GENETIC RISK FACTORS FOR SUICIDE IN MAJOR DEPRESSION: A FOCUS ON THE SEROTONIN TRANSPORTER AND TRYPTOPHAN HYDROXYLASE-2 GENES

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"One should guard against preaching to young people success in the customary form as the main aim in life. The most important motive for work in school and in life is pleasure in work, pleasure in its result, and the knowledge of the value of the result to the community"

Albert Einstein

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Abstract

Growing evidence suggests a partial genetic determination of suicide predisposition, with most studies focusing on serotonergic genes. Inconsistent results have characterized the serotonin transporter (5-HTT) gene, whereas few studies have investigated the recently discovered brain specific tryptophan hydroxylase 2 (TPH2) gene. At the same time, most of these studies have not controlled for associated psychopathology, an important confounder. Therefore, association studies were conducted to investigate the role of the 5-HTT and TPH2 genes in suicide susceptibility in the context of major depression and their effect on impulsive-aggressive behaviors (IABs), a possible intermediate phenotype. Finally, suicide predictors were investigated while controlling for genetic, clinical and behavioral risk factors. Certain 5-HTT and TPH2 genetic variants were significantly implicated in the vulnerability to suicide, however, no effect on levels of IABs was observed. Genetic and clinical suicide predictors were identified while controlling for other risk factors. Further studies in larger samples are necessary to replicate these findings and detect possible small genetic effects.

Résumé

De plus en plus d'études suggèrent l'existence d'une prédisposition au suicide partiellement déterminée par nos gènes, avec une majorité d'études sur les gènes du système sérotonergique. Des résultats inconsistants ont été observés sur le gène du transporteur de la sérotonine (5-HTT), tandis que peu d'études ont étudié le gène codant pour la tryptophane hydroxylase 2 (TPH2) récemment identifié. Cependant, la majorité de ces études n'ont pas tenu compte des psychopathologies associées, un important facteur confondant. Ainsi, des études d'association ont été conduites pour investiguer le rôle des gènes 5-HTT et TPH2 sur la prédisposition au suicide dans le contexte de la dépression majeure et leurs effets sur des comportements impulsifs agressifs (CIA), un possible phénotype intermédiaire. Aussi, des facteurs prédisposant au suicide ont été explorés en tenant compte de facteurs génétiques, cliniques et comportamentaux. Certaines variantes génétiques des ces deux gènes ont été impliquées dans la vulnérabilité au suicide sans effet cependant sur les CIA. De plus, des facteurs génétiques et cliniques prédisposant au suicide ont été identifiés. Des études additionnelles avec un échantillon plus vaste sont nécessaires pour confirmer ces résultats et identifier des possibles effets génétiques mineurs.

Chapter 1

Introduction

DEFINITION OF SUICIDAL BEHAVIOR

Suicidal behavior covers a spectrum of self-harming behaviors, which differ in their degree of severity, ranging from suicidal ideation to suicide completion, with suicide attempts in between. Completed suicide lies in the most severe end of this spectrum, being defined as a death resulting from a self-inflicted act with a clear intention to die (O'Carroll et al. 1996). At the mildest end, there is suicidal ideation, which refers to experiencing thoughts about suicide (O'Carroll et al. 1996). Attempted suicide may be considered in the middle of this continuum, and it poses a greater challenge in terms of its definition, since it represents a more heterogeneous group. Suicide attempts can be determined in the basis of two dimensions—suicide intent and medical lethality, which have also been proposed for their classification (Beck et al. 1975; Beck et al. 1976). The lethality dimension can range from individuals who made a very serious suicide attempt resulting in great medical damage to individuals whose suicidal act did not lead to serious injuries. On the other hand, the dimension of suicide intent refers to the individual's true intentions to die, and it can be assessed by inquiring about the extent of premeditation, the conception of the method's lethality, and the likelihood of discovery, among other characteristics (Beck et al. 1976). Furthermore, a high correlation between suicide intent and medical lethality has been noted when the suicide attempter has accurately conceived the lethality of

his act (Beck et al. 1975). Therefore, among suicide attempters, several distinctions are possible based on these two dimensions. As a result, defining suicide attempts still remains a controversial issue; however, the following relatively broad most commonly used definition has been: "A potentially self-injurious behavior with a non-fatal outcome, for which there is evidence that the person intended at some level to kill himself/herself" (O'Carroll et al. 1996). Taken together, completed suicide represents not only the most serious but also the most likely homogeneous group of the spectrum of suicidal behaviors.

THE EPIDEMIOLOGY OF SUICIDE

Over the last decades, suicide has become a very important public health problem worldwide. The recorded suicide rates from 1950 to 2000 reveal that this phenomenon continues to increase, particularly among males (World Health Organization, 2002b). In 1989, mortality data from 39 countries unveiled a total of 208,349 deaths by suicide (Diekstra, 1993). Eleven years later, in 2000, and with available mortality data from additional countries, suicide accounted for approximately 815,000 deaths around the world (World Health Organization, 2002c). This translates into a nearly four-fold increase in the figure reported in 1989, which might be attributed not only to a genuine increase in the number of suicides, but also to a greater availability of mortality data on suicide by other countries. Furthermore, whereas in 1950 the majority of the suicide victims were above 45 years old; in 2000, there was a noticeable change in the age distribution of suicide cases, affecting predominantly those below 45 years old (World Health Organization, 2002a).

Although suicide is a problem seen around the world, suicide rates vary widely among countries. In general, the highest rates are commonly observed in Eastern European countries—around 40.0 per 100,000 people—whereas low rates are mainly found in African and Latin American countries. In Canada, the most recent year age-standardized suicide rate has been estimated to 15.0 per 100,000 people (World Health Organization, 2002c) and, as for most of the developed countries, it is among the leading causes of death for individuals of all ages, and particularly among young adults (Public Health Agency of Canada, 2000). It is noteworthy that the province of Quebec has one of the highest suicide rates, not only within Canada but also in North America, and also one of the highest among other industrialized countries (Choinière, 2003). In accordance with the worldwide evolution of suicide rates, Quebec has also seen a significant increase over the last decades, from 14.8 per 100,000 people in the period of 1976-1978 to 19.1 per 100,000 people for the period of 1999-2001. This increase has been even more astounding for males, from 22.0 per 100,000 people to 30.7 per 100,000 people for the same time periods, whereas in the rest of Canada this figure is about half of that observed in Quebec (16.1 per 100,000 people) (St-Laurent and Bouchard 2004). There are few countries with data on suicide attempts; nonetheless, they have shown that attempted suicides occur up to 20 times more often than completed suicides (World Health Organization, 2001a). Rates of suicide attempts are generally higher among women than men (Diekstra, 1993).

The problem of suicide extends far beyond the individual's death; it has tremendous consequences on families and friends who have to deal with a considerable emotional and social burden. Because suicide affects mainly young

people, the economic repercussion on society is evident. It is not surprising that suicide accounts for one of the highest rates of years of potential life lost (Statistics Canada, 1996).

THE BURDEN OF MAJOR DEPRESSION

Major depressive disorder, also known as unipolar depression, is a common and severe mental disorder characterized by a minimum of a 2–week clinical course, in which either depressed mood or loss of interest is present on a constant basis and associated with at least four other symptoms of depression (American Psychiatric Association, 2000). These symptoms include changes in weight or appetite, excess or lack of sleep, psychomotor agitation or retardation, loss of energy, feelings of worthlessness or guilt, difficulty concentrating or making decisions, recurrent thoughts of death or suicidal ideation, and attempts or specific plans of suicide. Unlike bipolar disorder, patients diagnosed with major depression do not have at any time episodes of mania or hypomania (American Psychiatric Association, 2000).

Major depression is usually episodic, but it can also be chronic, and it has a greater distribution among middle-aged women than men (World Health Organization, 2001a). Two of the most recent epidemiologic surveys conducted in the US have reported lifetime and 12-month prevalence estimates of major depression in the range of 13-16% and 5-7%, respectively. These figures reflect the wide spread of major depression in the population (Kessler et al. 2003; Hasin et al. 2005). Of note is that this is a condition associated with a considerable functional impairment (Kessler et al. 2003), which results in a loss of productivity.

Also important is the stressful situation in which families that have to cope with a depressed member are immersed. The quality of life of these families and the affected individual are greatly disturbed.

According to the world health report issued in 2001 (World Health Organization, 2001a), the global burden of depression on society is very high, ranking fourth among all diseases, and particularly in the group of age of 15-44 years, where it is the second cause of disease burden. Moreover, among all diseases, major depression is the leading cause of years of life lived with disability. It is astounding that about 31% of all years lived with disability is accounted for by mental and neurological disorders, and particularly depression, which accounts for almost 12% (World Health Organization, 2001a).

Without doubt, suicide is the most terrible complication of major depression, and it definitely contributes to a greater extent to the already high price that society pays in relation to the consequences of depressive psychopathology. As it has been previously pointed out, both conditions have an enormous impact on young people, by either taking their lives or leading to serious role impairment on society.

DEPRESSION AND SUICIDE

Mortality and lifetime suicide risk

There are a number of studies carried out mostly in hospitalized patients showing that affective disorders, particularly major depression, have an elevated mortality, which is mainly explained by suicide, among other causes (Harris and Barraclough 1998; Angst et al. 2002). However, there are other studies that have

examined the mortality rates in outpatients, and which have not found an increased mortality for primary affective disorders. Rather, they have shown that the excess mortality is related to secondary affective disorders that have pre-existent psychiatric psychopathology such as substance use disorders, antisocial personality and others (Martin et al. 1985a; Martin et al. 1985b). The fact that hospitalized patients are usually the most severely ill, and thus, with probably a higher risk of suicide, should be taken into account when investigating the mortality rates of affective patients.

It has been reported that major depression has the highest risk of suicide among mood disorders (Harris and Barraclough 1997). For many years, the lifetime suicide risk for major depression was believed to be around 15% (Guze and Robins 1970); however, this figure has recently been under revision for a number of reasons; among them, the fact that it was calculated from studies investigating, again, mostly hospitalized patients, and that no distinction was made between unipolar or bipolar disorder. The most recent revision of the suicide risk in major depression has led to a much lower estimated risk of 3.4% with a strong gender effect of 7% for men and 1% for women (Blair-West et al. 1999). The gap observed between male and female suicide rates is more pronounced in young people below 25 years (Blair-West et al. 1999). This figure is similar to that reassessed in the previous year for all affective disorders by Inskip et al. (1998) (6%). On the other hand, and as pointed out above in this section, it has been argued that the lifetime prevalence of suicide in affective disorders varies according to the treatment setting. As such, the highest risk has been related to suicidal hospitalized patients (8.6%), followed by non-suicidal

hospitalized patients (4%), and by mixed hospitalized patients and outpatients (predominantly) (2.2%). The lowest rate has been associated to the general population where it is less than 0.5% (Bostwick and Pankratz 2000).

Of interest is that, although the suicide risk is greatest in early stages of the disorder, there are some depressive patients who are under a constant risk that persists over their lifetimes (Black et al. 1987; Angst et al. 2002), which prompts for a constant screening of all depressive patients regardless the onset of depression. Even though the lifetime risk of suicide in major depression has been re-estimated to a lower value, it is still quite significant, especially when the reported high prevalence of major depression in the general population is taken into account.

Psychological autopsy studies

The relationship between mental disorders, in particular major depression, and suicide has been the focus of numerous studies. To this end, some researchers have conducted prospective follow-up studies of affected patients, whereas many others have relied on the psychological autopsy method, which has become one of the most helpful research tools for suicide completion (Hawton et al. 1998; Isometsa, 2001). The purpose of the psychological autopsy approach is to gather as much complete and accurate information as possible about the circumstances behind the individual's death in order to define the mental and psychosocial characteristics of the suicide victims. This method consists in conducting a series of interviews with relatives or friends who were closest to the deceased, using structured instruments, and complementing the obtained information with medical

or other available records. Since the psychological autopsy approach is carried out retrospectively, it usually relies on the accuracy and reliability of the information obtained through the informant (Hawton et al. 1998). However, it has been well validated for determining clinical information including psychiatric diagnoses (Brent et al. 1993; Kelly and Mann 1996; Conner et al. 2001b; Schneider et al. 2004), a past history of suicide attempts and levels of suicidal intent, as well as behavioral measures (Dumais et al. 2005b) in addition to certain aspects of social support and stressful life events (Conner et al. 2001a).

Several psychological autopsy studies have repeatedly indicated a strong link between major depression and suicide completion. Results from two recent meta-analyses generally agreed that approximately 90% of the suicide cases had suffered from a mental disorder prior to death; and from those, affective disorders—particularly depressive disorders—accounted for the majority of cases (Cavanagh et al. 2003; Arsenault-Lapierre et al. 2004). According to the World Health Organization up to 60% of all suicides are accounted for depressive disorders and schizophrenia (World Health Organization, 2001b). Data derived from a large study on suicide victims in Finland, revealed that 31% had a diagnosis of major depression before death (Henriksson et al. 1993). A more recent study on exclusively male suicide completers from the Quebec population, found a prevalence of major depression of 35.6% (Kim et al. 2003).This figure was higher than that calculated for male suicide victims from the study in Finland (26%) (Henriksson et al. 1993). However, overall, both are consistent with the estimates reported by Lonnqvist (2000), which ranges from 29% to 88%. Elevated

comorbidity with substance use disorders and/or personality disorders seems to be a common finding in suicide victims (Cavanagh et al. 2003; Kim et al. 2003).

Overall, it is clear that major depression constitutes an important risk factor for suicide; however, as it was previously noted, not all patients with major depression do actually commit suicide. Thus, there must be other factors, apart from a diagnosis of major depression, which might be contributing to the suicide susceptibility. Reaching a better understanding of these factors would definitely help in the detection of high risk suicidal patients, and thus, in the implementation of effective preventive measures for this complex behavior.

CLINICAL, DEMOGRAPHIC AND ENVIRONMENTAL RISK FACTORS FOR SUICIDE IN MAJOR DEPRESSION

Several studies have focused on finding clinical symptoms or variables such as social or demographic characteristics that would help clinicians to differentiate depressed patients at risk of suicide from those that are not at risk. A history of previous suicide attempts is probably the strongest predictor of suicide in patients with major depression (Barraclough and Pallis 1975; Roy, 1983; Nordstrom et al. 1995); in particular, those rated as high in intent or lethality (Coryell et al. 2002). However, not only suicide attempts but also suicidal ideation has emerged as predictor of suicide in patients with affective disorders (Goldstein et al. 1991; Schneider et al. 2001; Angst et al. 2002).

Some of the depression-related symptoms that have been linked to an increased risk of suicide are insomnia, self-neglect, poor memory, loss of interest or pleasure and hopelessness (Barraclough and Pallis 1975; Beck et al. 1985;

Fawcett et al. 1987; Beck et al. 1989; Beck et al. 1990; Schneider et al. 2001). Furthermore, some investigators have suggested that some of these symptoms may be related to either a short- or long-term suicide risk. Accordingly, Fawcett et al. (1990), investigating a prospective population of patients suffering from major affective disorders for a period of 10 years, came up with two different sets of symptoms that differentiated patients with either an early or late risk of suicide. Thus, anxiety-related symptoms of panic attacks, psychic anxiety, insomnia, diminished concentration, and alcohol abuse, in addition to loss of interest and pleasure were associated to suicide completion within 1 year of assessment; whereas, hopelessness, suicidal ideation and a previous history of suicide attempts were associated with suicides taking place after 1 year. Although suicide attempts and ideation were not found as predictors of short-term suicide risk, these should not be excluded from the clinical assessment of risk of suicide. Rather, these results underscore the importance of assessing short and long term suicide risk in depressed patients. In support of the Fawcett et al (1990) study, a 21 year-follow-up study of patients with major depression, and no other axis I disorder or antisocial personality disorder found that the rates of suicidal tendencies—which quantify the intensity of suicide thoughts together with the seriousness of past suicidal behaviors—and a history of suicide attempts were also predictors of late suicide (Coryell and Young 2005).

Studies have highlighted the importance of comorbidity involving affective disorders, cluster B personality disorders and/or substance use disorders in the risk of completed suicide (Lesage et al. 1994; Isometsa et al. 1996; Kim et al. 2003; Hansen et al. 2003; Dumais et al. 2005a). This comorbidity has been

shown to vary in relation to the individual's age and sex in depressed patients. Thus, substance use disorders are mostly related to males, whereas physical illnesses are most frequently seen among the elderly. Additionally, personality disorders are suggested to be more common among younger suicide completers (Isometsa et al. 1994; Kim et al. 2003; Dumais et al. 2005a).

An inpatient status has also been implicated in the prediction of suicide completion (Coryell and Young 2005). Other issues related to social or medical support have also been involved. For instance, the year following discharge from psychiatric care has been related to a higher risk of suicide in depressed patients (Roy, 1983; Fawcett et al. 1987; Buchholtz-Hansen et al. 1993). Demographic characteristics such as being male, unmarried, living alone, as well as being an immigrant have been suggested as risk factors for suicide (Barraclough and Pallis 1975; Roy, 1983).

A family history of suicide has also been associated with an increased risk for suicidal behaviour in depressed patients (Roy, 1983; Linkowski et al. 1985; Roy, 1993). Early parental loss through either suicide or other causes of death seems to be an important correlate of suicidal acts in major depression as well as in other diagnostic categories (Roy, 1983; Malone et al. 1995; Botsis et al. 1995). Additionally, it has been reported that the experience of childhood maltreatment, which includes neglect, physical and sexual abuse, makes an adolescent or young adult more vulnerable to depression and/or suicidality (Brown et al. 1999). Moreover, adverse factors related to the milieu surrounding the child abuse also influence the risk of depression and suicidal behavior. Accordingly, depressed

adults with a past history of physical or sexual abuse are more likely to engage in suicidal behaviour than those without such a history (Brodsky et al. 2001).

It is worthy of note that despite the recognition of all these suicide risk factors over the last years, it appears that their contribution to the improvement of clinical detection of patients at risk is of little help. Studies that have attempted to predict suicide by building statistical models, which incorporate some of these risk factors, have not been successful (Pokorny, 1983; Goldstein et al. 1991). Therefore, these studies have come to the conclusion that it is currently unfeasible, based on presently recognized risk factors, to identify particular persons who will eventually commit suicide, even among a high-risk inpatient population. However, these risk factors have greatly contributed to build a bigger picture of the circumstances and specific situations that surround suicidal behavior. Taken together, it remains unclear what differentiates depressed patients who eventually decide to put an end to their lives from those who do not.

EXPLANATORY MODEL OF SUICIDE

It appears that the presence of major depression like other psychiatric disorders is a necessary but not sufficient factor to commit suicide. The lack of relationship between the severity of depression and ultimate suicide (Beck et al. 1985), along with an increased rate of suicide early in the course of illness (Black et al. 1987; Angst et al. 2002) suggest the existence of an underlying vulnerability for suicidal behavior that seems to be independent of coexistent psychopathology.

In an effort to have a better understanding of the mechanisms behind this complex behavior, a model called the trigger-threshold, or stress-diathesis model

has been previously described (Mann and Arango 1992; Mann, 1998; Mann et al. 1999). According to this model, suicide is determined by the interaction between individual's threshold and triggers, which ultimately precipitate the suicidal act. The threshold refers to the vulnerability or predisposition to suicidal behavior, whereas the triggers refer to stressors that determine the timing of the suicidal act. Thus, risk factors can be classified according to whether they influence the threshold or the trigger domain. Factors that affect the threshold are for the most part constitutive, and may be considered trait-related. However, some of these factors might also develop as a response to early life experiences (Mann, 1998). Among the factors that may affect the threshold are the genetic and biological makeup, behavioral traits and personality disorders. On the other hand, factors affecting the trigger may be considered state-related and they are variables commonly assessed at the clinical level, such as psychiatric or medical illness, comorbid substance use disorders, and familiy or social stress, for naming some of them. This model implies that depressed patients, who already have a stressor given by the psychiatric disorder, and perhaps some others, will not die by suicide unless they also have an increased susceptibility given by factors from the threshold domain.

PERSONALITY TRAITS AND SUICIDE RISK: IMPULSIVE AND AGGRESSIVE BEHAVIORS

Personality traits, particularly impulsivity and aggression are some of the factors that are believed to be part of the threshold domain. Personality traits are defined as "enduring patterns of perceiving, relating to, and thinking about the

environment and oneself that are exhibited in a wide range of social and personal contexts" (American Psychiatric Association, 2000). They are unique individual's characteristics that determine the pattern of interactions the person will have with other people. When these traits become inflexible and interfere with the adaptation process of an individual to certain environmental conditions, they are part of what is defined as personality disorders (American Psychiatric Association, 2000).

Personality traits are influenced by genetic and environmental factors, and the effect of these factors is variable (Benjamin et al. 1998; Reif and Lesch 2003). Since personality traits define our perception of the world and ourselves, they are very likely to contribute to the vulnerability for suicidal behavior. Among the most investigated traits for suicidal behavior are impulsive and aggressive traits (Baud, 2005).

Impulsivity has been differently defined across studies, including the inability to estimate the risk or danger inherent to a certain situation, acting on impulses without thinking, failure to plan ahead, the tendency to react rapidly to stimuli instead of inhibiting reactions, and the inability to keep oneself from responding, which results in punishment or a deficit in passive avoidance learning (Helmers et al. 1995). Yet, another definition that seems to cover most of the previous definitions refers to impulsivity as behaviors that "appear poorly conceived, prematurely expressed, unduly risky, or inappropriate to the situation and that often result in undesirable consequences" (Evenden, 1999). The variety in definitions for impulsivity reflects the wide range of behaviors that are covered by this term, and that ideally should be assessed when measuring impulsivity

levels in research studies. Different instruments have been designed to this end (Helmers et al. 1995; Barratt and Stanford 1995; Evenden, 1999; Moeller et al. 2001).

Aggression is manifested through several heterogeneous behaviors which may be differentiated based on whether or not there are existent causes or motivations, the nature of trigger, the mechanisms involved, the form of behavior in which they are manifested, the direction in which they are expressed (inward or outward), and function (e.g. intentional harm, injury or damage to either subjects or objects) (Lesch and Merschdorf 2000). In the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) (American Psychiatric Association, 2000), there is no specific definition for aggression, however, they refer to aggressive conduct as behaviors that are intended either to cause or threaten physical damage to people or animals. Aggression, together with rage and hostility is an anger-related trait that may be expressed inwardly or outwardly (Baud, 2005). Instruments aimed to reliably measure aggression have been put forth by different investigators (Barratt et al. 1997). Given the close relationship between impulsivity and aggression, which is supported by their frequent coexistence in the individual, they have generally been referred to as impulsive-aggressive behaviors. They also seem to be influenced, to some extent, by common genetic and environmental factors (Seroczynski et al. 1999).

Impulsivity and aggression have been found to correlate with suicidal and violent behaviors (Apter et al. 1993). A two-stage model describing these relations has been proposed by Plutchik and van Praag (1990). In this model, they suggest that an underlying aggressive impulse manifest itself only after a certain

threshold level of intensity is exceeded. They explain that variables that are positively correlated with suicidal and violent behaviors contribute, in this first stage, to increase the intensity of the impulse. Then, in the second stage, the direction of this impulse (inward or outward) is determined by variables that are positively correlated to either suicidal or violent behavior.

In major depression, there have been several studies reporting the association of impulsivity and aggression to the susceptibility for suicidal behavior. These studies have reported higher levels of impulsive and/or aggressive behaviors in suicide attempters than in non-attempters (Weissman et al. 1973; Malone et al. 1995; Pendse et al. 1999; Corruble et al. 1999). Moreover, in a two-year prospective study of patients with a major depressive episode, impulsive and aggressive traits were found to predict future suicidal acts (Oquendo et al. 2004). Further evidence for the association of personality traits to attempted suicide in major depression has been gathered indirectly from studies investigating the prevalence of diagnostic categories known to be associated with these traits. The impulsive and aggressive traits are part of the clinical features displayed by patients with cluster B (dramatic, emotional or erratic) personality disorders, especially borderline and antisocial personality disorders. Accordingly, patients with major depression and cluster B personality disorders, particularly borderline, have shown a higher risk of suicide ideation or attempts than patients with major depression alone (Malone et al. 1995; Corbitt et al. 1996). Similarly, depressed patients with substance use disorders, specifically alcohol abuse or dependence, have also been related to more impulsive suicide attempts as well as higher levels of lifetime aggression and impulsivity (Suominen et al. 1997; Sher et

al. 2005). Interestingly, greater rates of impulsivity and aggression have been found to differentiate suicide attempters from non-attempters, regardless of the psychiatric diagnosis (Mann et al. 1999).

In contrast, few studies have been carried out on suicide completers regarding levels of impulsivity and aggression. Most of the evidence supporting the role of impulsive and aggressive traits in suicide has come from studying unselected suicide completers and by reporting the prevalence of certain psychiatric diagnosis associated with these personality traits, as mentioned above, instead of measuring them directly. As previously pointed out, patients with affective disorders and comorbid cluster B personality disorders and/or substance use disorders have been found to be at higher risk of suicide completion (Lesage et al. 1994; Isometsa et al. 1996; Kim et al. 2003; Hansen et al. 2003; Dumais et al. 2005a). A significant association was found between aggression and older suicide victims (50 years and over); however, after adjustment for mood disorders, the association was no longer significant (Conner et al. 2004), which highlights the importance of controlling for effects related to underlying psychopathology. There have also been reports of higher levels of lifetime aggression in adolescent suicide victims compared to non-suicidal controls, even after controlling for axis I psychopathology. It has also been shown that adolescent suicide completers present an increased tendency to impulsive violence (Brent et al. 1994). The only direct evidence of the role of impulsivity and aggression in suicide completion while controlling for major depression has come from a study carried out in male suicide completers who died in the context of an episode of major depression compared to a group of living depressed males. This study indicated that suicide

completers had higher levels of impulsivity and lifetime aggression, and confirmed previous studies showing higher comorbidity with cluster B personality disorders—in particular borderline and antisocial personality disorders—and substance use disorders (Dumais et al. 2005a).

The observed correlations between suicide, major depression and impulsive-aggressive behaviors are further supported by evidence from neurobiological studies in suicide attempters and completers, which will be discussed in the following sections.

NEUROBIOLOGICAL STUDIES

Over the last years, a number of studies have been conducted to gain a better understanding of the complex neurobiology underlying suicidal behavior. Parallel to this increasing knowledge in suicide biology, our understanding of the biology of other closely related disorders such as major depression, as well as impulsivity and aggression has also advanced considerably. Most of these studies have focused on the serotonin system, and thus, an overwhelming amount of data has been generated, implicating overall this neurotransmitter in suicide and major depression, but also in impulsive-aggressive behaviors. Other neurotransmitter systems, such as the noradrenergic and dopaminergic have also revealed certain abnormalities although the data available on these systems is less extensive (Traskman-Bendz and Mann 2000).

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine that can be found in neurons, platelets, mast cells, and enterochromaffin cells. In the brain, the highest concentrations of serotonin are found in the brainstem raphe nuclei, where

it acts as a neurotransmitter sending projections to different areas of the brain such as the spinal cord, the cortex, the limbic system, hippocampus and hypothalamus. Thus, it is not surprising that the serotonergic system plays an important role in the modulation of diverse brain functions such as neuroendocrine rhythms, mood, sleep, appetite and cognition (Traskman-Bendz and Mann 2000).

With regard to major depression, there is strong evidence for serotonergic abnormalities. Patients who receive an antidepressant treatment with selective serotonin reuptake inhibitors (SSRIs) have an improvement of their symptoms. This effect is the result of blocking the reuptake of serotonin, which leads to an enhanced serotonergic activity, ultimately reversing the preexisting deficit (Asberg et al. 1986). Studies that have experimentally induced an acute depletion of serotonin in patients responding to SSRI treatment (Delgado et al. 1990), as well as patients in clinical remission with no antidepressant treatment (Smith et al. 1997) have led to a clear relapse of depressive symptomatology. A similar effect has also been observed in psychiatrically normal individuals who display decreased mood, but only in those with a history of major affective disorder in multiple generations (Benkelfat et al. 1994). Overall, these studies point to the involvement of serotonergic abnormalities in the development of major depression. On the other hand, suicide is also related to a serotonin deficit. It has been argued that, due to the wide range of symptoms presented in depressed patients, it is reasonable to think of a diffuse serotonergic deficit (Traskman-Bendz and Mann 2000), whereas in suicide a more localized abnormality would be expected.

Cerebrospinal fluid (CSF) studies

One of the biological markers of serotonin turnover in the brain that has been most extensively studied is the 5-hydroxyindolacetic acid (5-HIAA). 5-HIAA represents the final product of the metabolism of serotonin and it has been used as an indirect measure of serotonergic activity in the brain. Earlier studies carried out in depressed patients and healthy control individuals were characterized by significantly lower 5-HIAA concentrations in CSF of depressed individuals compared to controls; however, results from subsequent studies were not as supportive of these initial findings (Asberg et al 1997).

One of the earliest studies on suicidal behavior and levels of CSF 5-HIAA revealed a bimodal distribution of CSF 5-HIAA levels in depressed patients, which later showed to be related to suicidal behavior (Asberg et al. 1976). In that study, depressed patients with lower CSF 5-HIAA levels reported more suicide attempts than those with higher levels. Moreover, two suicide cases were reported among the patients with low CSF 5-HIAA levels, whereas no suicide cases were found among those with high CSF 5-HIAA levels (Asberg et al. 1976). These results were among the first to provide evidence of a serotonergic deficit in suicidal behavior. Since then, these findings have been replicated in a number of studies and in different diagnostic categories, with the exception of patients with bipolar disorder who apparently do not have differences in CSF 5-HIAA levels (Asberg, 1997). The results from all these studies suggest that the serotonergic alterations related to suicidal behavior are independent of accompanying psychopathology. Of further interest is the fact that low levels of CSF 5-HIAA are risk factors that may predict future suicide attempts or completed suicide (Roy et

al. 1989; Traskman-Bendz et al. 1992; Nordstrom et al. 1994), and hence they might be considered an enduring biochemical trait.

It is noteworthy that in the early study of Asberg et al. (1976), it was observed that depressed patients who used more violent suicide attempt methods showed lower CSF 5-HIAA concentrations, revealing an inverse correlation between violence of the suicide attempt and CSF 5-HIAA levels. These findings have received further support in subsequent studies (Traskman et al. 1981; Traskman-Bendz et al. 1992). Of note is that the choice of a violent method has been found as a behavioral marker of high levels of lifetime impulsive and aggressive behaviors (Dumais et al. 2005b). Several studies have found that CSF 5-HIAA concentrations are negatively correlated to higher levels of aggression (Brown et al. 1979; Brown et al. 1982; Linnoila et al. 1983; Virkkunen et al. 1994; Stanley et al. 2000; Placidi et al. 2001); and impulsivity (Linnoila et al. 1983; Virkkunen et al. 1994; Cremniter et al. 1999; Spreux-Varoquaux et al. 2001). These studies have examined individuals with different diagnoses. Taken together, these studies suggest that the relationship between high levels of aggressive and/or impulsive behaviors and low CSF 5-HIAA exists regardless of psychiatric diagnosis; similar to what was found in relation to suicidal behavior. Thus far, a common deficit in serotonin turnover seems clear for suicidal behavior, major depression and impulsive-aggressive behaviors.

Fenfluramine challenge studies

Fenfluramine has been the most commonly used serotonin agent in neuroendocrine studies of suicidal behavior and depression. It stimulates the

serotonin release from presynaptic storage granules, inhibits reuptake, and may also stimulate post-synaptic serotonin receptors (Carlton and Rowland 1989). This stimulation induces a dose-dependent increase of prolactin secretion (Quattrone et al. 1983), which can be measured and used as a biological marker that indirectly reflects the central serotonergic activity. Several, but not all studies on depressed patients have shown a blunted prolactin response to fenfluramine (O'Keane and Dinan 1991; Mann et al. 1995). A history of suicide attempts has been correlated to a reduced prolactin response in both patients with major affective disorders (Coccaro et al. 1989; Correa et al. 2000) and patients with personality disorders (Coccaro et al. 1989; New et al. 1997). Correa et al. (2000) found that among depressed hospitalized patients, those with a history of suicide attempts had a significantly lower prolactin response to fenfluramine than those without such a history. In addition, when depressed hospitalized patients without a history of suicide attempts were compared to a control group no significant differences were observed. These results have suggested the implication of prolactin response as a marker of suicidality rather than depression (Correa et al. 2000). It is noteworthy, that the decreased prolactin response is significantly lower in depressed patients with high-lethality suicide attempts than those with low-lethality (Malone et al. 1996; Correa et al. 2000).

In addition, an inverse correlation between reduced prolactin response to fenfluramine and impulsivity or aggression has been found in patients with personality disorders (Coccaro, 1989; Dolan et al. 2001) and unipolar depression (Sher et al. 2003).

Overall, these findings are consistent with those derived from studies on levels of CSF 5-HIAA, both implying a reduced serotonergic activity in relation to suicidal behavior, particularly more serious suicide attempts, major depression and impulsive-aggressive behaviors.

Platelet studies

Because of the several properties shared with neurons, platelets were thought to be an appropriate and convenient peripheral tissue for studies of the serotonin function in psychiatry, including suicidal behavior. Platelets contain serotonin 2A (5-HT2A) receptors and serotonin transporter (5-HTT) binding sites; and they also store serotonin (Pandey, 1997). Studies on 5-HTT in platelets have been carried out using the radiolabeled ligand-binding technique—either [^{3}H] imipramine or [^{3}H] paroxetine—and serotonin uptake measurement; however, the results have been mixed. In depressed patients, many of the studies carried out on platelets have shown fewer 5-HTT binding sites, but others have not reached the same conclusion. Similar inconsistent results have been observed regarding platelet 5-HTT binding sites as well as changes in serotonin uptake in suicidal patients compared to nonsuicidal patients (Pandey, 1997).

Studies on 5-HT2A binding sites in platelets have been more encouraging. These studies, using different ligands, have found higher 5-HT2A receptor binding sites in depressed patients, but mainly in suicidal depressed patients (Pandey, 1997). The increased 5-HT2A receptor binding sites in platelets have also been related to suicidal patients irrespective of diagnosis. Furthermore, it has been suggested that the increased density of 5-HT2A receptor sites is a state-

related phenomenon. This derives from the observation that suicidal patients with a current history of suicide attempts or ideation have a significantly higher density of 5-HT2A receptor binding sites than suicidal patients with a past history of suicide attempts (6 months or more), which in turn do not differ from the density of 5-HT2A receptors found in normal control individuals (Pandey, 1997). This has been further supported by the significant decrease of platelet 5-HT2A receptor binding after antidepressant therapy (Biegon et al. 1987). Overall, the results of the studies on 5-HT2A receptors indicate upregulation of these receptors in platelets of depressed and suicidal patients; however, the mechanisms responsible for these changes are unclear. Additionally, it should be considered that the observed changes in platelets might not reflect the same changes in neurons.

Postmortem studies

A large number of studies have been carried out investigating different receptors in brain tissue of suicide victims as a more direct approach to further understand the biological abnormalities behind suicidal behavior. In the serotonergic system, the most commonly studied receptors have been the presynaptic serotonin transporter (5-HTT) and the postsynaptic serotonin 2A (5-HT2A) and 1A (5-HT1A) receptors. They have been investigated in different brain regions with widely varying results (Gross-Isseroff et al. 1998).

Serotonin transporter

The 5-HTT sites are located on the axon terminals of serotonin neurons whose cell bodies lie in the dorsal and median raphe nuclei in the brain stem. The

serotonin transporter is responsible for the serotonin reuptake from the synaptic cleft into the axon (Arango et al. 1997). 5-HTT binding is one index of serotonin nerve terminal innervation of cortical areas (Zhou et al. 1995).

Several studies have investigated the 5-HTT in postmortem brain tissue of suicide victims. Most of them have focused on the cerebral cortex and in particular the frontal cortex (Purselle and Nemeroff 2003; Stockmeier, 2003). The results of these studies are mixed, with studies showing mostly decreases but also increases or no changes in 5-HTT binding in the brain regions tested. A number of methodological issues have been put forward to partly explain this lack of consistency, one of them is the choice of ligand used (Purselle and Nemeroff 2003). A large proportion of these studies, mainly the first ones, have relied on [^{3}H] imipramine, which is a nonspecific ligand that binds to both high and low affinity sites, most of them being nontransporter sites (Hrdina, 1984; Cash et al. 1985). Subsequent studies began to use other ligands such as [^{3}H] paroxetine, [^{3}H] citalopram and [^{3}H] cyanoimipramine, which have been identified as more selective for measuring the 5-HTT (Gurevich and Joyce 1996). Several other important confounders that have been suggested refer to the greater use in previous studies of homogenized tissue preparations rather than autoradiography techniques, which provide a more accurate neuroanatomical resolution of 5-HTT binding. Other relevant factors that might account for variability in postmortem studies are antemortem treatment, age, gender, psychiatric disorder and postmortem interval, among others (Purselle and Nemeroff 2003).

Despite the mixed results, it is interesting to note that recent evidence, using quantitative autoradiography, has suggested that suicide completers and

depressed patients might have distinct 5-HTT binding alterations. A reduction in 5-HTT binding was found to be most prominent in the ventrolateral prefrontal cortex (PFC) of suicide completers in comparison with control individuals (Arango et al. 1995). A subsequent study, using quantitative autoradiography as well, investigated 5-HTT binding sites in several prefrontal cortical regions in antidepressant-free suicide completers and deceased individuals with a lifetime history of major depression (Mann et al. 2000). In this study, it was demonstrated that individuals with a lifetime history of unipolar or bipolar depression had a reduced 5-HTT binding in the ventral and dorsolateral aspect of the PFC, whereas suicide completers showed a localized reduction in the ventral PFC. Thus, confirming the previous finding of a localized 5-HTT binding pattern in suicide completers.

These findings suggest that the diffuse reduction in 5-HTT binding in depressed patients might reflect a more extended serotonergic dysfunction, which is in accordance with the variety of symptoms manifested by these patients. On the other hand, the localized decrease of 5-HTT binding in the ventral PFC of suicide completers indicates a reduced serotonergic input specific to this brain region (Mann et al. 2000). The ventral PFC is considerably important because it is involved in behavioral inhibition (Shallice and Burgess 1996); therefore, injuries to this region may lead to disinhibition (Damasio et al. 1994). Thus, a reduced serotonergic input into this brain region might consequently translate in an increased propensity for suicidal or impulsive aggressive behaviors (Mann, 1998). Indeed, some studies including mainly violent suicide completers have reported reduced 5-HTT binding in the frontal cortex (Stanley et al. 1982; Arango

et al. 1995). Although the highest density of 5-HTT in the brain is found in the midbrain raphe nucleus, few studies have been carried out in this brain region, and thus far the existent studies have not shown alterations in 5-HTT binding (Purselle and Nemeroff 2003).

Overall, there is evidence indicating alterations in 5-HTT binding in suicide completers; however, in order to reach a definite conclusion, further studies are required.

Serotonin 1A receptor

5-HT1A is an autoreceptor that is found on the soma and dendrites of serotonergic neurons. It regulates the availability of serotonin in the synaptic cleft by controlling the firing rate of the serotonergic neurons. When serotonin binds to this receptor on the soma in the raphe nuclei, there is a reduction in the firing rate of serotonin neurons (Garlow et al. 1999). In a study of suicide completers, the 5-HT1A receptors were found in increased number in the prefrontal cortex of suicide victims who died from non-violent methods compared to those who died by violent methods, and controls (Matsubara et al. 1991). The increase in 5-HT1A receptor binding in suicide victims has been found to be localized to the ventrolateral PFC (Arango et al. 1995). However, other studies have also shown an increase of these receptors in other brain regions of suicide victims, such as the hippocampus (Joyce et al. 1993), as well as the dorsal raphe nucleus in the midbrain of suicide victims with major depression (Stockmeier et al. 1998). Of note is that 5-HT1A receptor binding has been reported to be negatively correlated with 5-HTT binding in the same brain areas (Arango et al. 1995),

suggesting that both receptors are subject to common regulatory factors that determine an increased binding in the former, and a decreased binding in the latter.

Serotonin 2A receptors

The 5-HT2A receptor is a post-synaptic receptor with a location in a vast number of cell types in the central nervous system (CNS), as well as in peripheral tissues. Chronic treatments of animals with different classes of antidepressants have been shown to down-regulate the 5-HT2A receptors in the CNS (Garlow et al. 1999). Additionally, a correlation between 5-HT2A binding and demographic variables such as age and sex has been detected (Arango et al. 1997). A number of postmortem studies, although not all of them have reported greater 5-HT2A receptor binding sites in the prefrontal cortex of suicide completers than normal control individuals (Stanley and Mann 1983; Mann et al. 1986; Arango et al. 1990; Hrdina et al. 1993; Turecki et al. 1999). A recent study on postmortem brains of teenage suicide victims (Pandey et al. 2002) provided further evidence for an increased 5-HT2A receptor binding sites in the prefrontal cortex, and they were also able to show increased levels of 5-HT2A receptor protein and mRNA expression in this brain region. Results from all the different studies on 5-HT2A receptor have not been uniform (Stockmeier, 2003); nevertheless, the general finding seems to be an increased number of 5-HT2A receptors in specific areas of the PFC of suicide victims.

The increased density in postsynaptic 5-HT1A and 5-HT2A receptors, as well as a decreased number of 5-HTT binding sites in the PFC suggests a

compensatory upregulation mechanism in response to decreased serotonergic activity in that brain region. The reduced serotonergic input in the PFC may contribute to the predisposition for suicidal behavior by facilitating the expression of impulsive and aggressive behaviors, which ultimately may lead to the suicidal act, when exposed to stressors such as major depression.

Overall, findings from neurobiological studies have generally implicated a common reduced serotonergic activity for suicidal behavior, impulsive-aggressive behaviors and major depression. However, it is interesting to note that the serotonergic alterations in suicidal behavior and impulsive-aggressive behaviors appear to be independent of accompanying psychopathology.

Of further interest is the fact that changes in the serotonergic system might be regulated by genetic effects. Animal studies in non-human primates (Higley et al. 1993) have investigated the contribution of genetic and environmental interactions to neurobiological changes in the central serotonergic activity. It has been suggested that a significant portion of the variance in the central serotonin turnover is under genetic mechanisms. Thus, it has been implied that behavioral traits in monkeys such as self-injurious behaviors—which correlates with low central serotonergic activity—might be genetically mediated. Higley et al. (1993) showed that genetic and environmental factors such as maternal deprivation influence the development of the central serotonergic system, and that the neurobiological changes set at early stages of life persist over long periods of time. Therefore, the serotonergic system may be considered a stable biochemical trait with an important genetic basis.

GENETIC FACTORS

There is no doubt that suicide is determined by the interaction of multiple factors which include psychiatric, behavioral, social, and neurobiological alterations. Additionally, increasing evidence has suggested that genetic factors have also an important role in determining the susceptibility for suicidal behavior.

Family studies

It has long been noted that suicide tends to aggregate in families. This has been demonstrated by a large number of studies that have either directly investigated families and the occurrence of suicidal behavior among them or examined a history of familial suicide in individuals who commited suicide (Turecki, 2001). These studies have indicated an increased risk of suicidal behavior for first- and second-degree relatives of suicide attempters or completers compared with the relatives of control individuals. However, since suicidal behavior occurs most of the time in association with a psychiatric disorder, it could be argued that the increased suicide risk observed in relatives of individuals who died by suicide may be the sole result of the genetically transmitted psychiatric disorder which is associated with suicide. Some studies have addressed this issue and have suggested that the familial aggregation of suicide is independent of the familial loading of psychiatric disorder (Egeland and Sussex 1985; Brent et al. 1996).

Egeland and Sussex (1985) examined the Old Order Amish community of Lancaster County, in Pennsylvania, over a period of 100 years. During this time 26 suicides were reported, with the majority of them coming from 4 families. Of

interest is the fact that whereas these families showed a high loading for affective disorders and suicide, other families with a similar loading for affective disorders did not report any suicide. Hence, these results suggested an independent familial segregation for suicide. A more recent study in adolescent suicide victims (Brent et al. 1996) supported previous findings of higher rates of suicidal behavior in the relatives of suicide probands compared to controls. But, more interestingly, this difference was found to remain significant even after adjusting for axis I and II psychiatric disorders, which again imply a suicide liability that is independent from that related to psychiatric disorders.

It is also worthy of note that some of these studies have found a relationship between a family history of suicide and the violence of the suicide method used (Linkowski et al. 1985; Roy, 1993). Consistently, depressed patients who have attempted suicide by violent methods have been found to report more frequently a family history of suicide than those who have used nonviolent methods (Linkowski et al. 1985; Roy, 1993).

Recent studies in offspring of mood-disordered suicide attempters by Brent et al (2002) showed that, as expected, the offspring with the highest genetic loading for suicidal behavior had the highest risk of suicide attempts. Furthermore, they found that the familial transmission of suicidal behavior was related, in part, to higher impulsive aggression in probands and offspring, which suggest a mediating role of these traits for the familial transmission of suicidal behavior (Brent et al. 2002; Brent et al. 2003).

Whereas these studies have shown a familial aggregation of suicidal behavior, this does not necessarily mean that it is due to genetic factors. The

clustering of suicide in families could also be occurring because of the influence of shared environmental factors, such as violence in the family, hostility, imitation of suicidal behavior by other family members, among others. Studies on twins and adopted children have been helpful to determine the genetic contribution to suicidal behavior.

Twin studies

Twin studies are considered a powerful approach to investigate the contribution of genetic factors to a certain condition. The rationale behind these studies is that identical or monozygotic (MZ) twins share the same genes, whereas fraternal or dizygotic (DZ) twins share half of their genes. However, it is assumed that they are under the same environmental influences, which might not necessarily hold true. Nevertheless, if there is a higher concordance rate for suicidal behavior among MZ than DZ twins, it is then possible to assume that the suicide susceptibility may have a genetic basis.

The concordance for suicidal behavior has been consistently found to be greater among MZ than DZ twin pairs by most of the studies published in recent years (Roy et al. 1991; Roy et al. 1995; Statham et al. 1998; Roy and Segal 2001). The most recent twin study (Roy and Segal 2001) confirmed previous results by examining a new series of 28 twin pairs. Additionally, this study indicated that across the reported twin studies to date, which include a total of 462 twin pairs with at least one report of suicide completion in one twin, there is a significant difference in concordance rates for suicidal behavior being greater among MZ twins (18.4%) than DZ twins (0.7%). This difference strongly supports the

involvement of genetic factors in the predisposition to suicidal behavior. Moreover, studying a group of MZ and DZ twins surviving the suicide of their co-twins, Roy et al. (1995) found that 38% of the MZ twins had attempted suicide compared to none of the DZ twins. They concluded that MZ twin pairs not only show higher concordance rates for suicide but also for attempted suicide, suggesting that both behaviors are influenced by genetic factors. One of the largest studies on twins published to date is that by Statham et al. (1998). This study addressed an issue that had not been investigated in other twin studies. That is the genetic transmission of psychiatric disorders as a possible confounder for the genetic transmission of suicidal behavior. This study was done on an Australian community-based sample of MZ and DZ twin pairs. They found a concordance rate for serious suicide attempts of 23.1% in MZ twin pairs compared to 0% in DZ twin pairs. It was also observed that a history of serious suicide attempts in one MZ twin translated into a 10-fold increase in the risk of making a serious suicide attempt by the other MZ twin. This association was significantly stronger than in DZ twin pairs. This study identified several risk factors for suicidal behavior such as psychiatric disorders, traumatic experiences, personality traits and certain demographic variables. It is interesting to note that after controlling for these factors, the MZ co-twin's history of suicidal behavior remained a strong predictor of suicidal behavior, whereas for DZ twin pairs a suicidal history on one twin was not a consistent predictor. They found that approximately 45% of the variance in suicidal behavior was accounted for by genetic factors. This study added further evidence for a genetic transmission of suicidal behavior while taking into account associated psychopathology. Thus,

suggesting a specific genetic transmission of suicidal behavior that is independent of that associated with related psychopathology.

Adoption studies

Adoption studies, although less common, are another method to separate genetic from shared environmental factors involved in the liability of suicidal behavior. Individuals, who have been adopted at birth, or very early in life, share their genes with their biological relatives but not similar environmental experiences. On the other hand, adoptees share common environmental experiences with their adopting relatives but no genes. Hence, if clustering of suicidal behavior occurs in adoption studies, it indicates that genetic factors must be responsible for this observation, rather than the family environment.

The two major adoption studies published so far have investigated the Danish case registry of adoptions. Schulsinger et al (1979) studied a group of 57 adoptees who died by suicide and 57 matched adopted controls. They found that more suicides had occurred among the biological relatives of adopted suicides (12 out of 269 biological relatives) than among the biological relatives of the adopted controls (2 out of 269 biological relatives). Furthermore, no reports of suicide were found among adopting relatives of either the suicide or control group. These results were consistent with the existence of a genetic component for suicide.

Wender et al (1986) also used the Danish case registry, but focused on individuals with affective disorders. They identified 71 adoptees with affective psychopathology—the index cases—and matched them with 71 control adoptees without affective disorders. The most remarkable findings in this study were the

8-fold greater frequency of unipolar depression among the biological relatives of affective disordered adoptees compared with their controls, as well as a 15-fold increase in the frequency of suicide, also among the biological relatives of the index cases. They did not find any difference in suicide risk between the adoptive relatives of the two groups.

From the evidence gathered from twin and adoption studies, it is possible to conclude that genes are involved in the predisposition to suicide. Moreover, these studies have also lent support to the role of environmental influences in the susceptibility for suicidal behavior. Although no studies on segregation of suicidal behavior have been published, a polygenic inheritance has been suggested based on a computational model applied to depressed patients with a lifetime history of attempted suicide (Papadimitriou et al. 1991).

Molecular genetic studies

Given the joint evidence from family, twin and adoption studies of a genetic component underlying the predisposition to suicide, what follows is the search for genes that might be implicated in suicide. Taking into consideration that suicidal behavior is a complex trait, the involvement of multiple genes of varying effect sizes are expected. Among the genetic approaches available for the study of suicidal behavior are linkage analyses and association studies (Lander and Schork 1994). There are important differences in these two methods; while linkage studies investigate the relationship between a disease phenotype and a gene allele in pedigrees, association studies investigate their relationship in unrelated individuals. Therefore, association studies do not look at concordant

inheritance, rather they focus on frequencies' differences between cases with the condition of interest and control individuals that do not exhibit it (Lander and Schork 1994). Due to the greater power of association studies to detect genes of modest effects compared to linkage studies (Risch and Merikangas 1996), the former constitutes a better and more practical method for the study of complex traits, despite some of its limitations (see Chapter 4).

A number of association studies have been conducted in suicidal behavior based on a candidate gene approach. The genes that have been included in these studies are those that code for components of biological systems that have been found to be related to suicidal behavior. Accordingly, a large number of these studies have been carried out on genes that code for components of the serotonergic system, since there is strong evidence of its implication in suicidal behavior. The most frequently studied serotonergic genes have been the tryptophan hydroxylase (TPH) gene, the serotonin transporter (5-HTT) gene, the serotonin receptor 1B (5-HT1B), 2A (5-HT2A) and 1A (5-HT1A).

The tryptophan hydroxylase genes

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the biosynthesis of serotonin. It hydroxylates the aminoacid tryptophan generating 5-hydroxytryptophan, which in turn is decarboxylated into 5-hydroxytryptamine or serotonin (Mockus and Vrana 1998). Two genes with different chromosomal locations have been found to encode two distinct isoforms, TPH1 and TPH2, which have different distributions in human tissues. While TPH1 is found in several peripheral organs such as the intestine, spleen and thymus; TPH2 has been

found to be present exclusively in the brain (Walther and Bader 2003). However, it was not until recently that the TPH2 gene coding for the brain specific isoform was discovered. Therefore, most of the studies published so far have looked at the TPH1 gene.

TPH1 gene studies have investigated primarily two polymorphisms in intron 7 (A779C and A218C) that are in tight linkage disequilibrium (Nielsen et al. 1997). Interesting results were found with earlier studies; however, the findings from subsequent studies were not consistent, and recently, by means of meta-analytic techniques, no overall association was found between suicidal behavior and TPH1 (Lalovic and Turecki 2002). Of note is that, despite the fact that this gene is not expressed in the brain, the less frequent alleles of these two intronic polymorphisms (A218 and A779) have been associated with a reduced prolactin response to fenfluramine (Manuck et al. 1999) and CSF 5-HIAA levels in healthy volunteers (Jonsson et al. 1997). The validity of these findings remains unclear.

Since the identification of a second TPH, with an exclusive expression in the brain, the focus of research on suicidal behavior and mood disorders has shifted to studies in the recently found TPH2 gene. Although a few studies have been published since then, a role for TPH2 variants located in this gene has been found in bipolar disorder (Harvey et al. 2004), attention-deficit hyperactivity disorder (ADHD) (Sheehan et al. 2005; Walitza et al. 2005), autism (Coon et al. 2005) and obsessive-compulsive disorder (Mossner et al. 2005). Furthermore, there is evidence suggesting that TPH2 variants have a modulatory role in the amygdala responsiveness, thus implicating this gene in disorders that display emotional dysregulation (Brown et al. 2005; Canli et al. 2005). As for major

depression and suicidal behavior, there have been only a few studies published, and, in general, they have been supportive of a role for TPH2 variants. These studies are further discussed in Chapter 3.

The serotonin transporter gene

The serotonin transporter (5-HTT) gene is probably one of the most extensively studied genes. It is located on chromosome 17q11.1-17q12 (Ramamoorthy et al. 1993), and due to its role in the reuptake of serotonin, it definitely constitutes an interesting candidate gene. It spans 31 kb and consists of 14 exons. Association studies between the 5-HTT gene and suicidal behavior have been characterized by inconsistent results that might be related, in part, to certain methodological issues. These studies, particularly those focusing on major depression, are reviewed in Chapter 2.

Other serotonergic candidate genes

The serotonin 1B receptor (5-HT1B) gene is a good candidate because the knockout mice model of this gene exhibits impulsive and aggressive behaviors (Ramboz et al. 1996). However, no evidence for the role of this gene in suicide has been found in humans. Huang et al. (1999) did not find any association between suicide, major depression, alcoholism or aggression with 5-HT1B gene, investigating the two polymorphisms identified in this gene (C129T and G861C). Similarly, Nishiguchi et al. (2001) found no association in suicide victims with the G861C polymorphism.

The results regarding the serotonin 2A receptor (5-HT2A) gene have lacked consistency. Zhang et al. (1997) reported an association between the TT genotype of the T102C polymorphism and suicide attempters with mood disorders. However, a later study investigating the same polymorphism in depressed suicide ideators found an association with the opposite allele (the C allele) and suicidal ideation (Du et al. 2000). Several studies have not detected any association between the 5-HT2A T102C polymorphism and suicide attempts or completion (Turecki et al. 1999; Du et al. 1999; Geijer et al. 2000; Bondy et al. 2000; Ono et al. 2001). The 5-HT1A gene has been less investigated in relation to suicidal behavior and no association has been reported thus far (Nishiguchi et al. 2002; Huang et al. 2004).

It is clear that more studies including these and other relevant genes are required to attain a better understanding of the molecular genetic mechanisms involved in suicidal behavior. Nonetheless, these studies have provided useful and promising findings, in particular for the TPH2 and 5-HTT genes. Despite some of the exciting results that have been obtained investigating these two genes in suicidal behavior, many of these studies have not taken into account underlying psychiatric diagnoses. Therefore, it is unclear to what extent the genetic associations found are, in fact, attributed to suicide rather than related psychopathology. Furthermore, the majority of studies on these two genes have not specifically investigated genetic risk factors for suicide in major depression—a condition that is the most commonly associated with suicide. Interestingly, impulsive-aggressive behaviors are strongly correlated to suicide, both showing

similar serotonergic alterations consistent with a reduced serotonergic activity. Moreover, the activity of the serotonergic system has been found to be partly under genetic control, which suggests that some genes may predispose to suicide by lowering the serotonergic activity, and thus facilitating the manifestation of impulsive-aggressive behaviors and ultimately suicide. These mechanisms may act as risk factors for suicide completion in individuals exposed to stressors such as major depression. Thus, impulsive-aggressive behaviors may be an intermediate phenotype linking the initial serotonergic alterations to suicide. The contribution of genetic and behavioral predisposing factors in the determination of the likelihood of committing suicide in patients with major depression deserves further study.

OBJECTIVES

The main goal of this research is to investigate genetic susceptibility factors for suicide completion in major depressive disorder. More specifically, the aims of this research are:

1. To investigate whether genetic variants at the serotonin transporter and tryptophan hydroxylase 2 genes are related to suicide predisposition.
2. To explore impulsive-aggressive behaviors as possible mediators of the relationship between genetic variation and suicide completion.
3. To find predictors of suicide while taking into account genetic, clinical and behavioral risk factors.

HYPOTHESES

We hypothesized that genetic variants of the serotonin transporter and/or tryptophan hydroxylase genes would have different allelic distributions between depressed suicide completers and depressed control individuals, and that the genetic variants thus identified would also be associated with increased levels of impulsive-aggressive behaviors.

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Chapter 2

Serotonin transporter intron 2 (STin2) variant and family history of suicide as significant predictors of suicide completion in major depression

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PREFACE

Given the increasing evidence suggesting the implication of serotonergic alterations in suicide predisposition, serotonergic genes are interesting candidates for molecular genetic studies of suicide. In particular, the serotonin transporter (5-HTT) gene, which codes for a presynaptic receptor that is responsible for the reuptake of serotonin, might play an important role in suicide susceptibility. However, inconsistent results have been reported by several studies investigating this gene. A factor that may account for inconsistencies is the lack of consideration for the confounding effect of psychopathology among suicide completers. In this chapter, the role of the 5-HTT gene in suicide susceptibility as well as other risk factors is studied in the context of major depression, a major risk factor for suicide.

ABSTRACT

Background: Suicide is the most serious outcome of major depression, yet not all depressed patients will commit suicide. Genes along with other factors may account for this difference. Serotonergic alterations have been observed in suicide and depression and impulsive-aggressive behaviors. Therefore, we aimed to identify predictors of suicide considering genetic variation at the serotonin transporter (5-HTT) gene.

Methods: We investigated the 5-HTT gene-linked polymorphic region (5-HTTLPR) and intron 2 (STin2) variants of this gene and their relationship to behavioral and clinical risk factors for suicide in a sample of depressed suicides (N=106) and depressed control subjects (N=152), diagnosed by means of proxy-based interviews.

Results: We found a significant association of suicide completion with having at least one copy of the STin2 10 allele (χ^2= 10.833, df = 1, p = 0.002). No differences were found for the 5-HTTLPR variable number of tandem repeats. After controlling for behavioral and clinical risk factors for suicide, the STin2 variant remained a significant predictor of suicide in major depression when jointly considered with a family history of suicide (odds ratio 5.560, 95% confidence interval 1.057-29.247).

Conclusions: The STin2 locus might account, at least in part, for the observed familial aggregation of suicidal behavior. These results should be further explored in families where clustering of suicidal behavior is observed.

INTRODUCTION

Suicide is the most serious outcome of major depressive disorder (MDD). Along with schizophrenia, depressive disorders account for as much as 60% of all suicide cases (World Health Organization, 2001). The lifetime suicide risk among MDD patients has been recently reassessed to 3.4%, with a higher risk among men (7%) than women (1%) (Blair-West et al. 1999). This figure is important considering the high lifetime prevalence of MDD, which affects an estimated 32.6 to 35.1 million adults in the US alone (Kessler et al. 2003). However, not all patients suffering from MDD will commit suicide and it is precisely this difference that is of great clinical relevance.

Several lines of evidence suggest that part of the risk for suicide is determined not only by the existent psychopathology but also by a certain predisposition (Mann, 1998; Mann et al. 1999). Studies also suggest that this predisposition results from the interaction of multiple environmental and biological factors (Mann, 2002; Turecki, 2001). Among these factors, the presence of impulsive-aggressive behaviors (IABs) has been of great interest as an intermediate phenotype for the study of suicidal behavior. Recently, we reported higher levels of IABs among suicide cases that died during an episode of MDD compared to controls, even after controlling for psychopathology (Dumais et al. 2005). The neurochemical alterations commonly associated with these behavioral traits (Coccaro, 1989; Manuck et al. 1998) are similar to those associated with suicide and depression (Asberg, 1997; Mann et al. 2001; Stockmeier, 2003) and point to a dysfunction of the serotonergic neurotransmission characterized by a reduced central serotonergic activity. Therefore, given the growing evidence

suggesting that genes mediate at least part of the suicide predisposition (Turecki, 2001), those genes coding for components of the serotonergic system, such as the serotonin transporter (5-HTT) gene, have been and remain interesting candidates.

Two polymorphic regions with transcriptional regulatory activities have been identified in the 5-HTT gene. The first one is the 5-HTT gene-linked polymorphic region (5-HTTLPR), which consists of a 44-bp insertion or deletion in the promoter region, resulting in a long (L) or short (S) variant, respectively. Lower 5-HTT expression and 5-HT uptake have been seen with the short variant of the gene, which has been associated with anxiety-related traits (Heils et al. 1996; Lesch et al. 1996). The second polymorphic region, a variable number of tandem repeats (VNTR), has been identified in the second intron (STin2) of this gene. The STin2 polymorphism comprises 9, 10 and 12 copies of a 17-bp length element. Studies have shown that the transcriptional regulatory activity of this variant is determined by the number of copies of the repeat, with the 12-repeat allele having a higher expression than the 10-repeat allele (Fiskerstrand et al. 1999; MacKenzie and Quinn 1999). A number of association studies have focused on these polymorphisms and their relationship to suicidal behavior with inconsistent results (Purselle and Nemeroff 2003). Two meta-analyses were recently carried out. One of them indicated a significant overall effect for the promoter polymorphism of the 5-HTT gene on suicide risk (Anguelova et al. 2003b), whereas the other one (Lin and Tsai 2004) reported a significant association with the S allele of the 5-HTTLPR polymorphism but only among suicide attempters when compared to suicide non-attempters with the same psychiatric disorder, and in violent suicides. On the other hand, results from a

meta-analysis (Anguelova et al. 2003a) investigating a possible effect of the 5-HTTLPR and STin2 variants in major depression did not provide evidence for such effect. It is noteworthy that most of the studies carried out so far, investigating the association between 5-HTT gene and suicidal behavior, have not controlled for the effect of psychopathology, an important confounder. Moreover, since suicide and major depression are associated to a certain extent with a serotonergic dysfunction (Mann et al. 2001), it is difficult to determine what effects result from either having major depression or a certain suicide predisposition. The purpose of this study was to investigate whether variation at the 5-HTT gene could mediate at least part of the genetic predisposition to suicide in MDD, and if so, whether phenotypically, this effect could be translated into an excess of impulsive-aggressive behaviors. To do so, we carried out a case-control study with individuals from the same ethnic origin and controlled for depressive psychopathology.

METHODS AND MATERIAL

Sample

We investigated a total sample of 258 subjects. Cases for this study were 106 suicide completers (92 males, 14 females) aged 18 years and older who committed suicide, as determined by the Quebec Coroner's Office, and who met DSM-IV diagnostic criteria for MDD or depression not otherwise specified (NOS). Suicides having a diagnosis of depression NOS were considered in this study because they most likely had MDD but were not recognized as such by the informants given the relatively low sensitivity of the psychological autopsy procedure for symptoms present

immediately before death (Ernst et al. 2004). Cases for this study were recruited from 2000 to 2004.

Controls were 152 living subjects (131 males, 21 females) suffering from MDD according to the DSM-IV criteria, and whose condition was severe enough to require follow up in a specialized psychiatric outpatient clinic. Suicide cases and control subjects that met criteria for bipolar or any psychotic disorder were excluded in order to increase the likelihood that the depressive episode might be the trigger of the suicidal act. Prior to inclusion in the study, controls and family members of the suicide completers provided written informed consent. The McGill University Institutional Review Board of the Faculty of Medicine approved this study.

French Canadian origin

Almost all subjects that participated in this study (98%); in both suicide and control groups, had French-Canadian ancestry determined by the presence of 4 grandparents of French-Canadian origin (see Discussion).

Suicide method

We classified suicide methods as violent or non-violent according to the classification used in previous studies (Coccaro, 1989; Denning et al. 2000; Dumais et al. 2005a). Drug overdose, carbon monoxide poisoning, and drowning were considered non-violent. All other methods were classified as violent.

Cases in our sample committed suicide using the following methods: hanging and strangulation (56.6%), carbon monoxide poisoning (14.2%), shooting (9.4%), drug overdose (8.5%), jumping (4.7%), drowning (3.8%), penetrating

lesions (1.9%), others (0.9%). These figures are similar to the distribution of suicide methods in the Quebec general population (St-Laurent and Bouchard 2004).

Relation of sample to other reports

Part of the sample used in this report has been previously described in studies investigating clinical risk factors (Dumais et al. 2005). However, the subjects included in this study have never been analyzed in our previous molecular genetic studies.

Psychiatric assessment

The presence of psychiatric illnesses and personality disorders in our sample was assessed by means of the psychological autopsy procedure, which has been well validated (Brent et al. 1993; Conner et al. 2001b; Conner et al. 2001a; Kelly and Mann 1996; Schneider et al. 2004). Briefly, this procedure consists in identifying a family member who will be interviewed as best acquainted with the deceased using a series of structured instruments adapted for use with a proxy. In this study, families were first contacted at the Montreal morgue and recontacted for interview after a period of approximately 4 months. To ensure comparability between the two groups, a best informant was also identified for each of our controls, and diagnoses were made by proxy-based interviews as for the suicide cases.

Information on axis I and axis II psychopathology was obtained using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (Spitzer et

al. 1992) and the Structured Clinical Interview for DSM-IV Personality Disorders (SCID-II) (First et al. 1995), respectively. After completion of the interviews, a case report was written taking into account the coroner's notes and medical records for the purpose of a best-estimate diagnosis. A panel of clinicians reviewed the case reports and best consensus for DSM-IV axis I and II diagnoses was reached. In addition, information on family history of suicide and history of physical or sexual abuse was investigated by means of structured questionnaires.

Assessment of personality traits

We also collected information on related behavioral and personality traits with informant versions of the following instruments: Barrat's Impulsivity Scale (BIS; Barrat, 1959) was used to assess impulsive behavior; Buss-Durkee Hostility Inventory (BDHI; Buss and Durkee 1957) and Brown-Goodwin Assessment of Lifetime History of Aggression (BGHA; Brown and Goodwin 1986) were employed to assess aggressive behaviors; and the Temperament and Character Inventory (TCI; Cloninger et al. 1994) was helpful in investigating temperament and character dimensions. Estimates of internal consistency (α) for the informant versions of most of the instruments used in this study have been previously carried out by our group (Dumais et al. 2005). The reported estimates were BGHA, 0.88; BIS, 0.89; and TCI, between 0.73 and 0.88 for the 4 temperament and 3 character scales, respectively. Overall estimates were satisfactory and similar to those reported in other studies using informant versions of some of these instruments (Brent et al. 1994).

Inter-rater reliability and validity of proxy-based personality assessments

Detailed description of reliability and validity of the proxy-based assessments are provided elsewhere (Dumais et al. 2005). Inter-rater reliability for key diagnoses was excellent: MDD, 0.96; alcohol abuse/dependence, 0.98; drug abuse/dependence, 1.0; and cluster B personality disorder, 1.0. The validity of proxy-based personality trait assessments was performed by interviewing two different informants using the above-mentioned instruments for each subject. No significant differences were found between the information provided by two different informants on the same subject (p-values ranged from 0.25 to 0.94). For controls, the information obtained with the informant was similar to that obtained with the subject (p-values ranged from 0.67 to 0.98).

Genetic variants

We genotyped two variants of the 5-HTT gene believed to have transcriptional regulatory activities (Fiskerstrand et al. 1999; Heils et al. 1996; Lesch et al. 1996; MacKenzie and Quinn 1999). The 5-HTT gene-linked polymorphic region (5-HTTLPR) consists in either a 44-bp insertion or deletion in the promoter region, resulting in a long (L) or short (S) variant, respectively. The short variant has been associated with lower transcription of the 5-HTT (Heils et al. 1996; Lesch et al. 1996). The second variant, a variable number of tandem repeats (VNTR), is located in the second intron (STin2) of this gene and comprises 9, 10 and 12 copies of a 17-bp length element. The transcriptional regulatory activity of this variant seems to be determined by the number of copies

of the repeat, with the 12 repeat allele leading to a higher expression than the 10 allele (Fiskerstrand et al. 1999; MacKenzie and Quinn 1999).

Genotyping

Genomic DNA was extracted from blood or frozen brain tissue according to standard procedures (Sambrook et al. 1989). Selective amplification of the genomic regions of interest was done by polymerase chain reaction (PCR) using a GeneAmp PCR system 9700 (Applied Biosystems). PCR was performed in a total volume of 10 μl consisting of 40-60 ng of DNA, 200 μM dNTP, 10pmol of the specific primers and 0.5 U of Platinum Taq (Invitrogen). The primers used for the amplification of the 5-HTTLPR were those used by (Gelernter et al. 1997). For the intronic variant we used the primers reported by (Ogilvie et al. 1996). Both sets of primers were obtained from Alpha DNA (Montreal, Canada). Cycle conditions consisted in an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at the specific temperature for each primer for 30 s, and extension at 72°C for 30 s as well. A final extension was carried out at 72°C for 7 min. The specific annealing temperatures for each pair of primers were: 5-HTTLPR, 58°C and STin2, 61°C. The genomic products obtained by the amplification procedure were then analyzed on a 2.5% agarose gel stained with ethidium bromide and visualized under UV light. All laboratory procedures were performed blind to subject status. For technical reasons, we did not have genotypic information for seven and four individuals for the 5-HTTLPR and the STin2 loci, respectively.

Statistical analysis

Tests for deviation from Hardy-Weinberg equilibrium were conducted using the chi-square test for goodness of fit. Allelic and genotypic frequency distributions between groups were compared using chi-square tests for independence. Differences in mean scores for each of the questionnaires used between suicide completers and controls grouped by genotypes were estimated by two-tailed student's t-tests. Mann-Whitney tests were performed when the data were not normally distributed. Prevalence of axis I and axis II psychopathology, as well as family history of suicide and history of physical or sexual abuse were compared between cases and controls by chi-square tests for independence. This test was also used for dichotomized data. Forward and backward conditional stepwise logistic regression was performed to find predictors of suicide and to control for the effect of age and other significant variables. All statistical analyses were carried out using the SPSS package (version 11.0 for Windows; SPSS, Chicago). Linkage disequilibrium between the two polymorphisms as well as haplotype analyses were performed using the GENECOUNTING support programs LDPAIRS and RUNGC, respectively (Zhao et al. 2002). Our alpha risk was set at 5%. Corrections for multiple testing were performed by the Bonferroni method. Our sample has over 80% power to detect in univariate comparisons a difference of 31% or over in exposure to a given factor that increases the risk of suicide by approximately 2.5 times (Fleiss, 1981). These figures are based on the assumption that type I error is not greater than 0.05 and that the exposure of normal subjects to the implicated factor is around 15%.

RESULTS

We studied a total sample of 258 subjects, 106 depressed suicides and 152 depressed controls. Demographic information of this sample is listed in table 1. There were no significant differences in age (t= 0.052, df = 175, p=0.958), sex (χ^2= 0.020, df = 1, p = 0.888) or marital status (χ^2= 1.394, df = 1, p = 0.238) between the groups. Cases and controls were almost from the same ethnic and religious background, consisting mostly of Caucasians of French-Canadian origin and of Catholic faith. Similar proportions of suicides and controls had at least one child. No differences in employment status (χ^2= 0.033, df = 1, p = 0.855) and living alone (χ^2= 0.452, df = 1, p = 0.502) were found between groups.

Table 2.1. Demographic characteristics of the study population

	Depressed Suicides	Depressed Controls	Statistic[a]	P-value
Age (Mean± SD)	41.8±14.7	41.7± 10.2	0.052[b]	0.958
Gender (%)				
Male	86.8	86.2	0.020[c]	0.888
Female	13.2	13.8		
Caucasian (%)	98.9	97.9	0.328[c]	0.567
Catholic (%)	94.9	88.4	2.251[c]	0.134
Married (%)	43.8	35.4	1.394[c]	0.238
Employed (%)	58.1	59.4	0.033[c]	0.855
Parent of at least one child (%)	61.5	51.6	1.897[c]	0.168
Living alone (%)	37.2	42.1	0.452[c]	0.502

[a] df=1 for all variables except age (df=175)
[b] Two-tailed student's t-test
[c] Chi-square test

Genotypic distributions for both loci among cases and controls were in Hardy-Weinberg equilibrium (data not shown). Allele and genotype frequencies for each of the loci investigated are shown in table 2. No significant differences in allelic (χ^2= 0.011, df = 1, p = 0.917) or genotypic (χ^2= 0.929, df = 2, p = 0.629) distributions for the 5-HTTLPR locus were found between depressed suicides and depressed controls. However, depressed suicide cases had a higher frequency of the allele 10 at the STin2 locus than depressed controls (χ^2= 7.382, df = 2, p = 0.025). This result remained significant (p = 0.05) after Bonferroni correction for two loci. Accordingly, there was a significant difference between groups in genotype frequencies at this locus, with depressed suicides showing a higher frequency of genotypes 12/10 (56.9%) and 10/10 (18.6%) (χ^2= 9.711, df = 3, p = 0.021). After correction for multiple testing this result was still significant (p = 0.042). This difference in genotype frequencies was significantly greater when genotypes were grouped according to the presence or absence of at least one copy of the 10 allele (χ^2= 10.833, df = 1, p = 0.001; Bonferroni corrected p = 0.002). Significant but not strong linkage disequilibrium between these two markers was found in our sample (D'=0.551, p=0.001). We also carried out haplotype analyses with these two 5-HTT polymorphisms; however, there were no differences in the distribution of estimated haplotype frequencies between groups (data not shown).

Table 2.2. Genotypic and allelic frequency distribution for the 5-HTT gene polymorphisms investigated in depressed suicide completers and depressed controls

Locus	Allele	Status		Genotype	Status	
		Depressed Suicide (%)	Depressed Control (%)		Depressed Suicide (%)	Depressed Control (%)
5-HTTLPR	L	115 (58.1)	178 (58.6)	L/L	31 (31.3)	53 (34.9)
	S[a]	83 (41.9)	126 (41.4)	L/S	53 (53.5)	72 (47.4)
				S/S[b]	15 (15.2)	27 (17.8)
STin2	12	101 (49.5)	184 (60.5)	12/12	21 (20.6)	58 (38.2)
	10	99 (48.5)	111 (36.5)	12/10	58 (56.9)	64 (42.1)
	9[c]	4 (2.0)	9 (3.0)	10/10	19 (18.6)	22 (14.5)
				Others[d,e]	4 (3.9)	8 (5.3)
				Allele 10	80 (78.4)	89 (58.6)
				No allele 10[f]	22 (21.6)	63 (41.4)

5-HTT, serotonin transporter; 5-HTTLPR, serotonin transporter gene-linked polymorphic region; L, long allele; S, short allele; STin2, serotonin transporter intron 2 variable number of tandem repeats.

[a] χ^2=0.011, df=1, p=0.917

[b] χ^2=0.929, df=2, p=0.629

[c] χ^2=7.382, df=2, p=0.050*

[d] χ^2=9.711, df=3, p=0.042*

[a] Others represent genotypes distributed in suicides and controls with frequencies less than 5.

[f] χ^2=10.833, df=1, p=0.002*

* Bonferroni corrected p-values

As reported elsewhere (Dumais et al. 2005), on a sample composed in its majority by subjects of the current sample, depressed suicide cases had higher scores than depressed controls on a series of measures of impulsive-aggressive behaviors. Suicide cases also had higher prevalence of axis II diagnoses, especially cluster B personality disorders characterized by high levels of these traits. However, no effect of variation at the STin2 locus was observed on levels of impulsive-aggressive behaviors (Table 3). Similarly, variation at this locus did not have an effect on cluster B personality disorders for all subjects (χ^2=0.004, df =1, p=0.950) and for only suicides (χ^2=0.098, df =1, p=0.754). Nevertheless, we found that suicide completers having genotypes with at least one copy of allele 10 used violent methods more frequently than those without any copy of this allele (χ^2 = 6.479, df = 1, p = 0.011).

As expected, depressed suicides had higher comorbidity with current (last 6 months) (χ^2=5.632, df =1, p= 0.018) and lifetime (χ^2=5.809, df =1, p=0.016) substance use disorder. In addition, reporting a family history of suicide was significantly more frequent for depressed suicide cases than for the controls [17.2% vs. 5.7%, respectively (χ^2=5.775, df =1, p= 0.016)].

We carried out forward and backward stepwise logistic regression to determine predictors of suicide completion and to investigate the effect of variation at STin2 locus, controlling for age and the effect of other significant variables. Variables included in this analysis were: age, last 6 months and lifetime prevalence of substance use disorder, cluster B personality disorder, BDHI scores, BGHA scores, BIS scores, family history of suicide, genetic variation at the STin2

locus and their interaction. The results of this analysis showed that last 6 months substance use disorder (odds ratio [OR] 3.281, 95% confidence interval [CI] 1.244-8.654), cluster B personality disorder (OR 5.170, 95% CI 1.542-17.327), and the interaction between family history of suicide and STin2 variation (OR 5.560, 95% CI 1.057-29.247) were significant predictors of suicide (Table 4). It is interesting to note that the STin2 variant remained a significant predictor of suicide only when a positive family history of suicide was reported.

Table 2.3. Measures of impulsive-aggressive behaviors and temperament and character dimensions in depressed suicide completers grouped by genotype at the STin2 locus

	STin2[a]	N	Mean score	SD	P value
BGHA[b]	Non-10	59	37.92	-	0.667
	10	17	40.53		
BDHI	Non-10	67	33.31	13.48	0.841
	10	18	34.03	13.54	
BIS	Non-10	69	66.08	14.26	0.281
	10	19	70.14	15.18	
TCI					
Novelty seeking	Non-10	68	20.47	7.47	0.238
	10	18	22.80	7.11	
Harm avoidance	Non-10	68	18.30	8.01	0.491
	10	18	19.75	7.43	
Reward dependence	Non-10	68	13.95	3.66	0.770
	10	18	14.24	4.18	
Persistence	Non-10	68	6.93	2.69	0.149
	10	18	5.83	3.35	
Self-directedness	Non-10	68	24.74	7.89	0.518
	10	18	23.36	8.35	
Cooperativeness	Non-10	68	25.90	6.44	0.698
	10	18	25.05	8.53	
Self-transcendence	Non-10	68	10.65	5.80	0.933
	10	18	10.78	5.65	

STin2, serotonin transporter intron 2 variable number of tandem repeats; BGHA, Brown-Goodwin Assessment of Lifetime History of Aggression; BDHI, Buss-Durkee Hostility Inventory; BIS, Barrat's Impulsivity Scale; TCI, Temperament and Character Inventory.

[a] Genotypes at the STin2 locus, dichotomized according to the presence or absence of at least one copy of the 10 allele.

[b] Mann-Whitney test was carried out, and the mean rank is reported instead of the mean score.

Table 2.4. Predictors of suicide completion in major depression

Predictor	B	Wald	P	OR	95% CI	
Last 6 months substance use disorder (alcohol and/or drugs)	1.188	5.768	0.016	3.281	1.244	8.654
Cluster B personality disorder	1.643	7.088	0.008	5.170	1.542	17.327
STin2 variation and family history of suicide	1.716	4.102	0.043	5.560	1.057	29.247

B, unstandardized logistic regression coefficient; Wald, Wald statistic; OR, odds ratio; CI, confidence interval; STin2, serotonin transporter intron 2 variable number of tandem repeats.

DISCUSSION

In this study we investigated genetic variation at the 5-HTT gene and its possible effect on intermediate phenotypes such as impulsive aggressive-behaviors. We studied a sample of 106 suicide cases and 152 controls, while controlling for the presence of MDD. Additionally, we also identified predictors of suicide taking into account genetic variation at this gene.

We provide evidence that variation at the 5-HTT gene mediates part of the genetic predisposition to suicide. Our results showed a significant association of suicide completion with the presence of at least one copy of allele 10 in the STin2 locus. However, we did not find evidence for an effect of variation in the more commonly tested 5-HTTLPR polymorphism on suicide completion. We also found that depressed suicides with the STin2 variant were more likely to use

violent methods. Although previous studies (Dumais et al. 2005) indicated that suicide method may be used as a behavioral marker of lifetime history of impulsive-aggressive behaviors, we failed to see a direct effect of STin2 on behavioral measures of these traits or on the presence of cluster B personality disorders in our sample. It is possible that we lacked sufficient power to detect such an effect, especially considering that measures of these behaviors and psychiatric assessments were obtained by means of proxy-based interviews (see limitations segment) and that not all subjects had this information available.

We have also shown that after controlling for the effect of behavioral and clinical risk factors for suicide, the STin2 variant remained a significant predictor of suicide outcome in major depression but only in subjects with a positive family history of suicide (17.2%) (see Table 4). The interaction between the effect of the STin2 10 allele and a positive family history of suicide increased the risk of suicide 5.56 times. This finding is interesting because it is independent of the presence of either axis I or II psychopathology and suggests that STin2 locus might account, at least in part, for the observed familial aggregation of suicidal behavior (Brent et al. 1996; Kim et al. 2005).

The allele-dependent differential enhancer activity of the VNTR in the second intron of 5-HTT has been previously reported in vitro studies in embryonic stem cells (Fiskerstrand et al. 1999), and in vivo studies in transgenic mice embryos (MacKenzie and Quinn 1999). Both studies suggested that this polymorphic region might act as a transcriptional regulator with the 12-repeat allele showing a stronger transcriptional activity than the 10-repeat allele. Recently, it was reported that not only the number of repeats could affect the

transcription of the gene but also individual repeat elements within the VNTR domain (Lovejoy et al. 2003). The fact that we found in depressed suicides a higher frequency of allele 10, which seems to result in lower transcription of the 5-HTT gene, might explain at least in part the reduced 5-HTT binding sites found in the prefrontal cortex of suicide subjects (Arango et al. 1995; Mann et al. 2000). This brain region is involved in behavioral inhibition, so that having reduced serotonin input would predispose individuals to suicidal behavior. Our findings imply a reduced 5-HTT function, however, which in principle would lead to increased serotonin concentration in the synaptic cleft. This seems, a priori, in contradiction with previous knowledge. A possible explanation is that the effect of this genetic variant could be more pronounced during brain development. Evidence from animal studies suggests that serotonin plays an important role during the development of the central nervous system (Lauder, 1990; Zhang, 2003) and genetic variants that lower 5-HTT expression may act at early stages of brain development by altering maturation of circuits involved in emotional and stress related responses(Ansorge et al. 2004). The genetic inactivation of the 5-HTT and the subsequent extracellular 5-HT excess has shown a profound negative impact in the development of certain cortical regions in mice (Persico et al. 2001; Salichon et al. 2001). Among other alterations that have been reported in 5-HTT knock-out mice models are a reduction in serotonergic neurons in the dorsal raphe nucleus of embryonic (Rumajogee et al. 2004) and adult mice (Lira et al. 2003), as well as reduced firing activity (Gobbi et al. 2001; Lira et al. 2003).

Over the last years, there have been at least 14 studies (Anguelova et al. 2003b; Purselle and Nemeroff 2003) investigating the 5-HTT gene

polymorphisms and suicidal behavior with inconsistent results and without definite conclusion on the role of these variants in suicidal behavior. Most of these studies have focused on the 5-HTTLPR variant and only three of them have either shown results on both variants (Hranilovic et al. 2003; Shen et al. 2004) or only on the STin2 variant (Yen et al. 2003). Ten out of 23 studies published to date have been positive (Baca-Garcia et al. 2002; Bayle et al. 2003; Bellivier et al. 2000; Bondy et al. 2000; Campi-Azevedo et al. 2003; Caspi et al. 2003; Courtet et al. 2001; Courtet et al. 2004; Du et al. 1999; Gorwood et al. 2000). Most of them showed an association of the short variant with suicidal behavior, especially violent suicidal behavior. None of the three studies with data on STin2 showed any positive results. However, a tendency to have an increase of the allele 10 frequency was seen in Croatian suicide victims (Hranilovic et al. 2003), which is in the same direction of the results obtained in our study. The vast majority of these studies have been performed on suicidal subjects having different psychiatric disorders, which does not allow separating the effect of suicide from that associated with underlying psychopathology. This confounder effect might be one factor contributing to the observed inconsistent results.

Studies have shown that allele frequency for both loci varies across different populations (Gelernter et al. 1997; Gelernter et al. 1999). Similarly, the patterns of linkage disequilibrium (LD) between 5-HTTLPR and STin2 loci are different depending on which population is being studied. For instance, no significant LD has been found for Japanese and African Americans. On the other hand, European Americans have shown a nearly significant LD (Gelernter et al. 1997). Following up on this study, (Gelernter et al. 1999) decided to study seven

different populations from 5 continents, and they confirmed the significant variation in allele frequencies and LD among populations. In this study a strong LD was observed in an African population (Mbuti Pygmies), whereas intermediate and no significant LD was seen in European (Danes and Adygei) and East Asian (Chinese) populations, respectively. Taken together, the previous results and those derived from our study in a French-Canadian population suggest that both loci are not in complete LD.

The limitations of case-control association studies are well known and well described (Lander and Schork 1994; Risch and Merikangas 1996). Arguably, the most important methodological bias leading to false positive results is the presence of population stratification effects. Our sample consisted of subjects of French-Canadian origin, which is an isolated and young population (approximately 12 generations) with a well-known founder effect (Heyer and Tremblay 1995). Because the French-Canadian population remained culturally isolated until recently (early 1900's), selecting subjects whose 4 grandparents are of French-Canadian origin guarantees French-Canadian ancestry. By doing so in both groups, we are more likely to select a group in which 1) the total genetic variance involved in suicide predisposition is likely reduced and 2) population stratification effects are less likely to occur.

Additional limitations of our study are inherent in the use of proxy-based assessments. The validity of such procedure, however, particularly with regard to the use of observable behaviors, has been well-demonstrated and discussed elsewhere (Conner et al. 2001a). In fact, the major problem of proxy-based interviews seems to be at the level of their sensitivity (Ernst et al. 2004).

Nevertheless, by using proxy-based techniques in both groups, this possible bias was controlled for.

In summary, we found that the allele 10 of the STin2 might play a role in the predisposition to suicide in subjects suffering from major depression. Furthermore, depressed subjects with at least one copy of allele 10 and a positive family history of suicide were at higher risk of suicide than subjects with a different variant at this locus, even after controlling for the effect of other suicide risk factors. These results should be further explored in families where aggregation of suicidal behavior is observed.

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Chapter 3

Effect of tryptophan hydroxylase 2 (TPH2) gene variants in the risk of suicide completion in major depression

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PREFACE

Since several genes are believed to be implicated in the predisposition to suicide, it is important to examine other serotonergic genes that might be involved. The recently discovered tryptophan hydroxylase 2 (TPH2) gene codes for a brain specific rate-limiting enzyme of serotonin synthesis. Since its discovery, the focus of research on suicidal behavior has shifted from the only previously known tryptophan hydroxylase 1 (TPH1) gene, which was found mainly expressed in peripheral tissues, to the TPH2 gene. Thus far, very few studies have been conducted examining the TPH2 gene and suicidal behavior, and as for studies on 5-HTT, most of them have studied unselected suicide attempters or completers. Following the same approach as in the previous study presented herein, the role of the TPH2 gene in the susceptibility to suicide completion was investigated while controlling for major depression.

ABSTRACT

Objective: The purpose of this study was to investigate whether variation at the TPH2 gene and upstream region may predispose to suicide in major depression (MDD); and if so, whether this predisposition is mediated by impulsive-aggressive behaviors (IABs). Predictors of suicide completion were also investigated considering genetic, clinical and behavioral risk factors for suicide.

Method: We genotyped 14 single nucleotide polymorphisms (SNPs) in 114 depressed suicides and 145 depressed controls diagnosed by means of proxy-based interviews. Single-marker and haplotype association analyses were conducted. Differences in behavioral and personality traits' scores according to genotypic variation in the total sample were examined. Suicide predictors were determined by hierarchical logistic regression.

Results: We found two upstream and two intronic TPH2 SNPs associated with suicide. No increased levels of IABs were related to genotypic variation at these SNPs. Additionally, two TPH2 gene variants in the upstream region and intron 1, respectively, had a significant contribution in the prediction of suicide along with cluster B personality disorders and family history of suicide.

Conclusions: TPH2 gene and upstream variants may play a role in the predisposition to suicide in MDD. However, it does not appear that this predisposition is mediated by increased levels of IABs. In addition, depressed individuals with certain TPH2 variation might be at higher risk of suicide. Further studies in larger and independent homogeneous samples are necessary. These future studies should also control for associated psychopathology, so that genetic risk factors attributable to suicide can be separated.

INTRODUCTION

Suicide is the most severe outcome of major depressive disorder (MDD). The lifetime suicide risk in MDD has been recently reevaluated to 3.4% with a higher risk in men than women (Blair-West et al. 1999). Considering the estimated high lifetime prevalence of MDD in the US alone (16.2%) (Kessler et al. 2003), the reported suicide risk is considerably important. Thus far, it is still unclear why some depressed patients do commit suicide whereas others with the same psychiatric condition do not.

Several lines of evidence suggest that biological and genetic factors are responsible for at least part of the variability in suicide risk (Mann, 1998; Turecki, 2001). Neurobiological studies on suicide, major depression and impulsive-aggressive behaviors (IABs) have been characterized by a reduced serotonergic activity (Coccaro, 1989; Mann et al. 2001). These findings have pointed out to serotonergic genes as interesting candidates in the study of this complex behavior. Additionally, the interest on IABs as an intermediate phenotype in the investigation of suicide has grown in recent years. Indeed, our group recently showed higher levels of these traits in male depressed suicide completers when controlling for depressive psychopathology (Dumais et al. 2005).

Among the serotonergic genes, the tryptophan hydroxylase 2 (TPH2) is probably the one that has gained most of the attention of researchers investigating mental disorders in the past few years. The TPH2 gene is located on chromosome 12q21.1, spanning a region of approximately 93.5 kb, and it is composed of 11exons. For many years, researchers believed that there was only one tryptophan hydroxylase (TPH) responsible for the widespread synthesis of

serotonin in the brain and peripheral tissues. It was not until recently that a second isoform of this enzyme, designated as TPH2, was identified in mice lacking the previously known TPH (now called TPH1). Furthermore, TPH2 has been found to be expressed exclusively in the brain of both mice and humans (Walther and Bader 2003; Zill et al. 2004b). It has also been shown a predominant TPH2 expression in the raphe nuclei, where the vast majority of serotonergic neurons are located (Bach-Mizrachi et al. 2005; Patel et al. 2004; Walther and Bader 2003; Zill et al. 2005).

Whereas several studies have supported a possible role of TPH2 gene variants in major depression (Zhang et al. 2005; Zhou et al. 2005; Zill et al. 2004a), few studies have been carried out exploring the TPH2 gene and suicidal behavior, with the majority of them investigating suicide attempters (De Luca et al. 2004; De Luca et al. 2005; Zhou et al. 2005) rather than suicide completers (Zill et al. 2004c). Furthermore, none of these studies have explored a possible role of TPH2 genetic variants on the individual's susceptibility to suicide through affecting IABs. Interestingly, a recent study carried out in mouse strains showed an association between a functional TPH2 single nucleotide polymorphism (SNP) and aggressive behaviors, which suggest that some variation at the TPH2 gene might be implicated in the regulation of these traits (Kulikov et al. 2005).

It should be noted that most of the studies on suicidal behavior have not controlled for existing psychopathology, which makes impossible to disentangle the effects attributable to either suicide or the underlying psychiatric disorder. Therefore, in this study we investigated whether genetic variants distributed across the TPH2 gene and 5'upstream region may predispose to suicide in the

context of MDD; and if so, whether this predisposition is mediated by intermediate phenotypes such as IABs. Furthermore, we sought to identify predictors of suicide completion in major depression taking into account genetic variation at the TPH2 gene, as well as clinical and behavioral risk factors. For these purposes, we carried out a case-control study with mostly French-Canadian individuals drawn from the same geographical area.

METHODS

Study population

Our sample consisted of 114 suicide completers (98 males, 16 females) and 145 control individuals (124 males, 21 females). Suicide cases included in this study were 18 years of age and older, whose cause of death by suicide was determined by the Quebec Coroner's Office. All cases met DSM-IV diagnostic criteria for MDD or depression not otherwise specified. We opted for the inclusion of suicide victims with the latter diagnosis because of the high probability that they had suffered from MDD, but due to the low sensitivity of the psychological autopsy procedure, especially for symptoms displayed immediately before death, they were not identified as such (Ernst et al. 2004).

The distribution of the methods of suicide used were: hanging and strangulation (56.1%), carbon monoxide poisoning (14.0%), drug overdose (9.6%), shooting (8.8%), jumping from a height or in front of a metro car (4.4%), drowning (4.4%), penetrating lesions (1.8%), and others (0.9%). These figures are similar to the distribution of suicide methods in the Quebec general population (St-Laurent and Bouchard 2004).

Participants in our control group were living subjects, also aged 18 years and older, suffering from MDD according to the DSM-IV criteria, and whose condition was severe enough to require follow up in a specialized psychiatric outpatient clinic. Suicide cases and control individuals that met criteria for bipolar or any psychotic disorder were excluded from the study in order to increase the likelihood that the depressive episode might have been the trigger of the suicidal act. All cases and controls were recruited from 2000 to 2004.

By means of structured questionnaires, information on specific demographic and clinical variables was obtained and compared between groups (Table 1). Both groups did not significantly differ on any of these variables except for family history of suicide, which was more frequently reported in suicide cases. Ninety-eight percent of the suicide completers and control individuals in our sample had French-Canadian ancestry determined by the presence of 4 grandparents of French-Canadian origin (see Discussion).

Prior to inclusion in the study, all control individuals and family members of suicide completers provided written informed consent. This study was approved by the McGill University Institutional Review Board of the Faculty of Medicine.

Part of this sample has been previously described in studies investigating clinical risk factors for suicide (Dumais et al. 2005); however, in molecular genetic studies, this sample, with fewer cases, has only been reported once in relation to the serotonin transporter gene (Lopez de Lara et al. 2005).

Table 3.1. Demographic and clinical characteristics of the study population

	Depressed Suicides	Depressed Controls	Fisher's Exact test (P-value)
Age (Mean± S.D.)	41.6±14.6	41.4± 10.2	0.90[a]
Gender %			
Male	86.0	85.5	1.00
Female	14.0	14.5	
Caucasian %	98.1	98.3	1.00
Catholic %	95.3	89.0	0.13
Completed college %	22.5	27.6	0.44
Employed %	55.9	59.8	0.58
Married (or common law) %	42.9	34.7	0.22
Parent of at least one child %	61.9	50.0	0.08
Living alone %	38.9	41.1	0.77
Family history of suicide%	16.5	5.6	0.02*
History of physical or sexual abuse%	28.4	20.7	0.28

[a] Student's t-test= 0.12, df= 195, p = 0.90 (two-tailed)
* Statistically significant at the 0.05 level.

Psychiatric assessment

Psychiatric illnesses and personality disorders were assessed by means of the psychological autopsy procedure, which has been well validated in earlier studies (Conner et al. 2001; Kelly and Mann 1996; Schneider et al. 2004). Briefly, a family member best acquainted with the deceased was identified and subsequently interviewed using a series of structured instruments adapted for use with a proxy. To ensure comparability between groups, the same proxy-based assessment procedure was also performed for each of the control individuals. In

this study, families of the suicide victims were first contacted at the Montreal morgue and recontacted for interview after approximately 4 months.

Axis I and II psychopathology was determined using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (Spitzer et al. 1992) and the Structured Clinical Interview for DSM-IV Personality Disorders (SCID-II) (First et al. 1995), respectively. Based on the information retrieved, coroner's notes and medical records, a case report was written and reviewed by a panel of clinicians who eventually reached a consensus on DSM-IV axis I and II diagnoses. In this study, depressed suicides had higher comorbidity with current (last 6 months) (32.5% vs 17.5%, p=0.01) and lifetime substance use disorder (43.9% vs. 29.1%, p= 0.03) than control individuals. Higher prevalence of cluster B personality disorders (cluster B PD) was also found in suicide cases than controls (23.9% vs 5.9%, p<0.001).

Personality traits' assessment

Information on related behavioral and personality traits was obtained with informant versions of the following instruments: Barratt Impulsiveness Scale (BIS-11) (Patton et al. 1995); Buss-Durkee Hostility Inventory (BDHI) (Buss and Durkee 1957); Brown-Goodwin Assessment of Lifetime History of Aggression (BGHA) (Brown and Goodwin 1986); and the Temperament and Character Inventory (TCI) (Cloninger et al. 1994). The BDHI has been commonly used as a scale of aggressive behavior. The TCI measures 4 temperament and 3 character dimensions, thus complementing the information given by the aforementioned questionnaires. Estimates of internal consistency (α) for most of these instruments,

as reported previously by our group (Dumais et al. 2005), were: BGHA, 0.88; BIS, 0.89; and TCI, 0.73 to 0.88 for the 4 temperament and 3 character dimensions. These estimates were overall satisfactory and similar to those reported in other study using informant versions of some of these instruments (Brent et al. 1994). Cases in this study exhibited higher levels of lifetime aggression than control individuals, as determined by the BGHA (11.2±11.7 vs. 7.9±9.1) [t(158.7) = 1.99, p= 0.048]; and the BDHI (33.3±13.4 vs. 29.8±11.7) [t(177.1) = 1.84, p= 0.068]. Mean scores on measures of impulsivity using the total BIS score did not differ between cases and controls. Additionally, depressed suicides showed slightly lower, but significant scores on the TCI reward dependence dimension in comparison to control individuals (14.2±3.9 vs. 15.4±4.5) [t(180.7)= -1.994, p = 0.048].

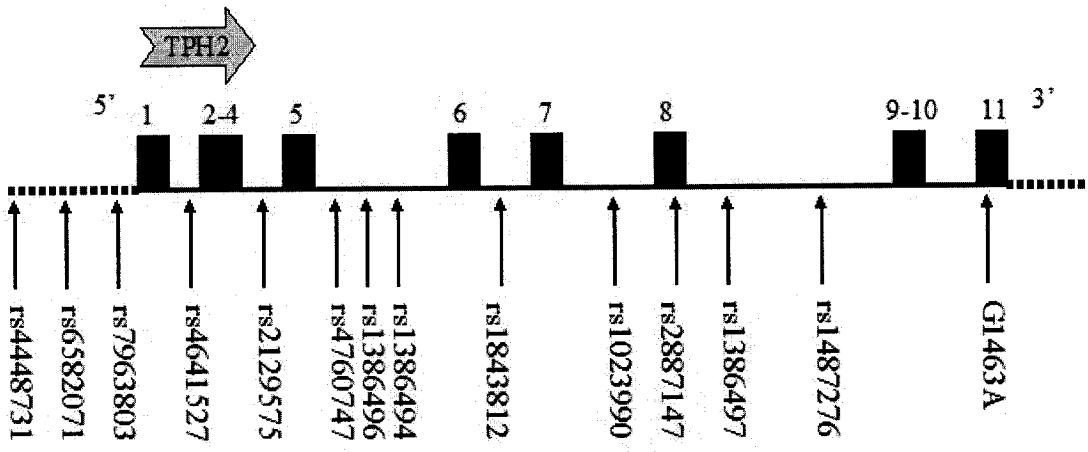
Inter-rater reliability and validity of proxy-based personality assessments

More detailed information on the reliability and validity of the proxy-based assessments are provided elsewhere (Dumais et al. 2005). The estimated kappa coefficients for inter-rater reliability were excellent: MDD, 0.96; alcohol abuse/dependence, 0.98; drug abuse/dependence, 1.0; and cluster B PD, 1.0. The information collected from two different informants on each individual in this study did not differ significantly (p-values ranged from 0.25 to 0.94). Moreover, no significant differences were found between the information gathered from the informant and that obtained from the individual himself in the control group (p-values ranged from 0.67 to 0.98). These results support the validity of proxy-based personality assessments.

Single nucleotide polymorphisms (SNPs) genotyped

We identified 14 SNPs from the publicly available SNP database (dbSNP) of the National Center for Biotechnology Information (NCBI) and from available literature. Selected SNPs were distributed across the TPH2 gene and within the 5'upstream region. These SNPs were chosen based on their location, average estimated heterozygosity and validation status reported in dbSNP. Additionally, we included the SNP previously found in association with major depression and suicide, in separate studies (rs1386494) (Zill et al. 2004c; Zill et al. 2004a), as well as the functional SNP described by Zhang et al (2005), associated to reduced serotonin synthesis in vitro and major depression. Of the 14 SNPs investigated, 9 were intronic (introns 1, 4, 5 [3], 6, 7, and 8 [2]), 2 were exonic (exons 8, and 11—nonsynonymous) and three were in the 5'upstream region of the gene (3.5 kb, 2.5 kb, and 1.3 kb from the transcription start site, respectively) (Figure 1). All SNPs are named according to the identification number given by dbSNP, except for the SNP in exon 11, which is named as G1463A.

Figure 3.1. Schematic representation of the distribution of the genotyped SNPs across the TPH2 gene and its upstream region.



Interrupted lines represent the upstream and downstream region of the TPH2 gene. Exons 1 to 11 are represented as vertical bars, and because of the proximity of exons 2 to 4, as well as 9 and 10, these are represented as one single vertical bar. Small arrows represent the approximate location of each of the SNPs investigated in this study.

Genotyping

Genomic DNA was extracted from blood or frozen brain tissue according to standard procedures (Sambrook et al. 1989). For genotyping the selected SNPs in this study, we used the commercially available SNaPshot method (Applied Biosystems, Foster City, CA, USA), which has been described elsewhere (Pati et al. 2004; Turner et al. 2002). Briefly, it consists in the extension of an unlabeled primer, which ends immediately before the interrogated SNP, by using fluorescently labeled dideoxy nucleotides (ddNTPs). The fluorescently labeled extension primers are then visualized by electrophoresis on an ABI PRISM 3100

Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Initial amplification of the target genomic regions was carried out as previously described by our laboratory (Lopez de Lara et al. 2005). Specificity of the primers designed (Alpha DNA, Montreal, Canada) to TPH2 sequences were verified by a BLAST search using *Ensembl.* The generated data was analyzed using the GeneMapper software v.3.7 (Applied Biosystems), which automatically determines the sample genotypes. All genotypes were determined blind to subject status. Additional information on amplification and extension primer sequences and reactions' conditions can be obtained upon request. The genotype completion rate for all markers ranged from 90% to 99%, except for the SNP G1463A (88%). Of the 14 SNPs genotyped, we found two non-polymorphic SNPs located in exon 8 (rs2887147) and exon 11 (G1463A). Hence, these SNPs were excluded from further analyses. All genotypic distributions in the control group for the SNPs investigated in this study were in conformance with Hardy-Weinberg equilibrium.

Data analysis

Data on demographic (except age) and clinical variables as well as on prevalence of axis I and II psychopathology between groups were compared by Fisher's exact tests (two-tailed). Differences in age and mean scores for each of the behavioral and personality traits questionnaires between groups were compared by two-tailed student's t-tests. Means and standard deviations are reported. The BGHA scores distribution was not normal; however, the student's t-test was still used since it has been shown that this test is robust enough when the normality assumption does not hold (Sawilowsky and Hillman 1992; Sullivan and

D'Agostino 1992). Deviations from Hardy-Weinberg equilibrium (HWE) were examined using an exact test implemented in the program Haploview version 3.2 (Barrett et al. 2005). This software was also used for testing allelic and haplotype associations, as well as determining linkage disequilibrium (LD) between pairs of SNPs. Haploview uses an expectation-maximization (EM) algorithm to estimate haplotype frequencies. Differences in the distribution of genotypic frequencies between groups were tested by chi-square tests for independence. We applied the Bonferroni method for multiple testing corrections. Hierarchical logistic regression analyses were carried out to evaluate the unique contribution of identified risk factors in the prediction of completed suicide, adjusted for each other, and for possible confounders. We also investigated the presence of possible interactions between genetic variants and other suicide risk factors, by logistic regression analyses. Correlations between variables were calculated by Pearson's r. Statistical analyses were carried out using the SPSS package (version 11.0 for Windows; SPSS, Chicago, Illinois). Our alpha risk was set at 5%.

RESULTS

A sample of 114 depressed suicide completers and 145 depressed control individuals was studied. To determine any relationship of the 12 investigated SNPs with depressed suicide victims, we carried out allelic and genotypic association analyses. Our results showed four SNPs significantly associated with depressed suicide cases, two in the TPH2 5' upstream region (at 3.5 and 2.5 kb from the transcription start site, respectively) and two in introns 1 and 8 (Table 2). For the upstream region SNPs, we observed a significant higher frequency of the

T allele of SNP rs4448731 (p=0.003) and G allele of SNP rs6582071 (p=0.004) in the suicide group. As for the intronic SNPs, we found more frequently the G allele of SNP rs4641527 (p <0.001) and the C allele of SNP rs1386497 (p < 0.001) in depressed suicides than control individuals. After multiple testing corrections, these four SNPs remained statistically significant (Bonferroni corrected p-value=0.004, for 12 loci tested). Accordingly, the distribution of genotype frequencies between groups at these four loci was also significantly different (Table 3). We observed a higher frequency of homozygotes for the T allele of SNP rs4448731 in depressed suicides than controls (p=0.002). In addition, homozygotes for the G allele of SNPs rs6582071 and rs4641527 were found more frequently in the suicide group (p=0.02 and p=0.001, respectively). In contrast, homozygotes and heterozygotes for the C allele of SNP rs1386497 were more frequent in depressed suicides than control individuals (p<0.001). Furthermore, the indicated differences in genotype frequencies for these SNPs were significantly greater when genotypes were grouped based on their significant association with suicide. After Bonferroni correction, the results of the analyses on genotypic frequencies were still significant, except for the SNP rs6582071 (Table 3).

Table 3.2. Allelic frequencies for SNPs in the THP2 gene and its upstream region between depressed suicide cases and depressed control individuals

Marker	Location	Position[a]	SNP	Allele Frequency		Chi-Square	P-value
				Depressed suicides	Depressed controls		
rs4448731	Upstream	70615373	C/T	0.37/0.63	0.51/0.49	8.73	0.003*
rs6582071	Upstream	70616379	A/G	0.14/0.86	0.24/0.76	8.13	0.004*
rs7963803	Upstream	70617585	A/C	0.14/0.86	0.09/0.91	3.12	0.08
rs4641527	Intron 1	70620950	G/T	0.88/0.12	0.75/0.25	12.30	<0.001*
rs2129575	Intron 4	70626340	G/T	0.82/0.18	0.76/0.24	2.26	0.13
rs4760747	Intron 5	70632487	A/G	0.14/0.86	0.09/0.91	2.88	0.09
rs1386496	Intron 5	70637057	C/T	0.16/0.84	0.13/0.87	0.94	0.33
rs1386494	Intron 5	70638810	A/G	0.12/0.88	0.12/0.88	0.00	0.99
rs1843812	Intron 6	70653922	A/G	0.19/0.81	0.13/0.87	2.98	0.08
rs1023990	Intron 7	70668514	C/T	0.30/0.70	0.27/0.73	0.39	0.53
rs1386497	Intron 8	70678557	A/C	0.72/0.28	0.85/0.15	14.59	<0.001*
rs1487276	Intron 8	70691326	A/G	0.19/0.81	0.17/0.83	0.51	0.48

[a] Chromosomal position (NCBI)

* Significant after Bonferroni correction for multiple testing (corrected p-value=0.004)

Table 3.3. Genotypic distributions of the four TPH2 SNPs associated with suicide.

Locus	Genotype	Status	
		Depressed Suicide (%)	Depressed Control (%)
rs4448731	C/C	23 (22.5)	40 (27.6)
	C/T	30 (29.4)	67 (46.2)
	T/T[a]	49 (48.0)	38 (26.2)
	T/T	49 (48.0)	38 (26.2)
	C/T, C/C[b]	53 (52.0)	107 (73.8)
rs6582071	A/A	2 (1.9)	10 (7.0)
	A/G	25 (23.8)	49 (34.3)
	G/G[c]	78 (74.3)	84 (58.7)
	G/G	78 (74.3)	84 (58.7)
	A/G, AA[d]	27 (25.7)	59 (41.3)
rs4641527	G/G	85 (80.2)	84 (57.9)
	G/T	16 (15.1)	50 (34.5)
	T/T[e]	5 (4.7)	11 (7.6)
	G/G	85 (80.2)	84 (57.9)
	G/T, TT[f]	21 (19.8)	61 (42.1)
rs1386497	A/A	53 (46.9)	106 (73.6)
	A/C	56 (49.6)	34 (23.6)
	C/C[g]	4 (3.5)	4 (2.8)
	C/C, C/A	60 (53.1)	38 (26.4)
	A/A[h]	53 (46.9)	106 (73.6)

[a] χ^2=13.0, df=2, p=0.002*
[b] χ^2=12.509, df=1, p<0.001*
[c] χ^2=7.697, df=2, p=0.02
[d] χ^2=6.458, df=1, p=0.01
[e] χ^2=14.051, df=2, p=0.001*
[f] χ^2=13.791, df=1, p<0.001*
[g] χ^2=19.590, df=2, p<0.001*
[h] χ^2=19.145, df=1, p<0.001*
* Significant after Bonferroni correction (corrected p-value=0.004)

We determined the LD pattern and haplotype frequencies among groups for the SNPs analyzed in this study. We identified two blocks of LD in our sample. The first block was composed of SNPs: rs4448731, rs6582071, rs7963803, rs4641527 and rs2129575. D' values ranged between 0.50 and 1.0, except for SNPs rs7963803 and rs4641527, where the D' value was of 0.43. The second block consisted of SNPs: rs1386496, rs1386494, rs1843812, rs1023990, rs1386497 and rs1487276. In this block, D' values ranged between 0.55 and 1.0. Haplotype frequencies were estimated and association tests performed on the mentioned blocks of LD. Only haplotypes with frequencies above 1% were analyzed.

Our results (Table 4) for the first block initially revealed four haplotypes associated with suicide; however, after correction for multiple testing (Bonferroni corrected p-value=0.003, for 15 haplotypes tested), there were only two haplotypes that remained significant [TGCGG (p=0.002) and TGAGG (p=0.001)]. These two haplotypes contained the previously two SNP alleles in the 5'upstream region and the SNP allele in intron 1 associated with suicide completion (rs4448731-T, rs6582071-G, and rs4641527-G, respectively). Analysis of the second block of LD showed two haplotypes initially associated with depressed suicides (TGGTCG and TGGCCG), which contained the suicide associated SNP allele in intron 8 (rs1386497-C). However, these haplotypes were not significant after multiple testing corrections by the Bonferroni method.

Table 3.4. Haplotype frequencies between depressed suicide cases and depressed control individuals for SNPs in linkage disequilibrium in the first and second block identified

Haplotype[a]	Frequency		Chi-square statistic	P-value
	Depressed suicides	Depressed controls		
Block 1				
CGCGG	0.34	0.50	12.55	<0.001
TGCGG	0.36	0.23	9.87	0.002*
TACTT	0.05	0.14	11.54	0.001
TAATT	0.02	0.07	6.25	0.01
TGCGT	0.04	0.01	7.34	0.01
TGAGG	0.05	0.003	11.39	0.001*
TACTG	0.02	0.02	0.001	0.98
CGAGG	0.03	0.01	4.43	0.03
TGCTT	0.02	0.01	0.36	0.55
Block2				
TGGTAG	0.46	0.54	3.209	0.07
TGGCAG	0.21	0.26	1.462	0.23
CAATCA	0.11	0.11	0.030	0.86
TGGTAA	0.03	0.05	1.351	0.24
TGGTCG	0.04	0.004	7.879	0.005
TGGCCG	0.03	0.004	6.775	0.009

[a] Haplotypes with frequencies above 1% were analyzed
*Significant haplotypes more frequently found in the group of depressed suicides than control individuals after Bonferroni correction for multiple testing (corrected p-value=0.003)

We then asked whether the two upstream region (rs4448731 and rs6582071) and two intronic SNPs (rs4641527 and rs1386497) associated with depressed suicides could increase the susceptibility to suicide through affecting levels of IABs, and temperament and character dimensions. To do so, we

compared the mean scores of the corresponding questionnaires according to genotypic variation at these four SNPs in the total sample (Table 5). We did not find any significant differences on levels of aggression or impulsivity measured by BGHA, BDHI and BIS scales for any of the individuals with the associated genotypes. However, homozygotes for the G allele of SNP rs4641527 were related to lower levels of reward dependence compared to heterozygotes and homozygotes for the non-associated allele ($p=0.04$).

We proceeded to investigate the unique contribution of risk factors that were individually identified in this study. To do so, we carried out a hierarchical logistic regression analysis, which included 7 steps. First, because of the high correlation between SNPs rs6582071 (upstream region) and rs4641527 (intron 1) ($r=0.85$, $p<0.001$), we decided to include in the analysis the SNP rs4641527, which had showed a stronger association with suicide, compared to the SNPrs6582071. Similarly, current and lifetime prevalence of substance use disorders showed a high correlation ($r= 0.63$, $p<0.001$); therefore, we created a variable called history of substance use disorders, which included both. All categorical variables included in the regression analyses were coded as 0 or 1 indicating absence or presence of a particular risk factor. With regard to the variable sex, females were coded as 0 and males as 1.

In the first step of the hierarchical logistic regression, we controlled for possible confounders by entering age and sex in the model. The order in which other important variables were entered in consecutive steps was as follows: step 2, family history of suicide; step 3, BGHA and BDHI scores (measures of aggression); step 4, BIS scores (measures of impulsivity); step 5, reward

dependence scores (TCI temperament dimension); step 6, cluster B PD and history of substance use disorders; and step 7, TPH2 genetic variants in the upstream region (rs4448731), intron 1 (rs4641527) and intron 8 (1386497). The results of this model (Table 6), which correctly predicted 78% of the suicides in MDD and explained 41% of the variance, revealed the upstream and intron 1 TPH2 genetic variants, as well as a family history of suicide and cluster B PD, as significant and independent predictors of suicide. It is noteworthy that once accounting for the effect of other risk factors, the SNP in intron 8 was no longer significant. Measures of aggression added significantly to the model, particularly BGHA scores that were significant at this step (B=0.05, p=0.04). However, when cluster B PD and substance use disorders were added, BGHA scores were no longer significant (Table 6). Age, sex and the measures of impulsivity (BIS scores) and reward dependence did not contribute to the predictive value of the model. No interactions were found between genetic variation and levels of IABs or cluster B personality disorders (data not shown).

Table 3.5. BGHA, BDHI, BIS and TCI reward dependence measures in the total sample according to genotypic variation at the four TPH2 SNPs associated with suicide.

SNPs[a]	Questionnaire Mean±SD (*n*)	T-test statistic	df	P-value
	BGHA			
rs4448731- T/T	9.5±11.3 (63)	0.31	157	0.76
C/T, C/C	10.0±10.6 (96)			
rs6582071- G/G	9.5±11.0 (108)	0.13	158	0.89
A/A, A/G	9.8±10.3 (52)			
rs4641527- G/G	9.9±11.0 (114)	-0.43	162	0.67
G/T, T/T	9.1±10.0 (50)			
rs1386497- A/C, C/C	8.3±10.0 (72)	1.36	165	0.18
A/A	10.6±11.1 (95)			
	BDHI			
rs4448731- T/T	30.3±11.6 (61)	0.90	171	0.37
C/T, CC	32.2±13.2 (112)			
rs6582071- G/G	31.8±13.0 (119)	-0.25	171	0.80
A/A, A/G	31.3±12.5 (54)			
rs4641527- G/G	32.1±12.7 (126)	-0.47	174	0.64
G/T, TT	31.2±12.4 (50)			
rs1386497- A/C, CC	29.5±12.3 (76)	1.79	177	0.07
A/A	32.9±12.7 (103)			
	BIS			
rs4448731- T/T	65.9±13.4 (67)	0.44	183	0.66
C/T, CC	66.8±13.3 (118)			
rs6582071- G/G	66.5±13.1 (125)	-0.35	184	0.72
A/A, A/G	65.8±14.4 (61)			
rs4641527- G/G	66.7±13.2 (133)	-0.13	187	0.90
G/T, TT	66.5±14.0 (56)			
rs1386497- A/C, CC	65.4±13.5 (79)	0.62	191	0.53
A/A	66.7±13.6 (114)			
	Reward dependence			
rs4448731- T/T	15.0±4.2 (62)	-0.09	176	0.92
C/T, CC	14.9±4.1 (116)			
rs6582071- G/G	14.7±4.1 (123)	1.13	177	0.26
A/A, A/G	15.4±4.4 (56)			
rs4641527- G/G	14.4±4.0 (131)	2.08	180	0.04*
G/T, TT	15.8±4.5 (51)			
rs1386497- A/C, CC	14.3±4.6 (78)	1.38	184	0.17
A/A	15.2±3.9 (108)			

[a] SNPs previously found in association with suicide are shown.
BGHA, Brown-Goodwin Assessment of Lifetime History of Aggression; BDHI, Buss-Durkee Hostility Inventory; BIS, Barrat's Impulsivity Scale; TCI, Temperament and Character Inventory. * Significant at the 0.05 level

Table 3.6. Hierarchical logistic regression analyses predicting suicide completion (n =124)

Step	Variable	B [a]	SE	Wald	P-value	OR	95% CI	
1	Age	-0.19	0.02	1.01	0.32	0.98		
	Sex	-0.24	0.69	0.001	0.97	0.98		
2	Family history of suicide	1.47*	0.69	4.50	0.03	4.35	1.12	16.95
3	BGHA	0.01	0.03	0.19	0.67	1.01		
	BDHI	-0.003	0.02	0.02	0.90	1.00		
4	BIS	-0.01	0.02	0.08	0.77	0.99		
5	Reward dependence	-0.05	0.06	0.73	0.39	0.95		
6	ClusterB PD	2.05*	0.90	5.20	0.02	7.75	1.33	45.12
	History of substance use disorders	0.86	0.51	2.89	0.09	2.37	0.88	6.41
7	Upstream variant [b]	1.66**	0.58	8.18	0.004	5.26	1.69	16.44
	Intron 2 variant [c]	1.65**	0.57	8.33	0.004	5.22	1.70	16.04
	Intron 8 variant [d]	0.33	0.53	0.40	0.53	1.39		

B, unstandardized logistic regression coefficient; Wald, Wald statistic; OR, odds ratio; CI, confidence interval.

[a] Unstandardized logistic regression coefficients (B) from the last step in the analysis are shown.

[b] SNP rs4448731

[c] SNP rs4641527

[d] SNP rs1386497

* Statistically significant at the 0.05 level

** Statistically significant at the 0.01 level

DISCUSSION

In this study, we have shown that genetic variants in the TPH2 gene and 5'upstream region may predispose, at least partially, to suicide completion in major depression. However, this predisposition does not appear to be mediated by increased levels of IABs. Moreover, TPH2 genetic variation, cluster B PD, and family history of suicide are significant and independent predictors of suicide when adjusted for each other and other risk factors.

Evidence for the implication of TPH2 gene variants in the susceptibility to suicide came from our results showing a significant association of two SNPs in the TPH2 5'upstream region (rs4448731, and rs6582071) and two SNPs in introns 1 (rs4641527) and 8 (rs1386497) with depressed suicide cases (Table 2). More specifically, we observed a significant higher frequency of alleles T, G, G and C of SNPs rs4448731, rs6582071, rs4641527 and rs1386497, respectively, in depressed suicide completers. In addition, our results from the genotypes' analyses showed that homozygotes for the T allele of SNP rs4448731, as well as the G SNPs rs6582071 and rs4641527 were more often found in suicide cases. Nevertheless, after Bonferroni correction, the genotype analysis on the SNP rs6582071 was no longer significant. With regard to the SNP rs1386497, carriers of the C allele were associated with suicide completion (Table 3). Further support came from the haplotype analysis where two significant haploytpes in the first block of LD (Table 4), containing the associated SNP alleles in the upstream region and intron 1, were more frequently found in the suicide group.

In comparison with the only previous TPH2 study conducted in suicide completers, at least the only one that we are aware of (Zill et al. 2004c), none of

the SNPs examined herein were investigated in that earlier study, except for a single SNP in intron 5 (rs1386494), for which they found an association with suicide. We deliberately included this SNP in our study, but we could not replicate this association. It is possible that the association found was not related to suicide but to comorbid psychopathology, since the study was carried out in unselected suicide victims. This explanation is further supported by the fact that the same intronic SNP was linked to MDD in another study by the same group (Zill et al. 2004a). Alternatively, true biological differences between populations may account for this lack of replication. Whereas in the previous study SNPs between exons 5 and 7 were included, in the present study a larger region of the gene was covered by including SNPs distributed across the gene and upstream region. The previously identified TPH2 functional SNP (G1463A) in exon 11, which was associated with a reduced serotonin synthesis and major depression (Zhang et al. 2005), was not polymorphic in our sample. This result agrees with the observed lack of variability for this SNP in other studies (Garriock et al. 2005; Walitza et al. 2005).

Although it is reasonable to hypothesize that these genetic variants could affect levels of impulsivity and aggression, and thus, increase the vulnerability to suicide; we did not find any significant differences in any of these measures between the SNPs genotypes found overrepresented in suicide and those that were not. These results suggest that the effect of the TPH2 variants on suicide is not mediated by increased levels of IABs. Nevertheless, it is possible that other TPH2 variants, which were not examined in this study, are the ones involved in the regulation of IABs. It is also reasonable to think that we lacked power to

detect such effects, especially taking into account that the assessment of these behaviors and personality traits were carried out by means of proxy-based interviews (discussed further on) and that this information was not available for all individuals. Unfortunately, no studies in humans have been previously carried out looking at the TPH2 genetic effect on IABs. The only evidence for a possible regulatory role of TPH2 genetic variation comes from a study on mice, which found a functional polymorphism that was shown to affect the TPH activity in the brain and the intensity of intermale aggression (Kulikov et al. 2005).

Our results showing a significant association between homozygotes for the G allele of SNP rs4641527 and lower scores of the reward dependence TCI dimension (Table 5), suggests that this particular SNP may increase the vulnerability to suicide by limiting the individual's beneficial social affiliations (Cloninger et al. 1994).

Finally, the finding of a strong and unique contribution of the TPH2 upstream region (rs4448731) and intron 1 (rs4641527) SNPs, while adjusting for the effect of each other and other risk factors and confounders, adds further evidence to the involvement of these variants in suicide (Table 6). Individuals that are homozygotes for the T and G allele of the upstream and intron 1 variants, respectively, had approximately a 5-fold increase in the risk of suicide than those individuals with different genetic variation. Cluster B PD and family history of suicide—which have been previously identified as risk factors for suicidal behavior in MDD (Dumais et al. 2005; Roy, 1993)—also appear to significantly contribute to a higher risk of suicide. A nearly 8-fold increase in suicide risk was found in individuals with cluster B PD, while an approximately 4-fold increase

was related to having a family history of suicide. Our analysis also indicates that measures of aggression, in particular BGHA scores, seem to contribute to the prediction of suicide when cluster B PD are not included in the predictive model. The reason for this may be that the contribution of cluster B PD is greater than that of BGHA scores, which is reasonable, since cluster B PD covers a wide range of clinical features, including aggression and impulsivity. Although suicide is the result of a complex interplay of genetic and environmental factors, we did not find any interactions between genetic variants and measures of IABs or cluster B PD. However, this negative result may be accounted for by a lack of sufficient power to detect possible interactions, mainly because of 1) the observed unequal proportion of individuals having the hypothesized risk factor versus those without it (Frazier et al. 2004), and 2) the relatively small sample included in these analyses due to the lack of complete behavioral and psychiatric information for all individuals.

In this study, we have been able to identify two TPH2 SNPs in the upstream region and intron 1 as strong predictors of suicide in MDD. Both SNPs are found in the same block of LD. However, the functionality of these SNPs is unknown. Whereas it is possible that the upstream SNP—located at 3.5 kb from the transcription start site—may be a causal one, there is also the possibility that these SNPs are in LD with other variants in this gene or other genes that are the ones responsible for the association with suicide. The latter is especially true for intronic SNPs, which are spliced out during transcription. However, recent studies have suggested that 1) regulatory elements necessary for correct gene expression can be found as much as 1 Mb either upstream or downstream from the

transcription unit; and 2) some of these elements can also be found within introns (Kleinjan and van Heyningen 2005). Further work in this field is required in order to increase our understanding of the mechanisms involved in the control of gene expression, and the role of genetic variants, especially those located in introns. Nonetheless, it is interesting to note that recent studies have found that variants in the upstream regulatory region of the TPH2 are implicated in the reactivity of the amygdala—an important brain region in emotional regulation—and have further supported the role of the TPH2 gene in disorders characterized by emotional dysregulation (Brown et al. 2005; Canli et al. 2005).

The limitations of this study, apart from the ones mentioned above, are those typically related to case-control association studies (Lander and Schork 1994; Risch and Merikangas 1996). Arguably, the most important methodological bias leading to false positive results is the presence of population stratification. However, our sample consisted in its majority of individuals of French-Canadian origin (98%), which is an isolated and young population (approximately 12 generations) with a well-known founder effect (Heyer and Tremblay 1995). Because the French-Canadian population remained culturally isolated until recently (early 1900's), selecting individuals whose 4 grandparents are of French-Canadian origin greatly improves our chances of including individuals with true French-Canadian ancestry. In addition, by following the same procedure in both groups, we are more likely to select a group where the total genetic variance involved in suicide predisposition is likely reduced, and population stratification effects are less likely to occur.

The use of proxy-based assessments represents another limitation in this study. However, the validity of such procedure, particularly with regard to the use of observable behaviors, has been well-demonstrated elsewhere (Conner et al. 2001; Schneider et al. 2004). The main concern appears to be related to the level of their sensitivity (Ernst et al. 2004). Nevertheless, by using proxy-based techniques in both groups, this possible bias was controlled for.

In summary, our results indicate that variants in the TPH2 gene and upstream region may be implicated in the predisposition to suicide completion in major depression. However, this predisposition does not seem to be mediated by impulsive-aggressive behaviors. Furthermore, TPH2 genetic variation, cluster B personality disorders and family history of suicide significantly and independently increased the risk of suicide completion in depressed individuals, while adjusting for the effects of other suicide risk factors and confounders. Further studies in larger and independent homogeneous samples are required in order to have stronger evidence of the implication of these variants in suicide, as well as to detect possible interactions between genetic variants and other risk factors. It is also important that future studies control for associated psychopathology, so that the effects attributable to suicide can be separated.

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Chapter 4

Discussion and conclusions

SUMMARY OF RESULTS AND DISCUSSION

The main purpose of this research was to investigate molecular genetic factors that might contribute to suicide susceptibility in major depressive disorder—a condition which on its own is already a well known risk factor for suicide. Specifically, the serotonin transporter (5-HTT) and the tryptophan hydroxylase 2 (TPH2) genes were investigated. These are genes that codify proteins whose function on the regulation of serotonergic activity is crucial. Additionally, their relationship to possible intermediate phenotypes of suicide, such as impulsive-aggressive behaviors, was examined. Finally, predictors of suicide were investigated considering the genetic variation at these genes, as well as other clinical and behavioral risk factors.

A brief summary of the overall results of this work and their implications follow. With regard to the 5-HTT gene, two variants that have shown transcriptional regulatory activity were studied (Fiskerstrand et al. 1999; Heils et al. 1996; MacKenzie and Quinn 1999). There was no association between the more commonly explored promoter variant (5-HTTLPR) and suicide; however, the 10 allele of the intron 2 variant (STin2) was found overrepresented in depressed suicide completers. This finding was in accordance with one of the very few previous studies on the STin2 variant, which found a trend for an association of the 10 allele with unselected suicide completers (Hranilovic et al. 2003). These

results indicate that the STin2 variant, rather than the one located in the promoter, may play a role in the predisposition to suicide while controlling for major depression. This provides further support for studying suicide cases and controls within a specific diagnostic category, a strategy that allows separating effects attributable to suicide from those linked to the associated psychopathology. This type of design has not been used in most of the genetic studies investigating the 5-HTT gene. Although the exact mechanism by which the 5-HTT gene ultimately predisposes to suicide is unknown, it might be related to alterations in its transcriptional regulation, which would result in a deficient serotonergic neurotransmission. No effect of this variant was observed on levels of impulsive-aggressive behaviors. Interestingly, the STin2 variant was found to significantly increase the risk of suicide completion, while adjusting for other risk factors, but only in individuals with a previous family history of suicide. Thus, suggesting that the STin2 variant may act as a moderator of the relationship between having a family history of suicide and ultimately committing suicide. In addition, cluster B personality disorders and last-6 months substance use disorders were also found as independent contributors to suicide vulnerability.

Regarding the TPH2 gene, out of 14 genetic variants examined in the upstream region and across the gene, there were four allelic variants that were significantly associated with suicide completion. In contrast with the 5-HTT gene variants, and perhaps due to its more recent discovery, there was no available information on the functional role of any of the 14 variants studied, except for one (Zhang et al. 2005) that was not polymorphic in the sample studied herein. Among the four genetic variants identified, two were located in the upstream

region of the gene and two were intronic. These findings, like those from the 5-HTT gene studies, support a role for TPH2 genetic variants in the susceptibility for suicide in depressed individuals. Since the associated genetic variants have not been investigated in previous studies in relation to suicide, further studies would be necessary to confirm their putative role in this complex behavior. These variants might be either functional—particularly in the case of the upstream variants—or in linkage disequilibrium with other variants which are in fact causal or linked to the causal one. The former is plausible since a regulatory activity for upstream variants on the reactivity of the amygdala, which is an important structure in emotional regulation, has been reported (Brown et al. 2005; Canli et al. 2005). There was no observable relationship between these variants and exhibited levels of impulsive-aggressive behaviors. Of interest, two of the four TPH2 genetic variants, one in the upstream region and one intronic, had a unique contribution in the risk of suicide while adjusting for the other genetic variants and other risk factors. Additionally, cluster B personality disorder was a strong predictor of suicide, which was consistent with our aforementioned findings studying the 5-HTT gene, along with a family history of suicide.

Taken together, these findings implicate genetic variation at both genes in the susceptibility to suicide that seems to be independent of that related to depressive psychopathology. However, they do not provide evidence that this susceptibility may be mediated through impulsive-aggressive behaviors exhibited by the suicidal individual. Also, it appears that certain 5-HTT and TPH2 gene variants have a significant contribution to the vulnerability for suicide, either in combination with other factors (i.e. the interaction between 5-HTT genetic variant

and family history of suicide) or independently. However, some considerations must be taken into account for an adequate interpretation of these findings and are briefly described here.

Candidate-gene association studies are a powerful, yet potentially limited approach to find genetic variants that have a small genetic effect (Risch and Merikangas 1996), which is generally expected for complex traits such as suicide. The complexity of this resides in the fact that they are not the result of classic Mendelian recessive or dominant inheritance that can be attributed to a single gene. Instead, they are determined by a number of genes with different effect sizes and their interactions with each other and multiple environmental factors (Lander and Schork 1994). However, this approach has received important criticism regarding the lack of replication of significant findings by subsequent association studies. There are several reasons that may explain the inconsistent reproducibility across studies. These are related to variability in the design of association studies, which might include different populations or phenotypes that are difficult to define—an extremely important issue in major psychiatric disorders (Tabor et al. 2002; Tsuang and Faraone 1990). Also, the size of the sample has been put forward as a determinant factor in the successful finding of genetic variants with small or moderate effect sizes (Lohmueller et al. 2003). In fact, the sample studied herein consisted of both suicides and controls with a diagnosis of major depressive disorder exclusively, limiting the size of our sample. Nevertheless, our total sample size was approximately 250 subjects, which is above the minimum sample size that has been suggested for genetic association studies (Conneally and Sparkes 1998). As such, although some genetic variants

were actually found in association with suicide, other possible effects, such as those on impulsive-aggressive behaviors might have not been detected.

Since the population studied was mostly of French-Canadian origin, which is a young and isolated population, the limitation of genetic association studies regarding population stratification was greatly reduced (for further discussion see Chapters 2 and 3). Population stratification is a main concern in association studies of large mixed populations because of the possibility of finding an association between a trait and allelic variant that might be related to a specific ethnic group, and is thus not applicable to the whole population studied (Lander and Schork 1994). On the other hand, these results on the relatively genetically isolated French-Canadian population may not be generalized to other populations; therefore, further studies on preferably homogeneous samples are necessary to replicate the findings of this research work. All the limitations noted above should be taken into account in future studies intended to replicate positive findings.

In the studies presented here, impulsive-aggressive behaviors were tested as intermediate phenotypes of the relationship between genetic variability and suicide since previous studies have shown higher levels of impulsivity and aggression as risk factors for suicidal behavior (see Chapter 1). Although no support for an effect of genetic variants on levels of these traits were found, this should not dismiss future studies from using these traits and others as intermediate phenotypes of suicide. In this regard, as it was pointed out in the relevant sections of each of the studies in this thesis (Chapter 2 and 3), measures of impulsive-aggressive behaviors were obtained by proxy-based assessments which may have affected the power to detect genetic effects on these behaviors. Additionally, and

more importantly, the lack of complete information for some individuals within the sample, and thus their exclusion from the analyses, led to a further reduction of the sample size.

It has been argued that since personality traits are phenotypically less complex than, for example, suicidal behavior, they might be easier to define and characterize. Additionally they might also be more genetically homogeneous (Baud, 2005). Because they are more proximal to the genetic substrate, it has been suggested that there may be fewer genes and factors influencing them (Schork, 1997), thus facilitating the identification of related genetic factors. However, impulsivity is considered to be heterogeneous, and thus, in order to achieve a good characterization of this trait, laboratory investigations including psychophysiological measures and/or cognitive assessments, in addition to self-report questionnaires have been proposed for further studies (Baud, 2005). These intermediate phenotypes have also been called endophenotypes, although the latter is more specifically used for heritable components. An endophenotype may be any neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological measurable component of certain disease or behavior. Using endophenotypes may help to decompose suicide into the different components that actually define it; thus leading to more straightforward genetic analyses (Gottesman and Gould 2003).

Cases and controls in these studies were matched by age and sex; however, the proportion of males in both groups was much higher than that of females. Given the known differences in suicide risk related to gender (Blair-West et al. 1999), the results derived from these studies may reflect suicide susceptibility

linked mostly to male, rather than female suicide completers. Also, because of these unequal proportions, no specific effects on gender could be investigated. Finally, when the word "predictor" is used, it must be interpreted from a statistical point of view, and not from a causal one. In addition, the analyses to find predictors of suicide involved a relatively small sample which might have affected the ability to find other important interactions between genetic and behavioral risk factors.

There are some important issues that were raised from this research work and that should be addressed in future studies in order to have a better understanding of the contribution of these genes to suicide vulnerability.

The suicide and control individuals in these studies were matched according to the presence of major depression; however, it would be interesting to see if the results obtained are the same once they are matched based on the course of the illness. In other words, it remains to be known whether there are any differences regarding suicide genetic risk factors when individuals are characterized by a lifetime prevalence of major depression compared to those with a much more recent prevalence.

Whereas a functional activity has been demonstrated for the two 5-HTT gene variants studied herein, no functionality has been attributed to the TPH2 gene variants that were related to suicide in the present study. On the basis of previous studies, it would be interesting to test the effect of these TPH2 genetic variants—particularly the ones that are more likely to have a functional role—in

the regulation of the reactivity of important brain structures in suicide such as the amygdala.

It would be interesting to investigate differences in the expression of these genes in relevant brain regions of suicide completers with the associated genetic variants in comparison to control individuals with different variants at these loci. Furthermore, quantification of these gene products in suicide victims with the implicated genetic variation would also prove to be valuable. However, before undertaking these types of studies aimed to evaluate their functionality, it would be appropriate to first confirm their association with suicide by replicating these findings in independent samples.

Regarding the TPH2 gene, future studies could take advantage of the information that has been generated in recent years by the HapMap project to select additional SNPs that tag common genetic variants in the TPH2 gene for further analyses of association with suicide. This approach would maximize the efficiency in the laboratory and minimize loss of information (Altshuler et al. 2005). Much of the HapMap information that is now available was not at the time when our SNPs were selected, and thus, the approach based on the selection of tag SNPs could not be done in the study presented here. However, since one of our SNPs found to be associated with suicide has been genotyped also in the HapMap samples, it would be feasible to examine whether or not there are other SNPs that are well captured by this single SNP, and identify among those variants, the ones in the coding regions that might have a relevant role in suicide for further functional tests. However, it must also be taken into account that if less common

variants are the ones implicated in suicide; these might not be detected by this approach (Altshuler et al. 2005).

With regard to the investigation of the influence of these genetic variants on impulsive-aggressive behaviors, a good strategy to improve the detection of these effects would be to divide the sample studied according to age. Since it is known that younger suicides have higher levels of impulsive-aggressive behaviors, the power to detect them in a uniformly younger sample will be improved, assuming there is a real effect of these variants. Alternatively, we could select the most impulsive and aggressive individuals and then investigate the influence of these genetic variants on these extreme intermediary phenotypes.

Finally, considering that the suicide phenotype is affected by multiple genes, and that candidate-gene association studies are able to identify only a few genes, alternative genetic approaches that screen multiple genes at the same time may be more helpful to have a more comprehensive view of the genes implicated in suicide and thus, a better understanding of the neurobiology of suicide. In this regard, genome-wide association studies as well as microarray gene expression studies are promising approaches.

CONCLUSIONS

The study of the vulnerability to suicide represents a great research challenge because of the multiple genetic and environmental factors—as well as their interactions—that are believed to play a role in its etiology. Genetic factors may be involved in suicide susceptibility; however, no consistent and strong evidence implicating a specific gene or set of genes in suicide completion has yet

been found. Further research is necessary to identify genetic factors predisposing to suicide that may shed light on the mechanisms underlying this complex trait.

Overall, the studies presented in this thesis support the role of serotonergic genes in the predisposition to suicide, specifically the 5-HTT and TPH2 genes. This work has showed that the less studied intronic 5-HTT gene variant may provide important insight into the mechanisms responsible for suicide, and thus it should be included in future studies. In addition, the extensively studied promoter variant should be examined again, but in studies whose design allows the separation of suicide susceptibility factors from those of related psychopathology. This would help to clarify the association claimed in previous studies that have not followed this methodology. The study on the TPH2 gene adds strong evidence for the implication of this gene in suicide, and thus encourages further studies involving this promising gene, preferably in studies that control for confounders, as mentioned above. As such, the main contribution of these studies is related to the finding of serotonergic gene variants involved in the susceptibility to suicide while controlling for an important confounder, namely major depressive disorder.

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Appendix

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