s and l / l polymorphisms.

Results: We included 9 individuals aged 31 to 73 years, 8 male and 1 female, of Mexican origin and 5 individuals as a control group, who died due to non-suicidal vehicular collision. Of the group of suicides, in 7 (78%) the polymorphism l / l corresponding to the amplified product of 512 bp was observed and in 2 (22%) the s / s polymorphism corresponding to the amplified product of 468 bp. In the control group, the polymorphism l / l was amplified in 4 individuals (80%) and the s / s in 1 (20%).

Conclusion: No statistically significant differences were found between the prevalence of polymorphisms of the SLC6A4 gene in individuals with completed suicide compared to the control group. It is possible that SERT activity is only a marker of sensitivity to antidepressant treatment.

Palabras Clave: Violent death, homicide, suicide, rural community
INTRODUCTION

The nervous system, present in all vertebrates and in many invertebrates, is the anatomical and functional basis that allows organisms to respond to changes in the environmental stimuli. The stimuli can be internal and external. The reaction that occurs is known as response, which is an adjustment that brings well-being to the body. The stimulus-response reactions are usually rapid and are an uninterrupted mechanism for maintaining internal consistency in the face of environmental changes (Fried, 1990). All these reactions are accompanied by a strong affective component, which is often determinant, as well as very varied vegetative manifestations. Experimental observations show that the coordination of this vast complex of somatic, affective and visceral components is carried out through the functional mediation of the structures of the limbic system (Bustamante, 1996). The activity of these structures related to emotional control supports and generates not only our emotional feelings, but also a set of motor, autonomic and endocrine responses, which probably evolved to dispose them to action and as way of social signaling of intentionality (Llinás, 2002; Velasco, 1998).

A group of molecules that fulfill neurotransmitter function parameters in the nervous system have been identified. For a neuroactive molecule to be considered a neurotransmitter, it must: possess a mechanism for its synthesis in presynaptic neurons; have a presynaptic location; have a release mechanism; its synaptic activity must be replicable through the exogenous application of the molecule; and have an identifiable effector mechanism (receiver) and signal determination (Toro, 2010).

Of great interest in suicidal behavior, serotonin is a substance belonging to the biogenic amines that acts as a neurotransmitter and as a neuromodulator, plays an important role in mood, anxiety, sleep and is distributed throughout the body. In the case of mammals, it is 95% in the enterochromaffin system of the gastrointestinal tract and the rest in the platelets and triptaminergic neurons of the central nervous system and the enteric nervous system. (Gershon, 2004). It has been shown that 5-HT is involved in different functions, including sleep, appetite, temperature, anxiety, motor activity, biological rhythm, learning and memory. It is present in a wide variety of areas of the brain. The alteration of serotonergic activity in the CNS seems to be involved in the appearance of various neuropsychiatric pathologies, such as affective disorders, obsessive-compulsive behaviors, panic, depression and seasonal affective disorder (Yura et al, 1996).

As soon as serotonin is released into the synaptic space, it is recaptured and subsequently degraded to its inactive metabolites. The serotonin transporter (SERT) is the membrane protein responsible for introducing 5-HT into the cell, and consequently reduces the availability of 5-HT in the extracellular medium. This transporter has become the main pharmacological target for the treatment of psychiatric pathologies in which the serotonergic system is altered. This is the case of tricyclic antidepressants, which prevent the reuptake of noradrenaline and serotonin,
and selective inhibitors of serotonin reuptake (SSRI). These inhibitors inhibit the reuptake of serotonin immediately, however, the therapeutic effect is reached after a long and repetitive treatment for reasons still unknown (Franzer, 1997).

Regulation of the extracellular concentration of 5-HT is carried out by means of a high affinity transporter, dependent on Na+ and energy. This transporter allows to internalize part of the 5-HT released after the passage of electrical impulses through the serotonergic axons. Therefore, it is the most effective mechanism to regulate the accessibility of 5-HT to pre and postsynaptic receptors, and ultimately, to control the activity of the serotonergic neurotransmission system (Moya, 2013). By recapturing 5-HT, and thus regulating the magnitude and range of responses to the neurotransmitter, SERT participates in the fine-tuning of cerebral serotonergic synapses, as well as in their peripheral actions. Interestingly, the greatest expression of SERT is found in cortical and limbic regions involved in behavior and emotional states (Murphy, 2011).

The human SLC6A4 gene, which encodes SERT, is a region of ~ 40 Kb, located on chromosome 17q11.2 and consists of fourteen exons (Figure 1). The sequence of its transcript predicts a 630 amino acid protein containing twelve transmembrane domains (Murphy, 2012).

There are variants of the SLC6A4 promoter: The first polymorphism in the 5' region of SLC6A4 was described in 1996. The authors named this polymorphism as the "polymorphic region associated with the serotonin transporter (5-HT Transporter-Linked Polymorphic Region, 5-HTTLPR). The L and S alleles of 5-HTTLPR have different transcriptional efficiencies, with S being comparatively less effective than L. When the same authors discovered that the S allele of 5-HTTLPR is associated with personality traits related to anxiety and depression, this resulted in an advance in the area of psychiatric genetics (Lesch, 1996).

Suicide is the self-inflicted act to cause death voluntarily, deliberately, in which three stages intervene successively, called together a suicidal process: suicidal desire, the suicidal idea and the suicidal act itself (Durkheim, 1982; 2002). Suicide is considered a multifactorial event in which biological, individual and environmental social factors participate. Suicidal behavior is preceded by some risk factors, including the presence of psychiatric illnesses, such as anxiety disorders, depression, substance abuse, personality disorders, schizophrenia and panic disorders (Hernández, 2001; Purselley, 2003). Moderately severe affective anxiety

Figure 1. Microscopical structure of the SERT codifying gene (obtained from Murphy et al, 2012)
disorders, transient adjustment reactions, anxiety as a personality trait and obsessive characteristics are also considered suicide risk factors (Gutiérrez-García, 2006, Charlier, 2003, Perlis, 2007).

Suicidal behavior implies some neurobiological processes already identified. Alterations of the serotonergic neurotransmission system (5HT) play an important role in the pathogenesis of suicide. The content of the main metabolite of serotonin, 5-hydroxy-indole-acetic acid (5-HIAA) is decreased in the cerebrospinal fluid of some individuals with violent suicide attempts, although dysfunction of other neurotransmission systems has also been found, such as dopaminergic and noradrenergic (Weiss, 2010; Isung, 2012).

Depressive affective disorder is characterized by emotional dysregulation and is one of the clinical entities that is most often associated with suicide. On the other hand, antidepressants have actions on the neuronal activity of these structures, in addition to the lateral septal nuclei. And, finally, serotonin is one of the neurotransmitters involved in the actions of antidepressants and possibly in suicide. But the results have not been conclusive; it is mandatory to know the role of SERT gene polymorphisms in the cerebral cortex of suicide victims.

METHODS

To identify the presence of the SERT polymorphism, blood samples were taken at the Forensic Medical Service of the Jalisco Institute of Forensic Sciences, located in the city of Tlaquepaque, Jalisco. For the extraction of DNA, the protocol established by the Promega Wizard® Genomic DNA Purification Kit was followed.

To carry out the detection of the polymorphism, the PCR technique was carried out. The primers specific for SLC6A4 of SERT (GenBank: EU035982.1) were designed for the amplification of the insertion / deletion polymorphic sequence. The size of the product obtained is 468 bp for the deletion allele (s) and 512 bp for the insertion allele (l). The SERT polymorphism in the SLC6A4 gene; the shortest variant (short / short or s / s) results in less transcriptional activity and greater vulnerability to affective disorders. In contrast, the longer variant (insertion) (long / long or 1 / 1) results in a higher transcriptional activity (Heils et al, 1996). The amplification was performed under the following conditions: an initial denaturation cycle at 95 ° C for 3 minutes, 35 cycles of: 1 minute of denaturation at 95 ° C, 1 minute of annealing at 61 ° C and 1 minute of extension at 72 ° C and 10 minutes of final extension at 72 ° C.

The PCR amplification products obtained were subjected to electrophoresis in a 3% agarose gel at 120 V for 30 minutes. From the total reaction volume of 12.5 μl of the PCR, 5 μl were taken for the run, and a molecular weight marker of 1000 bp was loaded. It was treated with ethidium bromide at 20 mg / ml for 20 minutes. The amplified products were observed in a transilluminator with UV light.

RESULTS

The samples used in this study were taken within a period of 8 hours after death, both in patients and controls, to guarantee the reliability of the test, even
though the collection tube of the sample contains the anticoagulant EDTA. Natural decomposition of the body can alter the integrity of the sample as well as fragmentation in the DNA which affects the effectiveness of the PCR.

We included 9 blood samples of consummate suicidal individuals aged between 31 and 73 years, 8 of the male sex and 1 of the female sex. Of the group of suicidal individuals, in 7 (78%) the polymorphism l / l corresponding to the amplified product of 512 bp was observed and in 2 (22%) the s / s polymorphism corresponding to the amplified product of 468 bp was obtained (Figure 2).

![Figure 2](image1.png)

**Figure 2.** Alleles of suicide samples. Column 1, molecular weight marker; column 2, 5-10, presence of the insertion polymorphism (l / l) of 512 bp with higher transcription; columns 3 and 4, presence of the deletion polymorphism (s / s) of 468 bp with decreased transcription

As a control group, 5 blood samples from individuals with an etiology of death were included; their cause of death was overcrowding; ages between 30 and 74 years, 3 of the male sex and 2 of the female sex. In 4 (80%) of them the polymorphism l / l and 1 (20%) polymorphism s / s were observed (figure 3).

![Figure 3](image2.png)

**Figure 3.** Control sample alleles. Column 1, molecular weight marker; columns 2, 3, 5 and 6 presence of insertion polymorphism (l / l); column 4, presence of deletion polymorphism (s / s).
DISCUSSION

It has long been shown that antidepressant treatments modify the functioning of the serotonergic system (Blier, 1987, Contreras, 1993, Contreras, 1994). For this reason, the serotonergic system has been involved in the pathophysiology of depression. In this sense, it is important to reconsider the fact that about one third of these patients develop suicidal thoughts and, on many occasions, they try to achieve it. That is why it is relevant to study this system in individuals suffering from depression and particularly in the victims of suicide.

There are several limitations in the case of the study of the necropsy of suicide victims. Although the identification of the cause of death is evident, many other aspects are unknown. For example, it is relevant and it is a confusion factor when interpreting the results, the existence of a previous treatment and especially its duration. The same applies to the accuracy of obtaining information about a previous diagnosis. These observations always prevent us from having a clear interpretation of the results.

A fundamental characteristic of the nervous system is its plasticity, understood as a capacity of neural networks to make constant adjustments in their function that in some cases can be related to the development and manifestations of diseases. For the particular case of our suicidal individuals, it is unknown if there was a previous diagnosis of depression or any other diagnosis. It is not possible to identify if those changes could be attributed to the development of the disease or to any specific treatment.

It is important to take into account that serotonin is not the only neurotransmitter involved in suicide. In fact, there is an association between high levels of glutamate in cerebrospinal fluid and suicidal ideation; these levels decrease after the correct pharmacological treatment (Garakani, 2013). Apparently, certain epigenetic alterations in the spindle and the kinetocoro associated with the so-called protein-2 are a good biological marker of suicidal behavior; this gene is related to the transactivation of the nuclear glucocorticoid receptor (Kaminsky, 2015), which evidently will alter the function of the hypothalamic-pituitary-adrenal axis and modify cortisol secretion patterns, which in turn is related to suicidal ideation and behavior (Li, 2013); we cannot rule out the participation of other systems such as the prolactin system, whose increased release in conjunction with cortisol can promote the formation of cytosines, which have also been linked to suicidal behavior (Pompili, 2013). The regulating properties of the serotonergic system influence the GABA system, particularly in the regions of the frontal middle cortex (Zhong, 2004). The results of any study may also depend on the stage of the disease in which the analysis is performed. In this sense, it is notable that the actions of fluoxetine, a prototype antidepressant, may depend on age, which may be explained by differential actions of fluoxetine on neuroplasticity, especially in the amygdala of the temporal lobe (Homberg, 2011); there may be some differences in the expression of symptoms of depression and sensitivity to antidepressant treatments, genetically determined, especially when applied to models of depression that appears in the later stages of life, at least experimentally (Perez-Caceres, 2013). This data is of interest due to the high suicide rate that occurs in the
elderly.

In recent years, studies have been conducted in which the hypothesis was whether the polymorphisms in the SERT gene are associated with suicide. Some studies have not shown any relationship between suicidal behavior and the SERT genotype. Ohara et al in 1998 and Geijer et al in 2000 studied the relationship of the genotype with depressed patients and with different forms of polymorphism (l / s, heterozygous form of the gene) finding no significant differences between patients and controls, concluding that the presence of polymorphism does not affect affective disorders. In 1999 Du et al. Carried out a study that showed a higher frequency of allele I in depressed suicide victims compared to non-suicidal controls. In 2015, Yi-Wei Yeh and colleagues, through a positron emission tomography study, confirmed the reduction of SERT in depressed patients with suicide attempts compared to depressed patients without previous attempts, which relates to the pathophysiology of behavior suicide.

More recently and using positron emission tomography techniques, associated with radioligands, it has been established that the regions rich in SERT are the same as the antidepressant treatments, such as the cingulum, the amygdala and the raphe nuclei. This occupation is related to the content of antidepressants in plasma, which seems to be related to the sensitivity and efficacy of antidepressant treatments, since the first step in the actions of antidepressants is to establish a blockade of SERT (Baldinger, 2014). There is a possibility that deficiencies in serotonin neurotransmission are underlying only the depressive process. This has been demonstrated by the use of positron emission tomography and specific markers; the function of the 5-HT1A receptor is decreased in patients with major depression, about 26% in the mesiotemporal cortex and in almost half (43%) in the nuclei of the raphe (Drevets, 2007). The neonatal administration of chlorimipramine produces behaviors suggestive of despair in the adult stage (Limon, 2014). In suicide victims, a reduction in the number of somatodendritic receptors and postsynaptic 5-HT1A receptors has been found; these observations have been replicated in positron emission tomography studies, associated with reduction of the binding capacity of the 5-HT1A receptor binding in specific areas of the dorsal raphe nucleus, the medial prefrontal cortex (mPFC), the amygdala and the hippocampus, structures related to the control of emotional behavior. Therefore, there is the possibility that some mechanism related to episodes of major depression and other alterations related to stress are dysfunctions in the 5-HT1A receptor (Savitz, 2009). These effects that are found with pharmacological treatments are also observed with other techniques, such as electroshock (Lanzenberger, 2013).

In the present study, the I / I polymorphism was found in both experimental groups, which is relevant given that serotonin uptake is approximately twice as high in cells that contain the l / l form as in the s / s form (Lesch, 1998), but there was no difference between the groups. The relevance of studying polymorphisms lies in the fact that the existence of these peculiarities, can explain the difference in the responses associated with prolonged stress or affiliative behaviors, such as cortisol (Berger, 2016), which is regulated by the action of the 5-HT2C receptor, which is located in the hypothalamus (Way, 2016).
Moreover, the risk of hypersecretion of cortisol and other forms of psychopathology related to stress increase with the genotype in which variations occur (Sorenson, 2013). In the present study, a case of completed suicide, which presented the short polymorphism SLC6A4 of the SERT gene, had multiple previous suicide attempts; the possibility of a relationship with the presence of the polymorphism of the short gene (s/s) and suicide cannot be ruled out yet.

In fact, in those cases closer to suicide, as in treatment-resistant depression, the neuroplasticity of the serotonergic system seems to be compromised, possibly due to an excess in the secretion of glutamate, serotonin, noradrenaline and histamine, which can cause an inhibitory effect on the release of serotonin. A relevant aspect that may aggravate this scheme is the presence of the short arm of the serotonin transporter gene (Coplan, 2014), which would explain the resistance to treatment and the very possible aggravation of suicidal ideation.

However, these data seem to indicate that the efficiency of the serotonin transporter gene is more related to the possible efficacy of some antidepressant pharmacological treatment, rather than being a characteristic of the subject who will develop suicidal behavior.

REFERENCES


receptors in adult rats induced by neonatal treatment with clomipramine. Physiol Behav, 124, 37-44. doi:10.1016/j.physbeh.2013.10.026


