DISSERTATIONES NEUROSCIENTIAE UNIVERSITATIS TARTUENSIS

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ANNE MUST

Studies on molecular genetics of male completed suicide in Estonian population

TARTU UNIVERSITY
PRESS

Department of Physiology, University of Tartu, Tartu, Estonia

Dissertation is accepted for the commencement of the degree of *doctor philosophiae* in Neuroscience on April 23, 2009, by the Council of the Commencement of Doctoral Degree in Neuroscience

Supervisors: Eero Vasar, MD, PhD, Professor, Institute of Physiology, University of Tartu

Aavo Lang, MD, PhD, Associate Professor, Institute of Physiology, University of Tartu

Sulev Kõks, MD, PhD, Professor, Institute of Physiology, University of Tartu

Reviewers: Maido Remm, PhD, Professor, Institute of Molecular and Cell Biology, University of Tartu

Katrin Õunap, MD, PhD, Associate Professor, Department of Pediatrics, University of Tartu

Opponent: Björn Appelberg, MD, PhD, Professor, Department of Psychiatry, University of Helsinki

Commencement: June 10, 2009

Publication of this dissertation is granted by the University of Tartu

ISSN 1024–6479
ISBN 978–9949–19–123–9 (trükis)
ISBN 978–9949–19–124–6 (PDF)

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimus nr. 172

Me teeme, teeme ümber oma elu
üht hiigelmüüri inimtihedat.
Ka pikemad ei küüni üle ääre.

Kui mõni sinnapoole väljalangend kivi
on lahkumisel ruumi jätnud piluks,
siis see, kes juhtus välja nägema
on äranähtu nimetanud iluks.
Ta samal hetkel ise läbi nähtud

ja pole kohta, kus võiks tunda häbi ta.
Suur saladus saab tema ainueluks,
tal iseennast au on läbida.

Juhan Viiding (1948–1995)

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following publications:

I. **Must A,** Tasa G, Lang A, Vasar E, Kõks S, Maron E, Väli M (2009) Variation in tryptophan hydroxylase-2 gene is not associated to male completed suicide in Estonian population. Neuroscience Letters 453: 112–114. Epub 2009 Feb 10.

II. **Must A**, Kõks S, Vasar E, Tasa G, Lang A, Maron E, Väli M (2009) Common variations in 4p locus are related to male completed suicide. Neuromolecular Medicine 11(1): 13–9. Epub 2008 Dec 25.

III. **Must A,** Tasa G, Lang A, Vasar E, Kõks S, Maron E, Väli M (2008) Association of limbic system-associated membrane protein (LSAMP) to male completed suicide. BMC Medical Genetics 9: 34.

Author's contribution:

I. The author participated in extraction of DNA, designed the SNP assay, performed genotyping, carried out statistical analysis and wrote the manuscript.

II. The author participated in extraction of DNA, performed the genotyping, carried out statistical analysis and wrote the manuscript.

III. The author participated in extraction of DNA, designed the SNP assay, performed genotyping, carried out statistical analysis and wrote the manuscript.

ABBREVIATIONS

3'-UTR	three prime untranslated region
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine = serotonin
5-HTT	serotonin transporter
AC	adenylyl cyclase
ACTH	adrenocorticotropic hormone = corticotropin
Akt	protein kinase B
AMPH	alpha-methylphenethylamine = amphetamine
AMPK	5' adenosine monophosphate-activated protein kinase
ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factor
bp	base pair
cAMP	cyclic adenosine monophosphate
CCK	cholecystokinin
CNS	central nervous system
COMT	catechol-O-methyltransferase
CREB	cyclic adenosine monophosphate response element binding (protein)
CRH	corticotropin releasing hormone = corticotropin releasing factor = corticoliberin
CSF	cerebrospinal fluid
DA	dopamine
DAG	diacylglycerol
DAT	dopamine transporter
DBH	dopamine beta-hydroxylase
DDC	dopa decarboxylase, aromatic L-amino acid decarboxylase
DIDMOAD	diabetes insipidus, diabetes mellitus, optic atrophy, deafness = Wolfram syndrome
DNA	deoxyribonucleic acid
DOPAC	dihydroxyphenylacetic acid
DST	dexamethasone suppression test
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
EVC	Ellis-van Creveld syndrome protein
EVC	Ellis-van Creveld syndrome gene
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
GDP	guanosine diphosphate
GLUT4	insulin-regulated glucose transporter, isoform 4
GPRC	G-protein coupled receptor
GTP	guanosine triphosphate
HD	Huntington's disease
HPA axis	hypothalamus-pituitary-adrenal axis

HTT	huntingtin protein
HTT	huntingtin gene
HVA	homovanillic acid
ICD	International Classification of Diseases
IDO	indoleamine-2,3-dioxygenase
Ihh	Indian hedgehog protein in mouse
IL-3	interleukin-3
IL-4	interleukin-4
IP3	inositol triphosphate
kb	kilo base pairs = 1000 bp
LC	locus coeruleus
LPR	length polymorphism in the promoter region
LSAMP	limbic system-associated membrane protein
LSAMP	limbic system-associated membrane protein gene
Lsamp	limbic system-associated membrane protein gene in mouse
MAO	monoamine oxidase
MAP kinase	mitogen-activated protein kinase
MHPG	3-methoxy-4-hydroxyphenylglycol
mRNA	messenger ribonucleic acid
NE	noradrenaline = norepinephrine
NGF	nerve growth factor
NT-3	neurotrophin type 3
NT-4/5	neurotrophin type 4/5
PCR	polymerase chain reaction
PFC	prefrontal cortex
PI	phosphoinositide
PI 3-K	phosphoinositide 3-kinase
PKA	protein kinase A
PKC	protein kinase C
Shh	sonic hedgehog protein in mouse
SNP	single nucleotide polymorphism
TBC1D1	tre-2/USP6, BUB2, cdc16 domain family member 1 protein
	TBC1D1 TBC1D1 gene
TH	tyrosine hydroxylase
Th2	type-2 T-helper cytokine
TPH	tryptophan hydroxylase protein
Tph	tryptophan hydroxylase protein in mouse
TPH2	tryptophan hydroxylase type 2 gene
UPR	unfolded protein response
VNTR	variable number of tandem repeats
WFS1	Wolfram syndrome 1 (wolframin) gene
WHO	World Health Organisation

1. INTRODUCTION

Despite extensive research as well as educational efforts and strategies in early detection and prevention, self-inflicted death still ranks as one of the principal causes of premature mortality, thus remaining a major public health concern worldwide.

The fact that taking one's own life is perceived as one of the most serious sins in many religious communities indicates that the social problem of suicide has haunted humankind throughout history. However, some forms of self-inflicted death, such as honor suicide and martyrdom, are considered socially acceptable within certain subcultures. Whether to view the suicide bombers or cult-related mass suicides as a subgroup of the general suicide population is currently unclear.

Cross-cultural differences in religious beliefs can partly explain the wide geographical variation in suicide occurrence. However, religious factors are not likely to explain the 10-fold differences in suicide rates across Europe, which is considered one of the most secular regions of the world (Neeleman and Lewis, 1999).

In addition to geography, there exists a considerable temporal variability in suicide rates, both seasonal as well as over years. In contrast to the common belief that people commit suicide most often during the dark winter months, sociologist Emilé Durkheim claimed a century ago that the incidence of self-killing was highest during spring and early summer and lowest during winter (Durkheim, 1970). The origin of the spring peak in suicide remains poorly understood. While various meteorological factors, such as temperature or changes in photoperiods and light intensities, most often are proposed to contribute to seasonal suicide rates, other authors argue that the spring suicide peak precedes the peak photoperiod but coincides with the peak in aeroallergens (Postolache et al., 2008). Thus, a link between immune system and suicide has been suggested.

Over years, suicide rates tend to reflect macroeconomic fluctuations in societies. Dissolution of the Soviet Union made the suicide rates in former member states increase substantially in the early 1990s (Värnik et al., 2001). Even 20 years later, these Eastern European countries, Estonia among them, are among the top in suicide rates around the world (WHO, 2009).

Another striking feature of the epidemiology of suicide has been the fact that while women attempt suicide twice as often as men, male deaths by suicide outnumber that of females by 4 to 5-fold (Hawton, 2000). The trend is reversed only in China where there is less than one male suicide for every female suicide (Wang et al., 2008). Despite arguments about the burden on males to be the economic provider for the family, many lines of evidence suggest that the sex difference in suicide rates is not fully attributable to social factors only.

Although the underlying pathogenic mechanisms of the propensity to commit suicide are far from being understood, meanwhile it has been accepted that a combination of genetic predisposition and environmental factors lead an

individual to self-inflicted death. Neither of the factors operates independently from each other – it is important to recognize that environmental stressors are most likely to affect those with genetic predisposition.

It has long been known that suicidal behavior runs in families. Twin studies have demonstrated a significantly higher concordance rate for completed suicide in monozygotic twins as compared to dizygotic twins (Roy et al., 1995). The 6-fold higher rate of suicide in the biological relatives of suicidal adoptees strongly supports the importance of genetic factors (Schulsinger et al., 1979).

Since it has been established that psychiatric disturbances are main risk factors for suicide, candidate genes for suicidality typically are sought among loci previously associated with psychiatric disorders. The classical finding by Åsberg, indicating low levels of the serotonin metabolite 5-HIAA (5-hydroxyindoleacetic acid) in suicide victims' cerebrospinal fluid, initiated a whole new era of serotonin-related suicide studies (Asberg et al., 1976). Nevertheless, three decades later, due to discrepant results, there still is no consensus about whether serotonin system genes constitute the genetic component of susceptibility to suicide.

Recent studies have expanded the research to identify new mechanisms and candidate genes including studies of the neurochemical (dopamine, noradrenaline, gamma-aminobutyric acid, endopioids), neuroendocrine (hypothalamic-pituitary-adrenal axis), neuroimmunological (cytokines) and neurodevelopmental (neurotrophins) systems. Several genome-wide scans have been conducted in order to establish new targets. In comparison to studies of psychiatric disorders, a major limitation of suicide studies is the lack of an animal model. While depressive, anxious, or psychotic patterns of behavior are easily reproducable in mice or rats, it is not possible to artificially induce suicidal intent in any species other than humans.

So far, precise genetic characteristics associated with suicidal behavior remain unclear. Both linkage as well as association studies have proven difficult to replicate in subsequent cohorts. This phenomenon may highlight the genetic complexity of suicide, with different subtypes or etiologies of suicidal phenotype produced by different fundamental molecular defects. It is important to keep in mind that data about candidate loci related to suicide diathesis can so far be regarded as only suggestive since they do not provide us with markers readily usable for predicting an individual's behavior. The genes have a little meaning *per se*, only in context with other genes and in an environment that is cellular, extracellular and extraorganismic.

The aim of the current study was to contribute to the overall knowledge about suicide biology by investigating genetic variability in a number of candidate loci in three studies: *TPH2* (tryptophan hydroxylase isoform 2), previously considered as a candidate gene for suicide susceptibility; *LSAMP* (limbic system-associated protein), a novel candidate related to neurodevelopment and synaptic plasticity; and selected genes within the short arm of chromosome 4, a region implicated in suicide and related disorders by several genome-wide association studies.

2. REVIEW OF THE LITERATURE

2.1 Demographic features of suicide

2.1.1 Diversity of suicidal acts

Suicidal behavior represents a wide spectrum of self-destructive acts, ranging from attempted to completed suicides (The Merck Manual, 2008). Completed suicide is an intentional act that results in fatality, while attempted suicide is an act associated with at least some intent to die, but does not result in death or injury (Posner et al., 2007). Suicidal behavior should be distinguished from non-suicidal self-injury (NSSI), or intentional self-injurious behavior, which refers to the deliberate, direct destruction or alteration of body tissue without conscious suicidal intent. Suicidal thoughts and threats are referred to as suicide ideation and traditionally not considered as suicidal behavior.

Given that the level of activity, lethality and resuscitation success rate vary considerably among different methods of suicide, suicide victims are not considered a homogeneous group. Self-poisoning by gases, solid, or liquid substances (ICD-9 E950-E952, ICD-10 X60-X69) are considered as nonviolent methods of suicide, while all other methods such as hanging, shooting, cutting, jumping from high places, burning, etc. (ICD-9 E953-E958, ICD-10 X70-X82) are considered violent methods (Maes et al., 1993). Drowning is considered to be a nonviolent method in some studies (Heila et al., 1997; Dumais et al., 2005), but violent in others (Rasanen et al., 2002). There are no commonly accepted rules for the categorisation of suicide methods, and the variability in classification makes comparisons between the results of different studies problematic.

2.1.2 Epidemiology of completed suicide

Suicide represents 1.4% of the global burden of disease, accounting for more than 800,000 deaths per year (WHO, 2006). In Europe, the most recently reported rates of suicide vary widely, ranging from less than 3 in 100,000 persons per year in Mediterranean Europe and Muslim countries, to more than 30 in 100,000 persons per year in some former Soviet Union areas (WHO, 2009).

In Estonia, suicide rates have varied over time (Figure 1). The first years of regained independence in the 1990s coincided with a substantial increase of suicide rates in all former Soviet Union member countries (Värnik et al., 2001). This was explained by sudden increase in environmental stressors, namely challenges to cope with massive declines in per capita income, high unemployment, and a wide range of reform experiences that accompanied the birth of the new democracy. By 2007, Estonian suicide rates had shown considerable decline, reaching a frequency of 18.86 per 100,000 population,

which is still nearly twice as high as the European average (WHO, 2009). Considering the relationship between socioeconomic situation and suicide rates, the global economic depression that started in 2008 may bring along an increase in suicide occurrence in the near future.

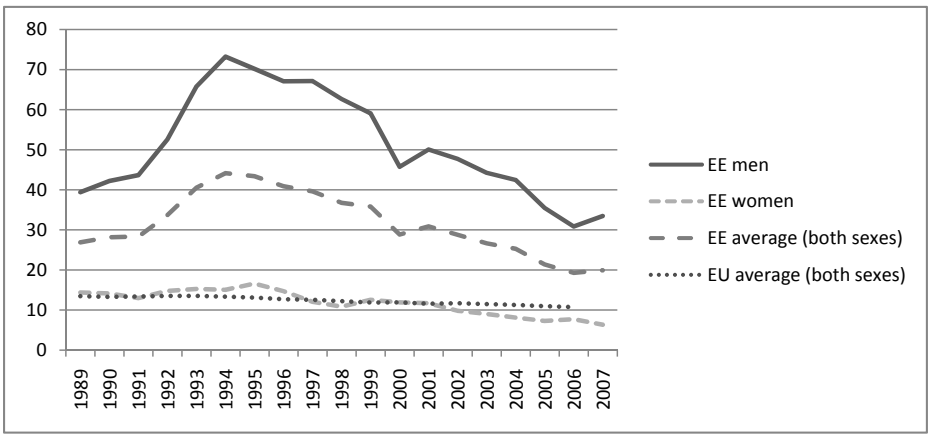

Figure 1. Suicide rates (per 100,000) in Estonia (EE) by sex as well as average, compared to European Union (EU, including all members as of 2007) average, 1989–2007 (Estonian Statistics, 2009; WHO, 2009).

Various demographic variables such as gender, age, marital status, social and occupational functioning, cumulatively contribute to suicide risk (Qin et al., 2003). In Europe, suicide rates have been highest among the elderly – in 2005, 33% of suicide cases were in individuals more than 65 years old (Belanger et al., 2008). In Estonia, 40–50% of the male suicide completers are 35 to 54 years old, while female suicide rates increase steadily with age (Estonian Human Development Report, 2002). Separated and divorced individuals are at higher risk for suicide, compared to married individuals (Wyder et al., 2009). Disadvantaged social and occupational functioning, such as living alone and being unemployed, represent additional risk factors (Johansson and Sundquist, 1997; Johansson et al., 1997; Blakely et al., 2003).

2.1.3 Sex differences in suicide rates

Suicide rates vary by gender, with males typically completing suicide four times more often than females, and females attempting suicide two to three times more often than males (Hawton, 2000). The only country in the world where female suicide rate exceeds that of males is China (Wang et al., 2008). Several explanations have been offered to explain the observed difference in completed suicide rates between genders.

One theory claims that the prevalence of males in successful suicidal acts is determined by gender differences in method preference. It is well documented that men tend to use violent methods for committing suicide, while women prefer nonviolent means (Schmidtke et al., 1996). The main difference between the two types of approach is reversibility, which has lead some researchers to claim that in females, the purpose of suicidal behavior is to communicate distress or to modify the behavior and reactions of other people (Hawton, 2000). On the other hand, there are arguments that women choose poisoning mainly because of limited access to other methods or lack of knowledge regarding these (Ajdacic-Gross et al., 2008).

On the other hand, it may be the hormonal profile that protects women from suicidal behavior. The steroid hormone estrogen has been shown to modulate several neurotransmitter systems, having both antidepressant as well as antipsychotic properties (Joffe and Cohen, 1998; Ostlund et al., 2003). Some studies have suggested a relationship between suicide attempts and the phases of the menstrual cycle when plasma estradiol falls to its lowest level (Fourestie et al., 1986; Baca-Garcia et al., 2000; Baca-Garcia et al., 2003; Caykoylu et al., 2004; Saunders and Hawton, 2006). However, others claim that suicide attempts occur more often during other phases of the menstrual cycle (Targum et al., 1991; Gisselmann et al., 1996) or that there is no relationship to the menstrual cycle (Ekeberg et al., 1986; Mann et al., 1999a). Regarding completed suicide, rates in US women tend to peak at menopause (Maris, 2002) when the drop in estrogen level affects the individual's cognitive and emotional functioning most sharply.

Concerning sex differences in temporal dynamics of suicide rates, male suicide appears to be highly sensitive to the state of the macroeconomy in their country of residence, while female rate remains relatively independent of changes in society (Värnik et al., 2001). The highest rate of male suicide in Estonia –73.24 per 100 000 males – was registered in 1994 (Estonian Statistics, 2009; WHO, 2009), three years after the country regained its independence. The male sensitivity to macroeconomical events appears to be highly age-specific: it is apparent in the cohort of working age (35–59) males but not detectable in younger or older groups (Estonian Human Development Report, 2002).

In modern Western societies, women and men are equally involved in the labor market and thus are exposed to the same socioeconomic challenges. Thus, it is not possible to explain by environmental factors only, why one half of the population is more vulnerable to stress-induced mental health problems than the other. Therefore, the precise nature of factors contributing to sex differences in suicide susceptibility remain to be established.

2.1.4 Suicide, psychopathologies and intermediate phenotypes

Increased suicide risk accompanies with virtually all psychiatric disorders (Harris and Barraclough, 1997). An extensive meta-analysis has been conducted,

pooling results from 27 independent studies regarding psychiatric diagnoses of over 3000 suicide victims (Arsenault-Lapierre et al., 2004). The study demonstrated that 87.3% of suicide completers suffered from a psychiatric illness, and the two single most common diagnostic categories are affective disorders (43%) and substance abuse disorders (25.7%). Moreover, there were significant gender differences in distribution of psychopathologies: the risk of substance abuse-related disorders, personality disorders, and childhood disorders were significantly higher in males, whereas the risk of affective disorders was greater in female suicide completers. This finding supports the hypothesis that the underlying etiology of suicide may be different between males and females.

Various lines of evidence suggest that the prevalence of psychiatric diseases remains underestimated in the suicide population. A considerable number of individuals commit suicide at the onset of their illness before a diagnosis is made or proper treatment is initiated (Angst et al., 2002). Again, gender differences in suicide rates are amplified by the fact that men with suicidal ideation are reluctant to seek help from mental health professionals (Luoma et al., 2002). Post mortem toxicological examination results have indicated that the majority of suicide completers were not using any prescribed psychotropic treatment at the time of their death (Isacsson et al., 1999; Henriksson et al., 2001), whereas it has been demonstrated that patients under long-term medication have a significant reduction of suicide mortality compared to untreated patients (Isacsson et al., 1996; Angst et al., 2002).

Alcohol and substance abuse are strongly related to suicide risk, since psychotropic substances impair judgement, induce impulsive behavior, and contribute to the choice of suicide method (Rich et al., 1998). Forensic data has demonstrated the presence of alcohol in more than 40% of male and nearly 20% of female suicide victims (Hayward et al., 1992; Ohberg et al., 1996; Värnik et al., 2006). Geographical variation in suicide rates is partly explained with different levels of alcohol consumption and heavy drinking between populations (Pridemore, 2006). One of the most famous success stories in history of suicide prevention has been the strict anti-alcohol policy introduced from 1984–1988 in the Soviet Union: 4 years of the „dry law“ coincided with a 40% decrease in male and 18% decrease in female suicide deaths in the Russian and Baltic areas (Wasserman and Värnik, 1998).

However, although the presence of a psychopathology is a strong predictor for suicide, only a minority of people with these diagnoses eventually commit suicide (Blair-West et al., 1999; Bondy et al., 2006). The question of why certain patients commit suicide while others with the same psychiatric problem do not, has puzzled clinicians for a long time. A hypothesis has been proposed about genetic transmission of a propensity for certain intermediate phenotypes and suicidal behavior, independent of transmission of a psychiatric disorder (Brent and Mann, 2005). This would be consistent with the fact that suicidal behavior runs in families independently of psychopathology but together with aggressive-impulsive personality traits (Brent and Melhem, 2008). Other possible intermediate traits include neuroticism (Roy, 2002) and hopelessness

(Beck et al., 1985; Beck and Steer, 1989; Young et al., 1994). Yet other indicators that increase the liability for suicidal behavior include a history of childhood abuse (Sfoggia et al., 2008) or past head injury (Mann et al., 1999b).

It follows from the studies discussed here that it is not the exclusive presence of a single psychopathology or expression level of a personality trait that leads to suicidal behavior. Rather, a complex endophenotype is formed by certain risk-posing behavioral traits and psychiatric comorbidity; the heritable predisposition is eventually triggered by stressful life events.

2.2 Genetic correlates to structural and functional alterations in the suicidal brain

Family, twin, and adoption studies provide strong evidence for a heritable component to suicidal behavior (Baldessarini and Hennen, 2004). Monozygotic twins have a significantly higher concordance rate for both completed suicide and suicide attempts than dizygotic twins (Roy et al., 1995; Roy and Segal, 2001). This has been confirmed by adoption studies, where a 6-fold higher rate of suicide was found among the biological relatives of suicidal adoptees, compared to the relatives of non-suicidal adoptees (Schulsinger et al., 1979). Results from family studies have been consistent, showing that the rate of suicide is elevated in the families of suicide completers (Qin et al., 2003; Runeson and Asberg, 2003). After statistical adjustment for psychiatric disorders, the familial effect for suicidal behavior still persists, indicating that suicidal behavior and psychopathologies comprise distinct genetic components that are transmitted independently (Brent and Melhem, 2008).

Suicidal behavior is a complex phenotype related to a vast number of liability genes interacting with the environment (Mann, 2003). In recent years, a number of loci have been revealed in whole-genome linkage scans regarding suicidal behavior, including 2p11 (Hesselbrock et al., 2004), 2p12 (Zubenko et al., 2004; Willour et al., 2007), 2q24, 4p16 (Cheng et al., 2006), 5q31–33, 6q12 (Zubenko et al., 2004), 6q24, 6q25 (Cheng et al., 2006), 6q26 (Willour et al., 2007), 8p21–22 (Zubenko et al., 2004), 10q25 (Cheng et al., 2006), 11q25 and Xq25–26 (Zubenko et al., 2004). Interestingly, several of these loci contain genes that have well-delineated neurobiological functions. In addition, a large number of association studies have been conducted and have produced an extensive body of data often not replicated or only partially replicated. In following sections, the current state of knowledge of suicide biology and a number of investigated candidate genes are summarized.

2.2.1 Serotonin (5-HT)

One of the first and most widely investigated neurochemical signaling systems in the suicidal phenotype is that of serotonin (5-hydroxytryptamine, or 5-HT), a monoamine transmitter involved in many neural functions, including mood and emotion.

The first work relating serotonergic neurotransmission to completed suicide was that of Åsberg and colleagues (1976). They demonstrated the reduction of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) of suicide victims.

In addition to this finding, the distribution of serotonin receptors is altered in the brains of suicide subjects, regardless of their psychiatric diagnosis. A compensatory reaction in response to 5-HT deficiency has been indicated by a decrease in presynaptic 5-HT uptake sites in the prefrontal cortex (Stanley et al., 1982; Mann et al., 2000; Arango et al., 2001), increased density of postsynaptic 5-HT_{1A} and 5-HT_{2A} receptors in the cortex and amygdala (Stanley and Mann, 1983; Mann et al., 1986; Arango et al., 1990; Hrdina et al., 1993; Stockmeier et al., 1998), and up-regulated tryptophan hydroxylase gene expression in dorsal raphe nuclei of suicides (Bach-Mizrachi et al., 2006). Furthermore, the increased number (Bach-Mizrachi et al., 2006) but decreased protein synthetic activity of dorsal raphe neurons (Gos et al., 2008b) also have been observed. Moreover, a blunted prolactin response to the fenfluramine challenge test has been described in suicide attempters (Coccaro et al., 1989), and has been associated with lethality of suicide attempts (Malone et al., 1996), thus indicating a higher probability for future suicide attempts (Keilp et al., 2008).

The assumption that serotonergic function is regulated by genetic factors has inspired numerous studies regarding variation in genes that are involved in serotonin synthesis, transport, recognition and degradation.

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the biosynthesis of serotonin. Two isoforms of the enzyme have been identified: TPH1, which is expressed preferentially in the periphery, and TPH2, which is expressed in the brain (Walther et al., 2003; Zhang et al., 2004). Several loci in both genes have been studied with regard to the suicidal phenotype. Two single nucleotide polymorphisms of *TPH1*, A218C and A799C, have been the subjects of numerous investigations regarding suicidal behavior (for review, see (Lalovic and Turecki, 2002)). Some studies have reported associations between *TPH2* genotype and suicidality (Zill et al., 2004; Zhou et al., 2005; Ke et al., 2006; Lopez de Lara et al., 2007; Yoon and Kim, 2009), although others disagree (De Luca et al., 2004; De Luca et al., 2005; Zill et al., 2007). Regarding neural correlates of genetic variation, a SNP in the *TPH2* promoter region has been associated with increased amygdaloid activity in a facial emotion recognition paradigm measured by MRI (Brown et al., 2005; Canli et al., 2005), as well as with increased event-related potentials in response to emotional stimuli (Herrmann et al., 2007), and impaired executive control in cognitive and affective processing (Reuter et al., 2007; Strobel et al., 2007; Reuter et al., 2008; Baehne

et al., 2008; Osinsky et al., 2008). In addition, a number of individual polymorphisms and haplotypes have been identified that predict the level of TPH2 mRNA expression in the human pons (Lim et al., 2007), as well as the level of 5-HIAA in the CSF (Zhou et al., 2005).

Regardless of the peripheral expression of TPH1 in the adult organism, it may still contribute to mental function by regulating neurodevelopment. A murine study has demonstrated that Tph1, rather than Tph2, is expressed in developing raphe neurons; thus, it is likely to be involved in fine-tuning and maturation of serotonergic networks (Nakamura et al., 2006).

Evidence about alterations in **serotonin receptor** distribution in brains of suicide victims has directed researchers' interest towards the genes for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C}. Despite numerous efforts, none of the serotonin receptor gene variants studied in relation to suicidal behavior have yielded a statistically significant association (Nishiguchi et al., 2002; Ohtani et al., 2004; Videtic et al., 2006; Wasserman et al., 2006; Serretti et al., 2007). These findings support the hypothesis that the up-regulation in receptor density observed in post mortem studies is not due to dysfunctional receptor variants but rather reflects compensatory reactions to other biochemical deficits in signal transduction pathways. An interesting study performed by Hsiung and colleagues (2003) reported attenuated activity of the phosphoinositide 3-kinase / protein kinase B (PI 3-K/Akt) pathway, a second messenger cascade downstream from 5-HT_{1A} activation, in the brain of suicide victims, compared with controls.

Serotonin transporter (SERT, 5-HTT) terminates the action of 5-HT via its uptake from the synaptic cleft into the presynaptic neuron. The human *5-HTT* gene has a common length polymorphism in the promoter region (LPR), where a 44 base pair (bp) insertion results either in a short (S) or long (L) allele. The L allele has been associated with up to 3-fold more efficient transcription of the gene, compared to the S allele, which would be less active resulting in reduced serotonin uptake (Lesch et al., 1996; Heils et al., 1996). Regarding neural correlates, the S allele of the *5-HTT* LPR has been associated with higher amygdala activation in response to fearful stimuli (Hariri et al., 2002), as well as functional coupling between the prefrontal cortex and amygdala, as measured by functional magnetic resonance imaging (fMRI) (Heinz et al., 2005). Recently, evidence for the robust influence of *5-HTT* LPR on brain anatomy has emerged. The S allele has been associated with a significant reduction in the volume of the anterior cingulate cortex and amygdala (Pezawas et al., 2005), and an increase in neuron number and volume in the pulvinar nucleus of the thalamus (Young et al., 2007; Young et al., 2008). The data are intriguing in the context of suicide since the pulvinar nucleus, cingulate cortex, and amygdala are intimately interconnected and involved in mediating emotional responses to environmental stimuli (Ohman, 2005).

Another polymorphism in the *5-HTT* gene is a variable number of 17 bp tandem repeats (VNTR) in the second intron (Ogilvie et al., 1996). There is evidence that the *5-HTT* VNTR may act as a transcriptional regulator, the

12-repeat allele displaying higher transcriptional activity than the 10-repeat allele (Fiskerstrand et al., 1999; MacKenzie and Quinn, 1999).

A few meta-analyses have summarized the majority of association studies regarding *5-HTT* polymorphisms and suicidal behavior (Anguelova et al., 2003; Li and He, 2007). In short, the data suggest that there is a tendency for a positive association with the S allele of the *5-HTT* LPR, while *5-HTT* VNTR does not appear to be related to suicidal behavior (Li and He, 2007).

Monoamine oxidase A (MAOA) is a mitochondrial membrane enzyme that catalyzes the degradation of several biological amines, including the neurotransmitters serotonin, noradrenaline and dopamine (Shih and Thompson, 1999). The general assumption of investigations of MAOA in relation to various behaviors is that low MAO activity results in elevated levels of its substrates in the brain.

Low platelet MAOB activity, indirectly reflecting variations in central MAOA levels, has been associated with suicidal behavior (Buchsbaum et al., 1977; Meltzer and Arora, 1986) as well as with many behavioral traits identified as suicide risk factors, namely impulsivity, aggression, and violence (Schalling et al., 1987). Several common polymorphisms have been described in the *MAOA* gene, including three functional polymorphisms. A VNTR is located 1.2 kbp upstream of coding sequences (*MAOA* uVNTR), resulting in a significant difference in transcriptional efficiency (Sabol et al., 1998). Two restriction fragment length polymorphisms, EcoRV and Fnu4HI, result in a 30-fold difference in enzyme activity (Hotamisligil and Breakefield, 1991). Concerning completed suicide, no associations have been detected with the *MAOA* uVNTR (Ono et al., 2002). However, the high-activity allele of *MAOA* uVNTR is reported to be related with violent suicide attempt in males (Courtet et al., 2005) and the high-activity allele of *MAOA* Fnu4HI has been weakly associated with a history of suicide attempts in female bipolar patients (Ho et al., 2000). The high-activity allele of *MAOA* EcoRV has been reported to be more frequent among depressed male suicide completers (Du et al., 2002).

2.2.2 Biomolecules related to catecholamine biosynthesis and catabolism

Compared to extensive research about serotonin, less information is available regarding the status of the catecholaminergic systems in suicide victims. Studies of the synthesis, signaling properties and catabolism of dopamine (DA) and noradrenaline (NE) have not identified any consistent alterations characteristic of suicide tendency.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis. Alterations of TH levels in the locus coeruleus (LC) of suicide victims have been described. However, the studies have yielded controversial results, with some researchers reporting decreased (Biegon and Fieldust, 1992), yet others increased (Ordway et al., 1994), levels of the enzyme. A recent study

has limited the finding of increased TH immunoreactivity in LC neurons to violent suicides only (Gos et al., 2008a). A tetranucleotide repeat has been described in the first intron of the *TH* gene (Polymeropoulos et al., 1991). A low prevalence of allele with one repeat (*TH*-K1) has been reported among suicide attempters, while a significant increase in the frequency of the three-repeat allele (*TH*-K3) was found in suicidal patients with adjustment disorders (Persson et al., 1997). In addition, reduced levels of the NE metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) have been reported in *TH*-K3 carriers (Jonsson et al., 1996).

Catechol-O-methyltransferase (COMT) is an enzyme involved in the inactivation of catecholamine neurotransmitters. A functional SNP has been described in the gene, which results in the substitution of valine (Val) with methionine (Met) at codon 158 (Lachman et al., 1996). Homozygotes for 158V have a significant reduction in enzyme activity, compared to individuals with two Met alleles and therefore, presumptively, more baseline synaptic dopamine (Chen et al., 2004). A number of studies have suggested an association between 158M and suicidal behavior (Nolan et al., 2000; Ono et al., 2004; Kia-Keating et al., 2007), as well as outward directed aggression in suicide attempters of varied psychiatric diagnoses (Rujescu et al., 2003). Other studies have not confirmed the association between suicidal phenotype and the polymorphism (Zalsman et al., 2008; De Luca et al., 2008a).

2.2.3 Dopamine (DA)

The interest in the role of the dopamine system in suicide etiology derives from the evidence about the involvement of this system in several psychiatric disorders as well as substance addiction and personality traits like impulsivity, which previously were related to suicide risk (Goto and Grace, 2008).

So far, unchanged (Crow et al., 1984; Arranz et al., 1997; Bowden et al., 1997b) or increased (Ohmori et al., 1992) concentrations of the DA metabolite homovanillic acid (HVA) have been described in several brain regions of suicide victims. Decreased concentrations of HVA have been reported in the CSF of suicide attempters (Roy et al., 1986; Engstrom et al., 1999). In addition, decreased concentrations of another DA metabolite, dihydroxyphenylacetic acid (DOPAC), have been found in the caudate, putamen, and nucleus accumbens of antidepressant-free non-violent suicides compared to controls (Bowden et al., 1997b).

A post-mortem study described decreased **dopamine receptor D2** (DRD2) affinity in the caudate nucleus of depressed suicide patients, while no differences were detected in the overall suicide group (Allard and Norlen, 2001). Two studies have not been able to confirm this finding. In one study, no differences in the number or affinity of **dopamine receptor D1** (DRD1) or DRD2 receptor in antidepressant-free depressed suicide patients and controls were found; instead an increased number of DRD1 in the nucleus accumbens of

antidepressant-treated depressed suicide patients was reported (Bowden et al., 1997c). In the second study, an increased density of DRD2 was demonstrated in the caudate nucleus of schizophrenic violent suicide victims (Ruiz et al., 1992). A SNP in exon 8 of the *DRD2* gene (Finckh et al., 1997) as well as an insertion/ deletion polymorphism upstream from exon 1 (Johann et al., 2005), have been associated with suicide attempts in alcoholics. However, the association with the latter polymorphism was not confirmed in a sample of suicidal mood disorder patients (Ho et al., 2000). In addition, a lack of association between variation in **dopamine receptor D4** gene and suicidality has been reported in two studies (Persson et al., 1999; Zalsman et al., 2004).

A brain imaging study found no differences in **dopamine transporter** (DAT) binding potential between suicide attempters and control subjects (Ryding et al., 2006). This is consistent with post-mortem studies where no differences in the density of DAT uptake sites were detected in the caudate nucleus of suicide victims and controls (Allard and Norlen, 1997; Bowden et al., 1997a), as well as with association studies where variations in the DAT gene were not related to the history of suicide attempt (Gerra et al., 2005).

2.2.4 Noradrenaline (NE)

Findings from postmortem studies provide data generally consistent with the hypothesis that a NE deficiency exists in depression, and possibly in suicidal behavior (Ordway and Klimek, 2001). A decreased number of noradrenergic neurons has been detected in the LC of suicide completers (Arango et al., 1996). There are reports about NE and decreased levels of its main metabolite, MHPG, in the urine of suicidal patients (Ostroff et al., 1985; Secunda et al., 1986). In addition, MHPG levels were negatively correlated with the lethality of suicide attempts in patients with mood disorders (Garvey et al., 1994; Sher et al., 2006). On the other hand, significantly higher levels of NE and MHPG in the CSF have been observed in suicidal patients with personality disorder (Brown et al., 1979). Moreover, a number of studies have not detected any alterations in NE or MHPG levels in suicidal patients (Beskow et al., 1976; Riederer et al., 1980; Roy et al., 1985; Roy et al., 1989).

Noradrenaline produces its functional effects by interacting with its various receptors. Adrenoceptor down-regulation has been described as a common biochemical effect of many antidepressant drugs, suggesting the possible involvement of noradrenergic receptors in depression, which is one of the risk factors for suicide (Subhash et al., 2003; Holoubek et al., 2004). Both increased (Meana and Garcia-Sevilla, 1987; Arango et al., 1993) and decreased (Gross-Isseroff et al., 1990a) densities of **α_2 adrenergic receptors** (α_2-AD) have been reported in the frontal cortex of suicide victims. One study reported an increased number α_2-AD in the temporal cortex of antidepressant-free suicide victims (De Paermentier et al., 1997). In summary, the majority of studies about α_2-AD are consistent about increased α_2-AD binding in the cortex and hippo-

campus of suicide victims compared to control subjects (Pandey and Dwivedi, 2007).

Relatively few studies have examined α_2-AD gene (*ADRA2*) variants in relation to suicidal behavior. One study found a lack of associations with three polymorphisms in the promoter region of the gene, but a rare 251K allele, associated with enhanced second messenger cascade activity, was observed only in suicides (Sequeira et al., 2004). However, another study failed to detect the 251K allele in either suicide or control group (Martin-Guerrero et al., 2006). A promoter region SNP, C-1291G, and its haplotype with another SNP, rs3750625, have been associated with suicide in Japanese females (Fukutake et al., 2008).

Three studies examining **β adrenergic receptors** (β-AD) found an increase in receptor binding in the frontal cortex of suicidal patients (Mann et al., 1986; Biegon and Israeli, 1988; Arango et al., 1990). One study reported a decrease in the frontal cortex (Little et al., 1993), and two studies showed a decrease in the temporal cortex and thalamus (De Paermentier et al., 1990; De Paermentier et al., 1991). To the author's knowledge, no studies have been conducted concerning the relationship between variation in β-AD genes and suicidal behavior.

2.2.5 Gamma-aminobutyric acid (GABA)

Many psychiatric disorders appear to involve an imbalance in excitatory and inhibitory processes in the central nervous system. Therefore, the gamma-aminobutyric acid (GABA) system is the target of a wide range of psychoactive drugs, including anxiolytics, sedative-hypnotics, general anesthetics, and anti-convulsants (Olsen, 2002). GABA is a critical neurotransmitter in circuits connecting the prefrontal cortex with the limbic system. These circuits are of relevance to impulsivity because of their central importance in behavioral inhibition (Horn et al., 2003) and affective processing (Phan et al., 2002). GABA-ergic neurotransmission modulates the activity of the noradrenergic, dopaminergic, and serotonergic systems (Bankson and Yamamoto, 2004). Therefore, it is reasonable to speculate that deficits in GABA-ergic inputs to the mono-aminergic pathways may be related to development of suicidal behavior.

An increase in CSF GABA levels has been reported in suicidal subjects with personality disorders (Lee et al., 2009). No difference has been detected in the density of GABA uptake sites in the frontal cortex (Sundman et al., 1997) or agonist binding to GABA type A receptors in the locus coeruleus of suicide completers (Zhu et al., 2006). A reduction in GABA receptor type A ($GABA_A$) subunit mRNA expression has been observed in the limbic system of depressed suicide victims (Merali et al., 2004; Sequeira et al., 2007), while expression of β subunits appears to be up-regulated (Sequeira et al., 2007).

Polymorphic variations in the gene coding for the $GABA_A$ α3 subunit (*GABRA3*) have not been associated to suicide attempts (Baca-Garcia et al., 2004). However, there is recent evidence about epigenetic mutations con-

tributing to differential expression of $GABA_A$ subunit genes in suicide victims. The increased expression of DNA methyltransferase isozyme 3 beta (DNMT-3B) mRNA and protein in the frontopolar cortex of suicide completers has been correlated with the increased DNA methylation of the $GABA_A$ receptor α1 subunit gene (*GABRA1*) promoter and reduced mRNA abundance of the α1 subunit (Poulter et al., 2008).

2.2.6 Cholecystokinin (CCK)

The neuropeptide cholecystokinin (CCK) has been investigated most often in relation to anxiety-related behaviors (Nair et al., 1982; de Montigny, 1989). However, several lines of evidence have suggested its role in suicidal behavior, as well. A higher level of CCK has been found in the CSF of suicidal depressive patients (Lofberg et al., 1998). In addition, a significantly higher level of CCK mRNA (Bachus et al., 1997), as well as the number and affinity of CCK type 2 receptors (CCK_2) have been detected in the frontal cortex of suicide victims (Harro et al., 1992). Further studies have confirmed the upregulation of CCK_2 expression in the cerebellum, cingulate cortex and prefrontal cortex of post-mortem brains of suicide victims (Sherrin et al., 2004). A single nucleotide polymorphism, -196G/A, has been described in the *CCK* gene promoter region (Fujii et al., 1999). The A allele has been associated with completed suicide in Japanese males but not in females (Shindo and Yoshioka, 2005).

It is not clear how CCK contributes to suicidal behavior, since the molecular targets of neuronal CCK are incompletely understood, but the evidence shows that the neuropeptide is colocalized with dopamine in mesolimbic neurons (Hokfelt et al., 1980) and in the nucleus accumbens (Lanca et al., 1998), which lends support for the hypothesis of CCK being related to modulation of motivated behaviors (Rotzinger et al., 2002).

2.2.7 Hypothalamic-pituitary-adrenal (HPA) axis

There is abundant evidence of disturbed hypothalamic-pituitary-adrenal (HPA) axis function in suicide victims and attempters. It was noted long ago that urinary corticosteroid level was elevated in patients who completed suicide (Fawcett and Bunney, 1967; Bunney et al., 1969; Krieger, 1970). Postmortem findings in suicide subjects have supported the proposed hypothesis about HPA axis hyperactivity: individuals who died from suicide were reported to have enlarged adrenal glands compared to controls who died from other causes (Dorovini-Zis and Zis, 1987; Szigethy et al., 1994), increased levels of corticotropin-releasing hormone (CRH) in the CSF (Arato et al., 1989), increased CRH expression in the hypothalamus and brain stem (Raadsheer et al., 1994; Austin et al., 2003; Bissette et al., 2003), and fewer CRH receptors in the frontal cortex, all of which has been interpreted as down-regulation

following CRH hypersecretion (Nemeroff et al., 1988). One study was unable to replicate the finding about alterations in CRH binding site density (Hucks et al., 1997), and another study has found a decrease in CRH receptor type 1 (CRHR1) but not type 2 (CRHR2) mRNA in frontal cortex of suicide completers (Merali et al., 2004).

Meta-analyses of dexamethasone suppression (DST) studies confirmed that non-suppression of cortisol has a significant predictive power with respect to completed suicide (Lester, 1992; Mann et al., 2006). DST is claimed to be the most powerful clinical tool for suicide prediction currently in use (Coryell and Schlesser, 2001).

Only a few studies have investigated HPA axis-related genes with regard to suicide. One demonstrated the association of haplotypic variation in the CRH receptor type 2 (*CRHR2*) locus and suicidal behavior in bipolar patients (De Luca et al., 2007). Another study reported a significant association between a SNP in the CRH binding protein gene (*CRHBP*) and suicide attempts in schizophrenic patients, as well as an association between an interaction between *CRHBP* and CRH receptor type 1 (*CRHR1*) gene SNPs and the severity of suicidal attempts (De Luca et al., 2008b). A family study demonstrated the linkage of a SNP in the *CRHR1* locus and suicidality in depressed males (Wasserman et al., 2008).

The mechanism by which the HPA axis influences suicidal behavior is not yet established. Various researchers investigating the pathophysiology of suicide have summarized findings that integrate HPA hyperfunction with disturbances in serotonin function (Lopez et al., 1997; Lanfumey et al., 2008).

2.2.8 Cytokines

Allergies, which are disorders of immune system hyperactivity, are associated with elevated risk for developing depression (Timonen et al., 2002; Timonen et al., 2003). The spring is the peak season for aeroallergens, as well as depression exacerbation, psychiatric hospital admissions, and suicides (Postolache et al., 2008). The impact of season on suicide risk is greater in individuals with a clinical history of atopic allergy, compared with non-atopic allergic suicide subjects (Timonen et al., 2004). These observations have led to a hypothesis about link between immune status and suicidal behavior.

Cytokine imbalance has been observed repeatedly in suicidal individuals. Various case reports have documented death by suicide in patients with no history of psychiatric disorders who were receiving cytokines to treat a variety of diseases like melanoma, hepatitis C, HIV, and multiple sclerosis (Baron et al., 1993; Janssen et al., 1994; Fukunishi et al., 1998; Lana-Peixoto et al., 2002).

In a recent post-mortem study, gender-dependent differential expression of type-2 T-helper cytokine (Th2) mRNA was detected in the orbitofrontal cortex of suicide subjects, independent of their psychiatric diagnosis (Tonelli et al., 2008). In this study, interleukin 4 (IL-4) mRNA levels were increased in female

suicides, compared with controls, while interleukin 13 (IL-13) mRNA levels were increased in males.

Several mechanisms have been proposed to explain the role of cytokines in suicidal behavior. First, proinflammatory cytokines are potent activators of the HPA axis; there is evidence that cytokines counteract the negative feedback action of corticosteroids on the HPA axis (Miller et al., 1999). Second, cytokines can upregulate the expression of the enzyme indoleamine-2,3-dioxygenase (IDO) which leads to peripheral depletion of tryptophan, resulting in diminished synthesis of serotonin (Wichers and Maes, 2002).

2.2.9 Endogenous opioids

Experimental and clinical evidence suggest that alterations in the endogenous opioid system may be involved in suicide. Postmortem brain studies of completed suicides have demonstrated a decreased concentration of β-endorphin (Scarone et al., 1990), increased expression of the μ-opioid receptor (MOR) gene (*OPRM1*) mRNA (Escriba et al., 2004), increased density (Gross-Isseroff et al., 1990b; Gabilondo et al., 1995) and altered binding affinity of MOR (Zalsman et al., 2005).

A common SNP, A118G, in the *OPRM1* gene that results in an asparagine (Asn) to aspartic acid (Asp) substitution at amino acid 40 has showed enhanced β-endorphin binding affinity of the receptor (Bond et al., 1998) and has been implicated in modulating the naloxone-stimulated HPA axis activation (Wand et al., 2002; Hernandez-Avila et al., 2003; Chong et al., 2006). The G allele of the SNP has been demonstrated to be less prevalent among suicide completers, suggesting that the protective allele may inhibit HPA axis responses and thus decrease suicide risk (Hishimoto et al., 2008).

2.2.10 Morphometric alterations: neurotrophins and limbic system-associated membrane protein (LSAMP)

Emerging evidence suggests that stress, psychiatric disorders, and suicidal behavior may be associated with structural abnormalities in the brain. For example, schizophrenic suicide completers have decreased right parahippocampal volume (Altshuler et al., 1990) and cortical laminar thickness (Rajkowska, 1997). Magnet resonance imaging studies have detected an increase in subcortical gray matter hyperintensities (Ahearn et al., 2001) as well as periventricular white matter hyperintensities in suicidal patients with mood disorders (Ahearn et al., 2001; Ehrlich et al., 2004; Ehrlich et al., 2005; Pompili et al., 2007). A study of depressed female patients revealed decreased orbitofrontal cortex gray matter and larger right amygdala in those patients who had attempted suicide, compared to non-suicidal patients and healthy controls (Monkul et al., 2007). The decrease of orbitofrontal cortex gray matter volume

was confirmed in a sample of suicidal schizophrenic males (Aguilar et al., 2008). In addition, reductions in temporal cortex volume were found in the same study.

Neurotrophins are a family of proteins essential for regulating neuronal differentiation in the developing brain, but also crucial for trophic support, neurogenesis, and regulation of synaptic connections in adult neurons as well as for activity-dependent plasticity (Schinder and Poo, 2000; Huang and Reichardt, 2001; Poo, 2001). Several studies have suggested that alterations in neurotrophin levels, especially brain-derived neurotrophic factor (BDNF), may contribute to stress-induced structural changes in the brain (Barbany and Persson, 1992; Smith et al., 1995). Expression of BDNF was shown to be lower in the hippocampus and prefrontal cortex of suicide subjects (Dwivedi et al., 2003b; Karege et al., 2005), and expression of nerve growth factor (NGF), neurotrophin type 3 (NT-3), and neurotrophin type 4/5 (NT-4/5) in suicide subjects is altered in a brain region-specific manner (Dwivedi et al., 2005). Expression ratios of different neurotrophin receptors in the brains of suicide subjects suggest possible activation of pathways that are apoptotic in nature (Dwivedi et al., 2009). Antidepressant treatment, on the other hand, is associated with an up-regulation of BDNF expression (Nibuya et al., 1995; Shirayama et al., 2002).

There seems to be a gender-specific interaction between the serotonin and BDNF systems. Double knockout mice for both 5-HTT and BDNF display higher 5-HT reduction in all brain regions compared with mice lacking either one of these genes (Ren-Patterson et al., 2005). Female double knockouts have lesser reductions in brain 5-HT, and thus appear to be protected by their gender (Ren-Patterson et al., 2006). In addition, double-mutant males demonstrate significantly increased plasma levels of adrenocorticotropic hormone (ACTH) after being exposed to a mild stressor, compared with female double-mutants and wild type animals. These findings demonstrate that the functional interaction between the serotonin and BDNF systems extends to a hormonally modulated hypersensitivity to environmental stressors.

BDNF G196A (rs6265) is a single nucleotide polymorphism, which results in a valine (Val) to methionine (Met) change at position 66 in the pro-BDNF sequence. The Met allele is associated with reduced expression of BDNF in vitro (Egan et al., 2003). In patients with mood disorders, the 66M allele has been associated with significantly higher risk of suicide attempts (Iga et al., 2007; Kim et al., 2008). However, there are studies that have not confirmed the link between the Val/Met polymorphism and suicidal behavior (Hong et al., 2003; Hwang et al., 2006).

Limbic system-associated membrane protein (LSAMP) is a highly conserved glycoprotein expressed on the somata and proximal dendrites of neurons in the cortical and subcortical regions of the limbic system (Zacco et al., 1990). During early stages of brain development, LSAMP acts as a selective homophilic adhesion molecule, facilitating formation of functional circuits between populations of limbic neurons (Keller et al., 1989; Zhukareva and Levitt, 1995;

Pimenta et al., 1995; Eagleson et al., 2003). The function of LSAMP in adult brain remains to be established. Rodent studies implicate the role of LSAMP in mediating behavioral response to novel environments (Nelovkov et al., 2003; Catania et al., 2008). Less is known about humans. A proteome analysis revealed the increased expression of LSAMP in the PFC of schizophrenic as well as bipolar patients (Behan et al., 2008). This evidence identifies the LSAMP as a novel candidate molecule for suicide studies.

2.2.11 Second messenger systems

Although several studies have demonstrated alterations in the expression of several types of neurotransmitter receptor in suicidal behavior, the involvement of functional changes in intracellular signal transduction pathways remains unclear. The research group of Dwivedi and Pandey has conducted an extensive series of investigations into this topic, paying particular attention to protein phosphorylation-dephosphorylation and activation-repression of transcription factors, which are key processes in signaling mechanisms that modulate the expression of genes involved in various neuronal functions.

The **adenylyl cyclase** *(AC)*-linked second messenger cascade follows the activation of a number of G-protein-coupled receptors (GPCR), including adrenergic, dopamine and 5-HT receptor subtypes. In the AC signaling system, cyclic adenosine monophosphate (cAMP), after its formation from adenosine triphosphate (ATP), stimulates the phosphorylating enzyme protein kinase A (PKA). Reduced [^{3}H]cAMP-binding to PKA, as well as decreased PKA activity was detected in prefrontal cortex (PFC) of depressed suicide victims (Dwivedi et al., 2002), however, another study attributes this finding only to the effect of antidepressant use (Lowther et al., 1997). In addition, a significant decrease in both mRNA as well as protein expression of certain PKA subunits (Dwivedi et al., 2004), which appears to be age-specific (Pandey et al., 2005), has been detected. The significance of the last finding is unclear, but is supported by the observation that teenage and elderly suicide victims have different psychiatric profiles (Turecki, 2005).

Other GPCRs exert their effects via **phosphoinositide** (PI) signaling pathway. Activation of protein kinase C (PKC) by diacylglycerol (DAG) is associated with phosphorylation of several proteins and transcription factors. It has been reported that activity and binding of PKC as well as mRNA and protein expression of several PKC subtypes are decreased in the PFC and hippocampus of teenage suicide victims (Pandey et al., 1997; Pandey et al., 2004). However, inositol trisphosphate (IP_3) levels have demonstrated to be increased in hippocampus of adult suicide subjects in an independent study (Rosel et al., 2000).

The AC-cAMP and PI pathways converge at many levels. For example, both PKA and PKC are able to activate the cAMP response element binding (CREB) protein. The activation of CREB causes the expression of wide array of genes,

such as *BDNF*, which has been implicated in suicide etiology (Dwivedi et al., 2003b). Furthermore, a significant reduction in mRNA and protein levels of CREB as well as its DNA binding activity in PFC and hippocampus of suicide subjects, irrespective of their psychiatric diagnosis has been shown (Dwivedi et al., 2003a). The finding appears to be age-specific, since no differences in CREB expression or DNA binding were detected in the hippocampi of teenage suicide victims compared with control subjects (Pandey et al., 2007). However, another study demonstrated a significant increase in CREB levels in the PFC of antidepressant-free depressed suicide completers (Odagaki et al., 2001).

Several types of cell surface receptors exploit the **Ras-mitogen-activated protein** (Ras-MAP) kinase signaling pathway. The final kinases in the MAP kinase cascade are a family of extracellular signal-regulated kinases (ERKs). Following activation, ERK kinases enter the nucleus and phosphorylate a variety of downstream proteins, including transcription factors. There is evidence that Ras-MAP kinase module can be activated by serotonergic (Launay et al., 1996; Watts, 1996; McDuffie et al., 2000), cholinergic and adrenergic receptors (Roberson et al., 1999). In addition, MAP kinase signaling is important for several neuronal functions that are regulated by neurotrophins (Segal and Greenberg, 1996). A post-mortem study revealed that MAP kinase activity is reduced in the PFC and hippocampus of depressed suicide victims, accompanied with a decrease in mRNA and protein expression of both ERK1 as well as ERK2 isoforms (Dwivedi et al., 2001). Decreased mRNA and protein level of ERK5 have been detected in the hippocampus of suicide subjects (Dwivedi et al., 2007).

To the author's knowledge, no association studies have been conducted regarding the candidate genes involved in second messenger systems and suicidal behavior. Moreover, the conclusions about the studies summarized in the above paragraph should be made with caution since there are very few, if any, studies that have replicated these findings and most of current results by Dwivedi and Pandey may have been obtained using the same study sample, which can lead to bias. To confirm the observations, a number of independent replication studies on distinct populations should be conducted.

2.2.12 Cholesterol

Cholesterol is an essential lipid in higher eukaryotic cellular membranes, playing a vital role in the function and organization of membrane proteins and receptors (Simons and Ikonen, 2000). Numerous studies have reported low serum cholesterol levels in suicidal patients with mood disorders (Sarchiapone et al., 2000; Sarchiapone et al., 2001; Guillem et al., 2002; Kim et al., 2002). Moreover, significant difference were observed in men, but not women, after gender stratification (Diaz-Sastre et al., 2007; Vuksan-Cusa et al., 2009). However, the only association study investigating various polymorphisms in genes

related to cholesterol metabolism failed to find any differences between genetic variants and completed suicide (Lalovic et al., 2004).

It has been proposed that low cholesterol and suicidality might be related to decreased serotonergic transmission (Engelberg, 1992). Low plasma concentrations of cholesterol have been associated with low plasma serotonin concentrations (Steegmans et al., 1996) and blunted neuroendocrine responses to fenfluramine (Muldoon et al., 1992). A reduction in the cholesterol content of cell membranes has been shown to decrease the binding affinity of a serotonin 5-HT_{1A} receptor agonist, alter G-protein coupling of the receptor, and decrease activity of the serotonin transporter (Scanlon et al., 2001; Pucadyil and Chattopadhyay, 2007).

Recent evidence suggests that the low cholesterol-related deficiencies in signal transduction may not be limited to the serotonin system but are characteristic to G-protein coupled receptors in general (Paila et al., 2009). It has been proposed that cholesterol can modulate the function of GPCRs either through a direct interaction, which induces a conformational change in the receptor, or by altering the physical properties of the membrane in which the receptor is embedded (Ohvo-Rekila et al., 2002; Lee, 2004). In addition to the 5-HT_{1A} receptor (Paila et al., 2009), a cholesterol binding motif has been detected in the structure of β_2-adrenergic receptor (Hanson et al., 2008).

Finally, a recent *in vitro* expression study revealed that the expression of many genes involved in the metabolism of cerebral cholesterol is regulated by neuronal CCK (Hansen et al., 2008), another biomolecule implicated in suicide pathophysiology.

2.2.13 Wolframin

Seventy years ago, a rare genetic disease characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) was described (Wolfram and Wagner, 1938). It has become clear that patients exhibiting the DIDMOAD syndrome share mutations in the wolframin (*WFS1*) gene that is located on chromosome 4 short arm (Strom et al., 1998; Inoue et al., 1998). In addition to various somatic symptoms, the patients often exhibit a number of psychiatric disorders including suicidal behavior (Swift and Swift, 2000). Close relatives to Wolfram syndrome patients also have been reported to have an increased incidence of depression, anxiety and suicide attempts, which confirms the hypothesis that variations in the wolframin gene may predispose individuals to psychiatric morbidity (Swift et al., 1991; Swift et al., 1998).

Wolframin is a transmembrane protein, localized in the endoplasmic reticulum of many tissues like pancreas, lung, heart, placenta and brain (Strom et al., 1998; Inoue et al., 1998). It has been suggested that wolframin modulates Ca^{2+} uptake by the endoplasmic reticulum (ER), thereby influencing intracellular Ca^{2+} homeostasis (Takei et al., 2006). Studies in cell cultures have demonstrated that wolframin deficiency is related to ER stress-induced apoptosis, both by

diminished Ca^{2+} uptake by the ER, as well as a maladaptive unfolded protein response (UPR) (Osman et al., 2003; Takei et al., 2006). Increased expression of UPR proteins by mood stabilizing drugs has suggested that the pathophysiology of mood disorders and suicidal behavior may be associated with alterations in the ER stress response (Wang et al., 1999). A post mortem study of depressed suicide victims has demonstrated up-regulation of ER stress proteins, possibly compensating for stress-related neuronal damage (Bown et al., 2000).

Despite the fact that maintenance of ER homeostasis is a common feature of all eukaryotic cells, only beta cells and neurons seem to be particularly vulnerable to ER stress caused by wolframin dysfunction. Post mortem studies of the pancreas and brain from subjects with Wolfram syndrome have shown beta cell loss as well as marked atrophy of several brain structures (Karasik et al., 1989; Rando et al., 1992). The link between these two distinct cell populations may be insulin. Although neurons are able to utilize glucose in an insulin-independent way, the hormone appears to play a considerable role in CNS function. It was recognized long ago that psychiatric patients have an increased prevalence of metabolic syndrome and its components as compared to the general population (Kooy, 1919; Raphael and Parsons, 1921; Lorenz, 1922; Kohen, 2004), and the number of new reports about the association has increased considerably in recent years (Holt et al., 2004; Vuksan-Cusa et al., 2009). In light of the evidence that metabolic syndrome also is linked to the 4p (Stone et al., 2002; Cai et al., 2004), a chromosomal region also linked to completed suicide (Cheng et al., 2006), the role of insulin in the etiology of psychiatric disorders merits further research.

Insulin receptors have been found to be concentrated in limbic system (Hill et al., 1986), and a number of studies have implicated the role of insulin in dopaminergic neurotransmission (Saller, 1984; Carvelli et al., 2002). Insulin deficiency reduces dopamine transporter (DAT) expression, thus increasing extracellular DA levels (Patterson et al., 1998; Williams et al., 2007). This has been confirmed by the fact that pharmacologically induced diabetic rodents are resistant to the motor stimulant properties of amphetamine (AMPH) (Rowland et al., 1985; Owens et al., 2005). The same applies to *Wfs1*-deficient mice (Luuk et al., 2008). AMPH is believed to compete with DA for DAT, and in the normoinsulinemic brain, AMPH administration reduces DA re-uptake and hence prolongs dopaminergic signaling (Koob and Bloom, 1988; Khoshbouei et al., 2003). DAT deficiency induced by hypoinsulinemia deletes the effect of AMPH.

Over 80% of the mutations associated with Wolfram syndrome are located within exon 8 of *WFS1* (Strom et al., 1998; Inoue et al., 1998). A common missense single nucleotide polymorphism, rs734312 (A1832G) produces a histidine-to-arginine change at position 611 (H611R). The functional consequence of this amino acid change is not known, but the SNP has been studied repeatedly in relation to psychiatric disorders (Furlong et al., 1999; Middle et al., 2000; Ohtsuki et al., 2000; Kato et al., 2003; Koido et al., 2005). Sequiera

and colleagues have reported an association of allele G with completed suicide in the general population (Sequeira et al., 2003).

2.3 Summary

In conclusion, evidence from numerous studies indicates neurochemical, neuroimmunological, neurodevelopmental and neuroendocrinological alterations in brains of suicidal individuals. Replication of original findings often fails due to several issues. For example, concerning association studies of candidate genes, many groups focus on single or very few polymorphic loci. However, more rather than fewer markers may be required to yield high probability of detecting a genetic risk factor. Modern high-throughput genotyping platforms allow the capture of the variation of whole genes or even a whole genome. Therefore, although many studies have failed to establish a genetic link between neurochemical dysfunction and psychiatric disorders, only very few have covered all the variation within the candidate gene and its vicinity. Thus, there is no reason to abandon any of the candidate genes; rather, more thorough scanning should be carried out.

Second, mechanisms of neurotransmission are not limited to the synthesis, catabolism and cell surface receptor-binding of first messengers. Less attention has been paid to intracellular signal transduction pathways that are equally important from the perspective of successful signaling function. Only a small number of studies have investigated second messenger cascades in relation to psychiatric disorders, and more are definitely needed.

Third, many interactive forces modulate neural function. The evidence about interactions between different neural pathways and possible modulatory effects of various endogenous and environmental factors is just beginning to emerge, providing novel starting points for future studies.

3. AIMS OF THE STUDY

Since previous studies have implied the contribution of multiple biological factors to suicide susceptibility, the aim of the current study was to investigate if the genetic variation in some loci previously associated with completed suicide, and in others novel to suicide research, are related to male completed suicide in the Estonian population. Estonian males were the subject of this work because the macroeconomic events following the fall of the Eastern block resulted in a sharp increase in the proportion of male suicides in Estonian population.

1. Impaired brain serotonergic function is hypothesized to contribute to the etiology of suicidal behavior. Tryptophan hydroxylase isoform 2 (TPH2) catalyzes the rate-limiting reaction in the biosynthesis of central serotonin (5-HT). Genetic variation in the *TPH2* gene may result in altered expression or catalytic properties of the enzyme, potentially influencing brain 5-HT levels. The aim of the association study was to investigate if 14 single nucleotide polymorphisms in the *TPH2* gene region were related to male completed suicide in the Estonian population.
2. The 4p chromosomal region has been implicated in a number of recent genome-wide linkage studies regarding suicide and related disorders. The goal was to estimate if 36 single nucleotide polymorphisms in the 4p11–16 were related to completed suicide in Estonian males.
3. Post mortem and imaging studies have suggested that the brains of suicide subjects exhibit certain volumetric abnormalities. The alterations most often are found in the limbic system, a set of brain structures involved in emotional processing and decision making. Limbic system-associated membrane protein (LSAMP) is an adhesion molecule that participates in the formation of functional circuits between limbic structures. Our investigation is the first association study addressing the possible link between genetic variants of the *LSAMP* gene and completed suicide. The aim of the study was to compare the frequency of 30 single nucleotide polymorphisms in *LSAMP* gene in male suicide completers and healthy volunteers.

4. MATERIALS AND METHODS

4.1 Subjects

Two samples of male subjects were investigated in the study: 288 suicide victims (mean age 42.8 years, SD = 13.69) and 327 healthy volunteers (mean age 40.5 years, SD = 14.49).

The definition of suicide was based on the results of medicolegal examination by the Estonian Forensic Examination Bureau. Kidney samples were collected by dissection at the autopsy. Diagnostic information about the occurrence of psychopathologies prior to death was available for a very small proportion of subjects, thus this data has not been presented in any of the papers.

The subjects committed suicide using the following methods: 88% by hanging, 10% by shooting, and less than 1% by each of other methods (jumping from height, cutting, poisoning, thermal trauma, drowning).

Healthy volunteers were recruited by newspaper advertisement. Subjects were interviewed using the Mini International Neuropsychiatric Interview (M.I.N.I. 5.0.0.) administered by experienced mental health professionals at the Psychiatry Clinic of Tartu University Hospital. The inclusion criteria involved no personal or familial psychiatric history, and good physical health.

Both cases and controls were unrelated individuals of Caucasian origin living in Estonia.

Study protocols were approved by the Ethics Review Committee on Human Research, Tartu University.

4.2 SNP selection

Single nucleotide polymorphisms (SNPs) were chosen as genetic variation markers due to their high abundance in the genome. Genotyping of all SNPs in an area of interest would provide the most accurate information about genetic variability but unfortunately would be very resource-demanding. Therefore, a number of tagging SNP selection algorithms have been implemented in publicly available software packages. The concept of tagging SNPs (tag SNP) is based on the assumption that there exists considerable variation in recombination rates throughout the genome, and a certain selection of markers are able to capture, by linkage disequilibrium, the variation that exists in the unexamined sites of the region.

4.2.1 Study I and III

We retrieved HapMap (http://www.hapmap.org) genotype information for CEU (Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain (CEPH) collection) population, and used

the Tagger algorithm implemented in Haploview to select tagging SNPs for genotyping. Tag SNPs were required to have a minor allele frequencies higher than 5%, and capture the variation of other SNPs by a minimum r^2 of 0.8.

Since *LSAMP* is a long gene, spanning over 635 kb, it would have been not possible to capture sufficient amount of variation within the whole sequence with less than 48 markers which is the upper limit for a single SNPlex assay. Therefore we focused on two fragments of 150 kb and 242 kb, from the 5' and 3' end of the gene and adjacent region, respectively, attempting to also cover putative regulatory regions.

Initially, 36 markers were chosen by Haploview but four markers were later exluded from the analysis due to reasons explained in Results paragraph (5.1 Genotype yield). Of the 32 markers actually used in analysis, 25 were intronic and 7 located in regions adjacent to the gene. According to estimations calculated by Haploview, approximately 70% of the variance in these regions was captured by the tag SNP set.

For a significantly shorter *TPH2* gene, initially, 16 tag SNPs were chosen from the gene and flanking regions by Haploview but two markers were later exluded from the analysis due to reasons explained in Results paragraph (5.1 Genotype yield). Of the remaining 14 markers, nine were intronic, one was located in the 3'-UTR and four in adjacent regions (three upstream and one downstream). According to estimations calculated by Haploview, approximately 60% of the variance in the gene region was captured by the tag SNP set.

4.2.2 Study II

Initially, 45 markers from the 4p11–16 region were chosen with the aid of SNPbrowser software by Applied Biosystems (De La Vega et al., 2006). Due to reasons explained in Results paragraph (5.1 Genotype yield), nine SNPs were later excluded from the analysis, thus only 36 markers were actually examined.

The selection of SNPs was based on functional criteria: the markers had to be validated and preferentially functional, had to be located in a coding region and result in an amino acid change (Table 1). Nevertheless, three SNPs were synonymous and four were located in the 3'-UTR of their respective genes.

Table 1. Study II: single nucleotide polymorphisms included in the 4p assay. According to the HapMap database (www.hapmap.org), a total of 35,319 SNPs are located in that region. Rows in italics designate markers that were excluded from further analysis (see Results section for explanation).

dbSNP ID	Major/ minor allele	Position on chromosome	Gene symbol	Gene name	MAF CEU	Function	Distance from previous
rs3796622	C/T	973060	SLC26A1	solute carrier family 26 (sulfate transporter), member 1	0.38	Arg/Gln	
rs1063743	A/G	1699786	TACC3	transforming, acidic coiled-coil containing protein 3	0.27	Gly/Ser	726726
rs2353552	A/C	2179863	POLN	polymerase (DNA directed) nu	0.15	Gln/His	480077
rs4961	*G/T*	*2876505*	*ADD1*	*adducin 1 (alpha)*	*0.21*	*Gly/Trp*	*696642*
rs2960306	G/T	2960297	GRK4	G protein-coupled receptor kinase 4	0.38	Arg/Leu	83792
rs362272	C/T	3204778	HTT	huntingtin (Huntington disease)	0.27	Val/Ile	244481
rs3213507	C/G	3387643	RGS12	regulator of G-protein signaling 12	0.25	Leu/Leu	182865
rs6811856	*A/C*	*3464624*	*DOK7*	*docking protein 7*	*0.17*	*Ser/Ser*	*76981*
rs2916414	A/T	4250535	OTOP1	otopetrin 1	0.25	Asp/Glu	785911
rs730469	C/T	5675571	EVC2	Ellis van Creveld syndrome 2 (limbin)	0.3	Thr/Ala	1425036
rs2302075	G/T	5806443	EVC	Ellis van Creveld syndrome	0.2	Lys/Thr	130872
rs1383180	C/T	5836343	EVC	Ellis-van Creveld syndrome	0.32	Arg/Gln	29900
rs16837960	A/G	6026439	FLJ46481	FLJ46481 protein	0.47	Arg/Trp	190096
rs2230720	*C/T*	*6354228*	*WFS1*	*Wolfram syndrome 1 (wolframin)*	*0.03*	*Ala/Val*	*327789*
rs734312	A/G	6354255	WFS1	Wolfram syndrome 1 (wolframin)	0.28	His/Arg	27
rs1805070	*A/G*	*6354581*	*WFS1*	*Wolfram syndrome 1 (wolframin)*	*0.07*	*Ile/Val*	*326*
rs2301790	C/T	6650913	MAN2B2	mannosidase, alpha, class 2B, member 2	0.41	Met/Val	296332
rs6841334	C/G	6936197	KIAA0232	KIAA0232	0.38	3' UTR	285284
rs16890979	*C/T*	*9531265*	*SLC2A9*	*solute carrier family 2 (facilitated glucose transporter), member 9*	*0.29*	*Val/Ile*	*2595068*
rs6820230	C/T	9636640	SLC2A9	solute carrier family 2 (facilitated glucose transporter), member 9	0.24	Ala/Thr	105375
rs3217	C/T	10053748	KIAA1729	KIAA1729	0.35	3' UTR	417108

dbSNP ID	Major/ minor allele	Position on chromosome	Gene symbol	Gene name	MAF CEU	Function	Distance from previous
rs1047389	*C/T*	*11010185*	*HS3ST1*	*heparan sulfate (glucosamine) 3-O-sulfotransferase 1*	*0.22*	*Asp/Asp*	*956437*
rs1971278	A/T	13215674	FAM44A	family with sequence similarity 44, member A	0.17	Leu/Ile	2205489
rs12644869	C/G	13722865	LOC152742	hypothetical protein LOC152742	0.17	His/Gln	507191
rs2302465	C/T	15318290	BST1	bone marrow stromal cell antigen 1	0.14	Arg/His	1595425
rs2240688	A/C	15579447	PROM1	prominin 1	0.26	3' UTR	261157
rs3795243	*C/G*	*17439088*	*NCAPG*	*non-SMC condensin I complex, subunit G*	*0.11*	*Ile/Met*	*1859641*
rs315675	A/T	24972999	ZCCHC4	zinc finger, CCHC domain containing 4	0.15	Leu/His	7533911
rs6448389	A/G	25287297	SLC34A2	solute carrier family 34 (sodium phosphate), member 2	0.14	Gly/Asp	314298
rs10440276	C/T	26818363	FLJ45721	hypothetical LOC401123	0.44	N/A	1531066
rs12507599	C/T	35986444	FLJ16686	FLJ16686	0.2	Arg/Cys	9168081
rs9654132	A/G	36017148	FLJ16686	FLJ16686	0.22	Arg/His	30704
rs2973275	A/G	37268523	C4orf19	chromosome 4 open reading frame 19	0.25	Ala/Thr	1251375
rs6811863	C/G	37638581	TBC1D1	TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1	0.42	Pro/Arg	370058
rs11096955	A/C	38452502	TLR10	toll-like receptor 10	0.41	Ile/Leu	813921
rs4833095	C/T	38476105	TLR1	toll-like receptor 1	0.3	Asn/Ser	23603
rs4975017	A/C	39126624	KLB	klotho beta	0.32	Gln/Lys	650519
rs17511668	G/T	39780162	N4BP2	Nedd4 binding protein 2	0.18	Ser/Ile	653538
rs10009228	A/G	40051179	CHRNA9	cholinergic receptor, nicotinic, alpha 9	0.21	Ser/Asn	271017
rs4861066	A/G	40505420	NSUN7	NOL1/NOP2/Sun domain family, member 7	0.35	3' UTR	454241
rs4861358	C/T	40710656	APBB2	amyloid beta (A4) precursor protein-binding, family B, member 2 (Fe65-like)	0.27	Gln/Arg	205236
rs6447368	C/T	44377222	GUF1	GUF1 GTPase homolog (S. cerevisiae)	0.45	Pro/Leu	3666566
rs4145944	*C/G*	*47288040*	*ATP10D*	*ATPase, Class V, type 10D*	*0.34*	*Thr/Ser*	*2910818*
rs13116684	A/G	47732683	NPAL1	NIPA-like domain containing 1	0.2	Ile/Val	444643
rs1051447	*A/C*	*48758629*	*FLJ21511*	*hypothetical protein FLJ21511*	*0.32*	*Asn/His*	*1025946*

4.3 DNA extraction and quantification

The kidney samples of suicide completers were obtained by dissection at the autopsy at the Estonian Forensic Examination Bureau. Whole blood samples from healthy volunteers were collected at the Psychiatry Clinic of Tartu University Hospital. Genomic DNA was isolated by the standard phenol-chloroform extraction method (Sambrook and Russell, 2001). Prior to that, kidney samples were homogenized in 2 ml of Tris-EDTA-NaCl buffer of pH 8.0 (10mM tris(hydroxymethyl)aminomethane, 10mM ethylenediaminetetraacetate, and 0.5M sodium chloride). Next, the homogenate was incubated at 37°C in 500 µl of 1.6% sodium dodecyl sulfate (SDS) lysis buffer for 15 minutes. After centrifugation, 1.5 ml of supernatant was transferred into new tube. Samples were digested by Proteinase K (20 µg/ml, Fermentas) overnight at 37°C. The next morning, samples were subjected to standard phenol-chloroform extraction.

Since it is critical for the success of SNPlex genotyping that all samples contain equal concentrations of functional DNA template, the DNA content of samples was estimated by the real-time PCR quantification assay for the housekeeping gene RNase P (Applied Biosystems). The main advantage of the Taqman® RNAse P assay over other quantifying methods is its accuracy in determining the amount of PCR amplifiable DNA in a sample. The ABI PRISM® 7000 Sequence Detecting System was used for running the assay. A five-point standard curve was generated using human genomic DNA with a known concentration. The DNA content of the samples was estimated in relation to the standard curve.

4.4 Genotyping

Genotyping was carried out using the SNPlex™ Genotyping System by Applied Biosystems. The methodology is based on oligonucleotide ligation, polymerase chain reaction and capillary electrophoresis, allowing the analysis of up to 48 bi-allelic single nucleotide polymorphism (SNP) genotypes at the same time (Tobler et al., 2005).

Ligation probe sets were constructed with the help of Applied Biosystems' web-based assay design application.

The genotyping workflow followed the standard protocol for the SNPlex platform as issued by Applied Biosystems. The Applied Biosystems 3730 DNA analyzer was used to perform capillary electrophoresis.

4.5 Statistics

Capillary electrophoresis data was analyzed and genotypes were assigned by Genemapper 3.7. The demographic characteristics of the study population were analyzed by SPSS 13.0 (III) and SPSS 16.0 (I, II). Comparison of allele and

genotype frequencies between cases and controls was performed by chi square test. Conformity of genotype frequencies to Hardy-Weinberg equilibrium was estimated by Haploview 3.32 (Barrett et al., 2005). Permutation tests with 10,000 permutations were performed to calculate corrected p values for multiple testing by the Haploview software.

In Studies I and III, linkage disequilibrium (LD) blocks of markers were defined using the confidence interval method of Gabriel implemented in Haploview 3.32 (Gabriel et al., 2002). Haplotype frequencies between cases and controls were estimated by chi square test implemented in Haploview.

In Study II, haplotype frequencies of cases and controls were compared using the maximum likelihood SEM (Stochastic Expectation-Maximization) algorithm implemented in THESIAS (Tregouet and Garelle, 2007).

The significance level of the probability tests was set for $p<0.05$.

5. RESULTS AND DISCUSSION

5.1 Genotype yield

In Study I (*TPH2*), 14 markers were examined. Initially, 16 markers were chosen for the assay but after genotyping, two were excluded from further analysis due to Hardy-Weinberg disequilibrium ($p<0.05$). Seven controls and 10 cases were removed from the analysis due to less than 50% genotype yield. From the rest, 93.7% of subjects reached >90% genotype yield.

In Study II (4p), 36 markers were examined. Initially, 45 markers were chosen for the assay but after genotyping, two were excluded from further analysis due to Hardy-Weinberg disequilibrium ($p<0.05$) and seven due to failure in genotype assignment. Eight controls and four cases were removed from the analysis due to less than 50% genotype yield. From the rest, 90.4% of subjects reached >90% genotype yield.

In Study III (*LSAMP*), 30 markers were examined. Initially, 32 markers were chosen for the assay but after genotyping, two were excluded from further analysis due to Hardy-Weinberg disequilibrium ($p<0.001$). Seven controls and 10 cases were removed from the analysis due to less than 50% genotype yield. From the rest, 93.7% of subjects reached >90% genotype yield.

Hardy-Weinberg disequilibrium, resulting in removal of some markers from the analyses, may be caused by failure to detect an allele in the study population, which may be related to low frequency SNPs, or by methodology-related bias in assigning allele calls in favor of a certain allele, e.g. the software's failure to detect heterozygotes.

Samples from individuals removed from analysis due to low genotype yield may have been a result of low quality DNA template. Tissue degradation is typically a problem with suicide studies, as well as the reduced quality of DNA that was extracted years before analysis.

5.2 Variation in *TPH2* gene (Study I)

A number of studies has suggested a relationship between serotonergic dysfunction and suicide. The main idea behind testing for an association between *TPH2* genetic variation and suicidal phenotype is that TPH2 is the first, as well as rate-limiting, enzyme in serotonin biosynthesis. Thus, a genetic variant may encode an enzyme with a decreased activity, resulting in less efficient serotonin production.

We investigated the frequencies of 14 SNPs in the *TPH2* gene and flanking regions in a case-control sample of male completed suicides and healthy male volunteers. According to Haploview, an approximately 60% of variance in gene region was captured by the tag SNP set.

No statistically significant differences were observed in any of the 14 loci, either at the allelic, genotype or haplotype levels. Our results are consistent with several other studies. In the first study that failed to confirm a link between *TPH2* locus and suicidality, three common SNPs, hCV245410, hCV8376173 and rs1487289, were investigated in parent-child trios of bipolar patients with a history of suicide attempt (De Luca et al., 2004). No significant associations were revealed either at the allele or haplotype levels. The next study by the same authors compared schizophrenic suicide attempters with non-suicidal schizophrenics, concluding that no differences between allelic or haplotype frequencies were observed between suicidal behavior and two SNPs, rs4131347 and rs11178997, in the promoter region of the *TPH2* gene (De Luca et al., 2005). A third study from the same group examined the same polymorphisms in a sample of suicide completers with a diagnosis of psychosis (De Luca et al., 2006). No associations were reported between the genetic markers and suicide. A recent study failed to detect any difference in *TPH2* promoter SNP rs4131347 allele frequencies between suicide attempters with a mood disorder and healthy volunteers (Mann et al., 2008). Furthermore, the study failed to detect any associations with the gene variant and CSF monoamine metabolite levels.

Despite of the fact that a number of other studies have succeeded to describe an association between *TPH2* gene variants and suicide (Zill et al., 2004; Zhou et al., 2005; Ke et al., 2006; Lopez de Lara et al., 2007; Yoon and Kim, 2009), our results do not confirm their findings. This is similar to various other studies about serotonergic system genes – the replication of an initial association often fails and eventually, no definite conclusion can be reached.

The mechanism underlying the alterations observed in brains of suicidal subjects has not been established. Considering our result, and the results of many other groups that have failed to detect any differences in *TPH2* gene variants in suicidal subjects, it seems plausible that the alterations in serotonin production, observed in the DRN of suicide completers, may be due to factors other than a structural variant of a single gene.

5.3 Variation in 4p region (Study II)

Several independent linkage studies have identified the short arm of chromosome 4 as a putative region of susceptibility for many disorders previously identified as risk factors for suicidal behavior (Blackwood et al., 1996; Ewald et al., 1998; Asherson et al., 1998; Ginns et al., 1998; Detera-Wadleigh et al., 1999; Als et al., 2004; Le Hellard et al., 2007; Christoforou et al., 2007; Vazza et al., 2007), as well as for completed suicide itself (Cheng et al., 2006). These observations have led to the hypothesis that the genomic region harbors genes that play a role in suicidal behavior.

We compared the frequencies of 36 SNPs in the 4p11–16 region between our cases and controls. We did not attempt to cover the whole area with tagSNPs, instead the selection of markers was based on functional criteria: the

majority of the SNPs were nonsynonymous and were located in the coding regions of their respective genes.

While the frequency of 32 allelic variants did not show any differences in frequency between suicide victims and controls, four SNPs – rs138310 in the *EVC* gene, rs734312 in the *WFS1* gene, rs362272 in the *HTT* gene and rs6811863 in the *TBC1D1* gene – showed allelic p values <0.05 (Table 2). Moreover, rs138310 was significantly associated with completed suicide even after permutation tests. Despite of the fact that we investigated polymorphisms all over the chromosome 4 short arm, all associated loci are located in the distal portion of chromosome arm, supporting the evidence from genome-wide linkage studies (Blackwood et al., 1996; Ewald et al., 1998; Asherson et al., 1998; Ginns et al., 1998; Detera-Wadleigh et al., 1999; Als et al., 2004; Le Hellard et al., 2007; Christoforou et al., 2007; Vazza et al., 2007).

However, we cannot claim with absolute confidence that the associated alleles are actually related to phenotype because they may fall near the true risk loci and thus, may be inherited together. Equally, we cannot completely exclude the possibility that the very loci may actually play a causal role in suicide etiology.

5.3.1 Ellis-van Creveld syndrome gene *(EVC)*

The strongest statistical association in this study linkes male completed suicide to a single nucleotide polymorphism, rs138310, in the *EVC* gene, where allele T encoding for glutamine instead of arginine at position 576 was more frequent among suicide subjects [$\chi^2(1)=10.8$, $p=0.001$] (Table 2). Together with rs2302075 in the same gene, a rare protective haplotype GT is formed [OR 6.89 (1.60–29.76), p=0.009].

The gene has been implicated in both human chondroectodermal dysplasia Ellis-van Creveld syndrome and Weyers acrodental dysostosis (Polymeropoulos et al., 1996; Ruiz-Perez et al., 2000; Ruiz-Perez et al., 2003). Homologues of its protein product have been discovered in many species but due to its unique structure, its functions remained unknown. However, it has been demonstrated that EVC protein contains putative transmembrane domains and a leucine zipper element, and is expressed in a wide range of tissues (GeneCards, 2009).

Murine knockout studies suggest that EVC facilitates Indian hedgehog (Ihh) as well as sonic hedgehog (Shh) signaling in the developing skeleton (Ruiz-Perez et al., 2007). Hedgehog signaling plays a crucial role in organogenesis by promoting proliferation and migration of stem cells in the body. In the developing brain, it is a crucial factor acting on the neuroepithelial precursors of the midbrain dopaminergic (mDA) neuron population to specify their neurotransmitter identity (Prakash and Wurst, 2006). Moreover, failure to establish this signaling cascade results in a fate-switch of mDA progenitors into other identities such as rostral hindbrain serotonergic neurons. It may be speculated that a dysfunctional EVC may attenuate the formation of proper dopaminergic

circuits by Shh in the developing limbic system, a set of brain structures associated with emotional behavior and memory.

Table 2. Differences between frequencies (MAF) and absolute numbers (N) of minor alleles in *LSAMP* and 4p loci between suicide completers and healthy volunteers. Displayed are only those SNPs which statistical significance level did not exceed p=0.05 in chi square test. For complete list of results, see Paper II and Paper III, respectively.

Gene symbol	Maj/min allele	Function	Case		Control		Chi square	P value*
			MAF	N	MAF	N		
HD	C/T	Val/Ile	0.37	208	0.31	187	5.74	0.017
EVC	C/T	Arg/Gln	0.43	241	0.34	211	10.8	0.001**
WFS1	A/G	His/Arg	0.46	253	0.53	296	5.56	0.018
TBC1D1	C/G	Pro/Arg	0.39	219	0.45	285	5.02	0.025
LSAMP	T/G	intron 1	0.27	147	0.32	202	4.19	0.041
LSAMP	T/C	intron 1	0.27	147	0.32	203	4.13	0.042
LSAMP	C/T	intron 6	0.32	178	0.38	238	3.98	0.046
LSAMP	G/A	intron 6	0.07	40	0.11	67	4.10	0.043

* Not corrected for multiple testing

** Remains significant ($p<0.05$) after correction for multiple testing

Less is known about hedgehog function in the adult brain. Compelling evidence from a recent study shows that Shh is expressed in the adult pituitary where it facilitates corticotrophin (ACTH) secretion in response to corticotrophin-releasing hormone (CRH), the key mediator of the central nervous system response needed to adapt to stress (Vila et al., 2005).

Considering the findings that implicate the role of neurodevelopmental, HPA axis-related and dopaminergic processes in the etiology of suicidal behavior, the function of EVC in the central nervous system deserves further investigations.

5.3.2 Wolframin gene *(WFS1)*

A higher prevalence of the ancestral A allele of rs734312 in the *WFS1* gene, coding for histidine instead of arginine at position 611 among suicide victims was observed in our study [$\chi^2(1)=5.56$, $p=0.018$] (Table 2).

The *WFS1* gene product wolframin is a transmembrane protein, localized in the endoplasmic reticulum of many tissues including brain (Strom et al., 1998; Inoue et al., 1998). Like EVC, wolframin shows no similarity with any other proteins that would indicate its functions. Currently it has been suggested that wolframin modulates Ca^{2+} uptake by the endoplasmic reticulum (ER), thereby influencing intracellular Ca^{2+} homeostasis (Takei et al., 2006).

The coincidence of beta cell loss and brain structure atrophy in Wolfram syndrome patients has revived the researchers' interest in the possible link between metabolic status and mental health (Karasik et al., 1989; Rando et al., 1992). The presence of insulin receptors in limbic system (Hill et al., 1986) suggests the role of insulin in dopaminergic neurotransmission (Saller, 1984; Carvelli et al., 2002). The widespread evidence supports the role of dopamine in impulsivity (Pattij and Vanderschuren, 2008), which has been shown to be one of the main behavioral risk factors in predisposition to suicidal behavior (Simon et al., 2001; Maser et al., 2002; Swann et al., 2005). Inherited individual differences in dopamine reuptake may affect dopaminergic signaling, and therefore contribute to behavioral inhibition (Congdon et al., 2008). Considering the role of insulin in dopaminergic circuits, it may be speculated that deficiencies in insulin-related signaling pathways may lead to failure in regulating extracellular dopamine levels and even excitotoxicity-induced apoptosis. This would explain vulnerability of neurons in Wolfram syndrome patients.

The rs734312 polymorphism, located in exon 8 of the *WFS1* gene, has been studied in relation to psychiatric disorders (Furlong et al., 1999; Middle et al., 2000; Ohtsuki et al., 2000; Kato et al., 2003; Koido et al., 2005). Sequiera et al. (2003) has found an association of the minor allele G with completed suicide in the general population (Sequeira et al., 2003). However, the authors acknowledged possible bias in the results because a marginal departure from the Hardy-Weinberg equilibrium for this locus was detected.

On the contrary, we observed a higher prevalence of the A allele in our sample of suicide victims. Interestingly, another member of our research group has associated allele A with bipolar disorder in the Estonian population (Koido et al., 2005). Similarly, a slight but not statistically significant prevalence of the same allele among bipolar patients has been shown by others (Furlong et al., 1999; Middle et al., 2000; Ohtsuki et al., 2000; Kato et al., 2003). Concerning bipolar disorder, it has been shown that the mood stabilizing agent valproate induces wolframin expression in neurons (Kakiuchi et al., 2009).

5.3.3 Huntingtin gene *(HTT)*

The rs362272 polymorphism is a missense nucleotide change in exon 61 of *HTT* gene, resulting in an isoleucine instead of a valine at position 2786. In our study, the minor T allele was more prevalent in the sample of suicide completers [$\chi^2(1)=5.74$, $p=0.017$] (Table 2).

The *HTT* gene encodes huntingtin, a protein involved in another neurodegenerative disorder, Huntington's disease (HD), characterized by a combination of motor, cognitive, and psychiatric symptoms (The Huntington's Disease Collaborative Research Group, 1993). Practitioners agree that suicide rates in HD patients remain higher than those found in other neurodegenerative diseases, although the *HTT* gene itself has never been considered as a candidate gene for suicide outside of the HD patient population (Cummings, 1995).

Huntingtin is expressed predominantly in the brain (Li et al., 1993). Its exact function is unknown but the pathogenesis of HD is related to abnormal cellular Ca^{2+} homeostasis (Panov et al., 2002). Development of the disease has been associated with a polyglutamine repeat in the first exon of the gene that causes the protein product to aggregate and cause apoptosis (Li and Li, 2004). Studies have demonstrated that expression of defective huntingtin initiates the UPR in the ER and increases Ca^{2+} uptake by mitochondria, subsequently inducing the release of pro-apoptotic factors and selective loss of striatal neurons (Reijonen et al., 2008). Recent studies have suggested altered cholesterol homeostasis in HD after it was found that cultured human and mouse cells expressing mutant huntingtin show reduced mRNA expression for cholesterol biosynthetic enzymes (Markianos et al., 2008). Considering the previously established link between low cholesterol and suicide, the impact of common *HTT* variants on cholesterol metabolism remains to be addressed.

Up to date, there are no data available about whether the rs362272 is a functional polymorphism or not, therefore the exact physiological role of this amino acid change remains to be elucidated. However, it may be speculated that the isoleucine variant may alter the protein function and in cooperation with *WFS1* rs734312 A or another causal variant, the effect on the ER stress pathways would become amplified.

5.3.4 *TBC1D1* (*tre-2/USP6, BUB2, cdc16 domain family member 1*) gene

Fourth, our study of the 4p region revealed an association between the rs6811863 in the *TBC1D1* gene and suicide. The allele C coding for proline instead of arginine at position 44 was more frequent in suicide group [$\chi^2(1)$= 5.74, p=0.017] (Table 2).

The protein product of *TBC1D1* is a member of the TBC1 Rab-GTPase family, which is expressed widely in the human body (Taylor et al., 2008). Recent studies have revealed its role as protein kinase B (Akt) and 5'AMP-activated protein kinase (AMPK) substrate in myocytes, regulating both insulin- and contraction-stimulated glucose uptake by the cell (Sakamoto and Holman, 2008). Presumably, TBC1D1 functions as its paralog AS160/TBC1D4, by inhibiting glucose transporter GLUT4 exocytosis in the GDP-bound state (Roach et al., 2007).

The phosphoinositide 3-kinase (PI 3-K)/Akt cascade has been implicated in numerous physiological functions including adrenergic, serotonergic and dopaminergic neurotransmission. For example, it has been demonstrated that insulin modulation of AMPH-induced trafficking of DAT exploits the PI 3-K/Akt pathway (Garcia et al., 2005). Phosphorylated Akt targets a number of substrates in both the cytoplasm and the nucleus, including TBC1D1. Attenuated activities of PI 3-K and Akt have been reported in the brains of suicide victims (Hsiung et al., 2003). In addition, it has been demonstrated that Akt is sensitive to changes in cholesterol levels (Adam et al., 2007), which is another variable previously associated to suicide risk (Sarchiapone et al., 2000). Considering the aforementioned findings, it would be interesting to investigate whether and how TBC1D1, distinct from upstream components of its activating pathway, participates in development of psychiatric disorders.

5.4 Variation in *LSAMP* gene (Study III)

Neuroimaging studies have demonstrated volumetric abnormalities of limbic structures in suicide victims (Monkul et al., 2007), suggesting that impaired neurodevelopment or synaptic plasticity might contribute to psychiatric disorders and suicidal behavior. Limbic system-associated membrane protein (LSAMP) facilitates formation of functional circuits between limbic structures (Keller et al., 1989; Zhukareva and Levitt, 1995; Pimenta et al., 1995; Eagleson et al., 2003), thus making a novel candidate for studies of suicide biology.

We examined the frequencies of 30 SNPs in the *LSAMP* gene and flanking regions in our case-control sample of male completed suicides and healthy male volunteers. Our study revealed that ancestral alleles of four polymorphisms in the *LSAMP* gene were found to be slightly overrepresented in suicide subjects: rs4831129 and rs9874470 from intron 1 [$\chi^2(1)$=4.19, p=0.041 and ($\chi^2(1)$=4.13, p=0.042, respectively] and rs2918213 and rs2918215 from intron 6 [$\chi^2(1)$=3.98,

p=0.046 and ($\chi^2(1)=4.1$, p=0.043, respectively] (Table 2). Moreover, the two intron 1 SNPs seem to increase the risk in a dose-dependent way: the differences between study groups were more prominent if only the predisposing allele homozygote frequencies were compared. However, none of the associations were resistant to the correction for multiple testing.

The limbic system is a set of brain structures involved in emotional processing, decision-making and predicting long-term outcomes of one's behavior. The relatively diminished cortical modulation of lower limbic structure activity might contribute to impulsive acts and heightened susceptibility to environmental stress; the traits often associated with autoaggressive behavior. The observed changes could be applied to several causative mechanisms, including impaired neurodevelopment or synaptic plasticity.

Limbic system-associated membrane protein (LSAMP) is a molecule that promotes cell adhesion and neurite outgrowth from specific populations of fetal neurons (Keller et al., 1989; Zhukareva and Levitt, 1995; Pimenta et al., 1995; Eagleson et al., 2003). The effect of the LSAMP on the development of limbic circuits has gained considerable attention in the last decade. Although its expression in CNS structures was demonstrated more than 20 years ago(Levitt, 1984), its molecular characteristics as well as specific mode of action currently are being revealed (Zhukareva and Levitt, 1995; Cote et al., 1995; Pimenta et al., 1995; Zhukareva et al., 1997; Mann et al., 1998; Gil et al., 2002; Eagleson et al., 2003).

Little is known about the role of LSAMP in the adult organism. In the brain of adult rats, LSAMP is expressed on dendrites and cell bodies rather than axons (Cote et al., 1995). Unpublished data from Philips and colleagues indicate that in the mouse brain, the pattern of LSAMP expression is not limited to limbic structures but appears widely all over the neocortex, particularly in sensory areas. A recent study by Catania et al. showed that *Lsamp* knockout mice exhibit a maladaptive response to novel environmental stressors, expressed as behavioral disinhibition (Catania et al., 2008). On the contrary, is has been demonstrated that high anxiety rats, as indicated by lower exploratory activity in the elevated plus-maze model, have up-regulation of the *Lsamp* gene in the amygdala and periaqueductal gray matter (Nelovkov et al., 2003; Nelovkov et al., 2006). Very preliminary data from the *Lsamp* knockout mouse study by Philips and colleagues suggests that these animals exhibit mild alterations in social interactions such as lack of whisker trimming. Earlier studies have indicated that barbering of cage mates is an essential form of dominant behavior in rodents and lack of it may represent a failure to establish a normal hierarchical social interaction between animals.

To the author's knowledge, our study is the first to regard the possible association of *LSAMP* with human behavior. Despite the fact that none of the associations were resistant to the correction for multiple testing, the results of the present study point to a novel factor in suicide etiology. Further replication studies are required to see if our initial associations occurred by chance. The potential functional consequences of the SNPs under study remain unknown at

present. All four of the initially associated SNPs lie within the intron region, so no direct change in amino acid sequence results in the polymorphism. However, considering their conserved nature, it is possible that they are linked to a nearby functional polymorphism or act as regulatory elements.

5.5 Limitations to studies of suicide biology

As described earlier, suicidality represents a very heterogenous range of behaviors. Whereas completed suicide refers to a distinct phenotype, suicide attempts range from acts with a very low level of lethality and intent to draw attention, to fortunate survivals of those who were actually determined to take their life. Nevertheless, the majority of studies consider suicide attempters as a homogenous group and generalize any results obtained to the rest of the suicidal population. One of the possible explanations why studies of suicide biology have led to discrepant results is the possibility that different types of suicidal behavior may not share the same etiological features. Attempted suicide, even in a manner that favors discovery and rescue, may be a strong predictor of completed suicide, but extrapolation of results from suicide attempters to suicide completers, and vice versa, may not be a reliable way of making conclusions. Considering the varying motivational background of suicidal acts, uniform diagnostic criteria need to be established in order to identify different subtypes of suicidal behavior. Until then, suicide attempters and suicide completers should be investigated as partly overlapping but separate phenotypes.

Due to large gender differences in suicidal rates, as well as different psychiatric profiles of suicidal men and women, these two groups should be considered separately in suicide studies. However, many studies still tend to pool both genders in their analyses (De Luca et al., 2005; Haghighi et al., 2008). In addition, various lines of evidence suggest age-related differences in underlying etiology. For example, the psychiatric profiles of different age groups do not overlap (Turecki, 2005). Thus, it remains to be investigated whether young and elderly suicides have different diatheses.

A number of studies involve one diagnostic group of suicidal patients, such as those with mood disorders, schizophrenia, or alcoholism, and compare these to healthy controls (Westrin et al., 1998a; Westrin et al., 1998b; Abbar et al., 2001). Such a design cannot discriminate whether the biological markers are specific to a single diagnosis or allow generalizations to the whole suicide population. Other case-control studies of suicidal psychiatric patients select the control group from other non-suicidal patients within the same diagnostic category (De Luca et al., 2005; Ke et al., 2006; Lopez de Lara et al., 2007). In order to exclude the likelihood that the association with markers are due to to concomitant psychiatric disorder rather than suicide itself, the latter seems to serve as a preferred option.

However, other studies include suicide attempters/completers regardless of their diagnosis (Gross-Isseroff et al., 2000; Zalsman et al., 2005). It certainly increases the probability that any observed alterations are characteristic to a mental illness rather than suicide, but as described earlier, many suicide victims lack diagnostic data because they have not been diagnosed with a mental illness during their lifetime. Often this data is obtained post mortem by psychological autopsy, the method for which validity and reliability have been questioned repeatedly (Pouliot and De Leo, 2006).

Technical problems working with suicide samples include tissue degradation that is caused by varying intervals between time of death and body discovery. This is extremely critical for mRNA and protein expression studies, where sample quality directly determines the outcome. In addition to postmortem delay, several other factors may contribute to bias in the RNA expression profile, such as direct cause of death, agonal factors, tissue pH, post-autopsy tissue handling, dissection procedures, and tissue storage conditions (Gwadry et al., 2005). In addition, the lack of toxicological data can lead to important bias in expression studies. However, these variables are less essential in genetic association studies because the DNA sequence is not as sensitive to the abovementioned variables and modern genotyping methods allow amplification of even a very small amount of intact DNA template. Nevertheless, gene studies have several limitations of their own, the main problem being the inability to predict which events are going to influence the path between gene sequence and the signaling protein it encodes, which are the factors affecting downstream steps of transcription, translation, protein processing etc.

Association studies have been advocated as the method of choice for the investigation of genetic factors in complex phenotypes such as suicide. However, they have a number of limitations. There is a considerable lack of sufficient statistical power in individual studies to detect anticipated small gene effects, even in relatively large samples. Another problem with case-control studies is that ethnic differences can contribute to observed differences in allele frequencies. Therefore, it is important to match cases and controls for ethnicity in order to avoid bias due to population stratification. The alternative option would be to use family-based strategies, as far as these are not sensitive to population stratification, but subjects are more complicated to recruit.

5.6 Limitations to our studies

The major limitation of our studies is the lack of psychiatric diagnoses of suicide subjects. Therefore, we cannot claim that the associations are characteristic to a concomitant psychiatric disorder or to suicide itself. Replication studies on samples with diagnostic data are required in order to confirm our observations.

Second, we did not capture the whole variance in the genes under observation. In Study I (*TPH2*), the tagSNPs covered approximately 60% of the

polymorphic variation, but two other studies either described only one SNP per gene (Study II, 4p) or covered only a small region of the gene (Study III, *LSAMP*). Ideally, to draw conclusions about the relationship between a genotype and a phenotype, the DNA of study population should be investigated by a whole genome assay. As yet, the methods are too resource-demanding, but the technology is advancing rapidly; this may become an option in the future.

Third, sample size is always a critical issue in association studies, directly determining the power of the results. The same applies for our study. Genes of a complex phenotype such as suicide are likely to be genes of modest effect, exerting small effects. The sample sizes may need to be large depending upon the gene effect and allele frequency. In our study, most of the significant associations obtained by chi square test were not retained after correcting for multiple comparisons. Often the correcting procedure can be overly conservative but on the other hand, omitting the correction step may result in Type I error.

6. CONCLUSIONS

1. The results of the present study do not support the hypothesis about the role of structural variants of the tryptophan hydroxylase second isoform (*TPH2*) gene in male completed suicide.
2. The data analyses suggest that the single nucleotide variation in the 4p chromosomal region may be associated with male completed suicide. Considering the results of the chi square test, the allele frequency of four single nucleotide polymorphisms in the Ellis-van Creveld syndrome (*EVC)* gene, in the wolframin (*WFS1)* gene, in the huntingtin (*HTT)* gene and in the *TBC1D1* gene, were significantly different between suicides and controls. In case of the SNP in the *EVC* gene, statistical significance was retained even after correcting for multiple comparisons.
3. The results suggest that genetic variants of limbic system-associated membrane protein (*LSAMP*) may be associated with male completed suicide. However, the results should be taken with precaution since none of the associations remained significant after correcting for multiple testing.

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SUMMARY IN ESTONIAN

Uurimusi geneetilise variaabelsuse rollist meeste enesetappudes Eesti populatsioonis

Suitsidaalse käitumise epidemioloogia ning olemasolevad teadmised selle etioloogiast

Viimastel aastakümnetel on teaduspõhine meditsiin teinud tohutuid edusamme erinevate haiguste tekkepõhjuste väljaselgitamisel ning nende avaldumise ennetamisel. Hoolimata sellest, ning rahvatervisespetsialistide jätkuvatest pingutustest suitsidaalse käitumise preventsioonis, on enesetappude sagedus üle maailma jätkuvalt kõrgel tasemel ning näitab osade epidemioloogide arvates isegi tõusutrendi (Mann, 2002). Mingil põhjusel on suitsiidisuremus eriti kõrge soome-ugri rahvaste hulgas ning endistes Nõukogude Liidu liikmesriikides (WHO, 2009).

Uurimused näitavad, et enam kui 90% suitsiidisooritajatest kannatab mingi psüühikahäire all (Arsenault-Lapierre et al., 2004) ning on alust arvata, et see number on isegi suurem, sest suur osa enesetappudest pannakse toime haiguse algfaasis, kus indiviid pole veel psühhiaatri juurde jõudnud (Angst et al., 2002). Hoolimata sellest, et psüühikahäire on oluliseks suitsiidiriski suurendavaks faktoriks, ei ole mitte kõik psühhiaatrilised patsiendid suitsidaalsed (Bondy et al., 2006). Selle põhjal saab järeldada, et psüühikahäire ei ole mitte ainus suitsiidi riskifaktor, vaid pigem üks osa suitsiidiriski vahendavast fenotüübist, mille teised komponendid võivad olla näiteks teatud isiksuseomadused (Brent and Mann, 2005).

Suitsidaalne käitumine on tugevalt pärilik, nagu nähtub kaksikute-, adoptsiooni-, ja perekonnauuringutest (Baldessarini and Hennen, 2004). Suitsiidi kui psüühikahäirest sõltumatu geneetilise komponendi hüpoteesi kinnitab asjaolu, et suitsidaalne käitumine pärandub sõltumatult sellega kaasnenud psühhiaatrilisest haigusest, ent koos impulsiivsete ja/või agressiivsete isiksuseomadustega (Brent and Melhem, 2008).

Üks huvipakkuvatest asjaoludest suitsiidi epidemioloogias on fakt, et kuigi naised sooritavad meestest oluliselt sagedamini suitsiidikatseid, on meeste suremus suitsiidi läbi neli kuni viis korda naiste omast kõrgem (Hawton, 2000). See annab aluse arvata, et eksisteerivad soolised erinevused suitsiidi bioloogias, ning põhjuse suitsiidiuurimustes sugusid eraldi käsitleda.

Suitsiidi bioloogilise tausta väljaselgitamiseks on läbi ajaloo lugematu hulk uurimusi läbi viidud. Kui mitme aastakümne jooksul tegeles uurijaskond peamiselt serotoniinisüsteemi alatalitluse hüpoteesi kinnitamise ja ümberlükkamisega, on viimase aja jooksul hakanud jõudu koguma ka alternatiivsed teemad: suitsidaalsust on püütud seostada katehhoolamiinide ning gamma-aminovõihappe süsteemi düsfunktsiooniga, hüpotalamuse-hüpofüüsi-neerupealiste telje hüperaktiivsusega, närvikasvufaktoritega seotud muutustega aju morfomeetrias,

immuunsüsteemiga tasakaalustamatusega, madala kolesteroolitasemega, reniini-angiotensiini süsteemi kuuluvate neuropeptiididega ja nii edasi. Kõige värskemalt tõstatunud teema on rakusiseste signaalikaskaadide roll psühhiaatriliste haiguste väljakujunemises ning esimesed tulemused selles vallas on paljulubavad.

Vasturääkivused erinevate autorite tulemustes pole seni võimaldanud suitsiidi põhjuslikke mehhanisme üheselt kirjeldada. Erinevad tulemused on seletatavad mitmete asjaoludega. Näiteks ekspressiooniuurimuste puhul on äärmiselt oluline uuritava materjali kvaliteet. Suitsiidimaterjali kogumisel ei ole alati võimalik tagada koe värskust, sest intervall surmahetke ning lahangule jõudmise vahel võib olla väga erinev. Geenide ekspressiooniprofiili võivad mõjutada veel enesetapumeetod, koe pH tase, lahangumeetodid, koematerjali lahangujärgne käitlemine ja selle säilitamise tingimused. Oluliseks tulemusi mõjutavaks faktoriks on toksikoloogilised faktorid: suitsiidiohvri organismis võib leiduda alkoholi, mõnuaineid, ravimeid või nende jääke, mis kõik potentsiaalselt geeniekspressiooni mõjutavad.

Kuigi genoomne järjestus eelpool loetletud faktoritest ei sõltu, ning tänapäeva võimsad genotüpeerimismeetodid võimaldavad amplifitseerida ka väga väikest kogust intaktset DNAd, on geneetilistel uuringutel omad piirangud. Näiteks valimi suurusega seotud nüansid: assotsiatsiooniuurimustes võib liiga väikese grupi puhul positiivne seos tänu mitmese testimise rangetele kriteeriumitele märkamata jääda, või siis vastupidi – ilmneb valepositiivne seos.

Erinevad tulemused võivad olla põhjustatud ka valimi koosseisust: osad uurijad vaatlevad meessoost ja naissoost suitsidaalseid indiviide ühtse grupina, kuigi mitmed tõendid lubavad oletada, et soolised erinevused suitsiidide esinemissageduses ei ole vaid sotsiaalsete põhjustega. Mõned autorid valivad uurimiseks kindla psühhiaatrilise diagnoosiga enesetapjad. Sellisel juhul ei ole päris selge, kas leitud seosed on põhjustatud kaasuvast vaimsest häirest või suitsidaalsusest. Osaliselt võib tulemuste vasturääkivus olla seotud ka suitsiidikäitumise heterogeensusega. On alust oletada, et suitsiidikatse on väga laia spektriga fenotüüp: alates indiviididest, kes madala letaalsusega meetodit rakendades otsivad vaid tähelepanu, lõpetades nendega, kes tõsise vigastuse kiuste elule tagasi võidetakse. Seega tuleks hoolikalt analüüsida, kas ellu jäänud suitsiidikatsetajatel saadud tulemuste põhjal saab teha järeldusi nende indiviidide kohta, kes on vabasurma läinud kõrge letaalsusega meetodi läbi.

Kokkuvõtteks võib öelda, et suitsiid on kompleksne fenotüüp, mis ei ole põhjustatud vaid ühe või paari geeni või nende produktide hälbest. Suitsidaalse käitumise aluseks on tõenäoliselt hulk kumuleeruvaid riskifaktoreid, millel kõigil on oma neuraalne substraat. Seega on õigustatud suitsiidi bioloogia mitmekülgne uurimine võimalikult paljude, sealhulgas kvantitatiivse geneetika meetoditega.

Uurimuse eesmärk

Kuna eelnevad uuringud on viidanud pärilike tegurite osalusele suitsidaalse käitumise väljakujunemisel, sai antud uurimuse põhieesmärgiks viia läbi assotsiatsiooniuuringud Eesti populatsioonis, võrdlemaks mõningates genoomiregioonides esinevate struktuursete variantide sagedust suitsiidi sooritanud meeste ning meessoost tervete vabatahtlike grupi vahel, et selgitada välja uuritavate lookuste võimalik seotus suitsiidiga.

Alaeesmärgid:

1. Serotoniinisüsteemi alatalitlus on üks suitsidaalse käitumise uuritumaid bioloogilisi korrelaate. Trüptofaan hüdroksülaasi teine isovorm (TPH2) katalüüsib esimest konversiooniastet kesknärvisüsteemis toodetava serotoniini biosünteesis, mistõttu on alust oletada, et geneetiline muutlikkus *TPH2* geenis võib põhjustada häireid ensüümi toimimises ning seeläbi aju serotoniinisünteesi võimekuse langust. Töö eesmärgiks oli võrrelda 14 üksiknukleotiidi polümorfismi (SNP) sagedust *TPH2* geenis meessoost suitsiidiohvritel ning meessoost vabatahtlikel.
2. Neljanda kromosoomi lühikest õlga (4p) on genoomiülestes aheldusuuringutes korduvalt seostatud psüühikahäirete ning suitsidaalse käitumisega. Töö eesmärgiks oli uurida 36 selles regioonis asuva SNP võimalikku seost suitsiidiga.
3. *Post mortem* uuringud on näidanud, et suitsiidiohvrite ajus esineb volumeetrilisi iseärasusi. Eriti sagedased on mahumuutused limbilises süsteemis, mida peetakse emotsioonide reguleerimise ja otsuste langetamise protsessidega seotud ajustruktuuride kogumiks. Limbilise süsteemiga seotud membraanivalk (LSAMP, *limbic system-associated membrane protein*) on adhesioonimolekul, mis osaleb limbilise süsteemi struktuuride vaheliste ühenduste väljakujunemises ja seega ilmselt ka limbilise süsteemi adekvaatses toimimises. Töö eesmärgiks oli kontrollida, kas 30 SNP-d *LSAMP* geeni algus- ja lõpuosas on seotud suitsiidiga.

Valim ja meetodid

Uurimismaterjalina kasutati DNA-d, mis oli pärit 288 meessoost suitsiidiohvrilt (keskmine vanus 42.8±13.69 aastat) ning 327 psüühiliselt ning kehaliselt tervelt meessoost vabatahtlikult (keskmine vanus 40.5±14.49 aastat). Suitsiid diagnoositi ning suitsiidiohvrid lahati Eesti Kohtuarstlikus Ekspertiisibüroos. Suitsiidimeetoditest prevaleeris poomine (88%), järgnes laskevigastus (10%) ja ülejäänud meetodid: kõrgelt hüppamine, lõikevigastus, mürgistus, termotrauma, uppumine (kokku 2%). Surmaeelne psühhiaatriline diagnoos oli kättesaadav vaid väga vähese hulga indiviidide kohta, seega neid andmeid artiklites ei kasutatud. Tervete vabatahtlike sobivust hinnati TÜ Kliinikumi Psühhiaatriakliinikus Mini rahvusvahelise neuropsühhiaatrilise intervjuuga (Mini International Neuropsychiatric Interview 5.0.0.).

Uuritavad markerid valiti välja andmebaasi HapMap (http://www.hapmap.org) ning programmide Haploview (Barrett et al., 2005) ning SNPBrowser (De La Vega et al., 2006) abiga. DNA puhastati klassikalisel fenooli-kloroformi ekstraktsioonimeetodil kas täisverest (kontrollgrupp) või neerukoest (suitsiidigrupp). Genotüpeerimine viidi läbi SNPlex platvormil (Applied Biosystems). Demograafiliste andmete analüüsimiseks kasutati SPSS paketti; alleelide sagedust, Hardy-Weinbergi tasakaalu ning mittetasakaaluliste ahelduste struktuuri hinnati Haploview abil, haplotüüpide esinemissagedust kas Haploview (artiklid I ja III) või THESIASe (artikkel II) abil.

Töö tulemused ja järeldused

Esimene töö käsitles *TPH2* lookuse strukturaalse variaabelsuse seost suitsiidiga. Kuivõrd suitsidaalset käitumist on korduvalt seostatud serotoniinisüsteemi alatalitlusega, on ka suitsiidi kandidaatgeene enim otsitud serotoniinisüsteemiga seotud lookuste hulgast. Hoolimata mõningatest varasematest seostest *TPH2* genotüübi ja suitsidaalsuse vahel (Zill et al., 2004; Zhou et al., 2005; Ke et al., 2006; Lopez de Lara et al., 2007; Yoon and Kim, 2009), ei õnnestunud meie uurimuses sarnaselt mõnede teistega (De Luca et al., 2004; De Luca et al., 2005; Zill et al., 2007) täheldada ühtegi assotsiatsiooni ei alleelide, genotüüpide ega haplotüüpide tasemel. Kokkuvõttes ei toeta meie tulemused hüpoteesi, mille kohaselt on *TPH2* geneetilised variandid seotud suitsiidi fenotüübiga.

Järgmises uurimuses võrdlesime 36 üksiknukleotiidse polümorfismi sagedust 4. kromosoomi lühikeses õlas. Antud regiooni on genoomiülestes aheldusuuringutes sageli seostatud mitmete suitsiidiriski suurendavate psüühikahäirete esinemisega (Blackwood et al., 1996; Ewald et al., 1998; Asherson et al., 1998; Ginns et al., 1998; Detera-Wadleigh et al., 1999; Als et al., 2004; Le Hellard et al., 2007; Christoforou et al., 2007; Vazza et al., 2007), samuti suitsiidi endaga (Cheng et al., 2006). Kolmekümne kuuest analüüsitud markerist nelja – rs138310 Ellis-van Creveldi sündroomi (*EVC)* geenis, rs734312 volframiini (*WFS1)* geenis, rs362272 huntingtiini (*HTT)* geenis ja rs6811863 *TBC1D1* geenis – esinemissagedus erines oluliselt suitsiidide ja kontrollide vahel ($p<0.05$), ning erinevus rs138310 alleelide esinemissageduses säilis isegi pärast 10 000 kordusega permutatsioonitesti rakendamist. Ühegi nimetatud geeni produkti funktsioon pole praeguse seisuga kindlalt teada.

Teatud EVC mutatsioonide tulemusena kujuneb välja Ellis-van Creveldi sündroom – kondroektodermaalne düsplaasia – ning Weyersi akrodentaalne düsostoos (Polymeropoulos et al., 1996). Transgeensete hiirte uuringud näitavad, et EVC soodustab *hedgehog* signaaliülekannet arenevas skeletis (Ruiz-Perez et al., 2007). On teada, et *hedgehog* signaalisüsteem on väga oluline ka arenevas ajus, näiteks osaleb see keskaju dopamiinergilise neuronipopulatsiooni väljakujunemisel (Prakash and Wurst, 2006). Täiskasvanud ajus ekspresseerub *hedgehog* hüpofüüsis, kus ta soodustab kortikotropiini sekretsiooni vastusena kortikotropiini vabastava faktori mõjule (Vila et al., 2005). Võttes arvesse tõen-

deid selle kohta, et suitsiidiohvrite ajus on leitud muutusi, mis viitavad närvisüsteemi puudulikule arengule, dopamiinisüsteemi ja hüpotalamus-hüpofüüsineerupealiste telje funktsioonihäiretele, tuleks EVC rolli suitsiidi fenotüübi väljakujunemises kindlasti edaspidigi uurida.

WFS1 geeni produkt on volframiin, unikaalse struktuuriga transmembraanne valk, mis paikneb mitmete eri rakutüüpide endoplasmaatilises retiikulumis. Närviteadlaste huviorbiiti on ta tõusnud tänu asjaolule, et *WFS1* mutatsioonide tulemusena kujuneb välja Wolframi sündroom, mille iseloomulikud sümptomid on magediabeet, suhkrudiabeet, nägemisnärvi atroofia ja kurtus (Strom et al., 1998; Inoue et al., 1998). Lisaks sellele kannatavad Wolframi sündroomiga patsiendid ning nende lähisugulased erinevate psüühikahäirete all ning sooritavad üldpopulatsiooniga võrreldes sagedamini enesetappe (Swift and Swift, 2000). Volframiini arvatavaks funktsiooniks on reguleerida kaltsiumi tagasihaaret tsütoplasmast endoplasmaatilisse retiikulumi (Takei et al., 2006). Rakusisene kaltsiumidünaamika on väga oluline raku eluliste funktsioonide täitmise seisukohalt ning häired selles võivad viia isegi rakusurmani.

Post mortem uuringud Wolframi sündroomiga patsientidel on näidanud mitmete ajustruktuuride märkimisväärset atroofiat ning pankrease beetarakkude kadu (Karasik et al., 1989; Rando et al., 1992). Neid kahte rakupopulatsiooni ühendavaks nimetajaks on insuliin, mis pole küll neuronite jaoks oluline glükoosi metabolismi seisukohalt, kuid etendab närvisüsteemis sellegipoolest märkimisväärset rolli: insuliiniretseptorid ekspresseeruvad ulatuslikult limbilises süsteemis (Hill et al., 1986) ning insuliinivaegus vähendab dopamiini transporteri ekspressiooni, pikendades sellega dopamiini toimet postsünaptilistele retseptoritele (Patterson et al., 1998). Võttes arvesse, et seost metaboolse sündroomi ja psühhiaatriliste haiguste koosesinemise vahel kirjeldati juba 90 aastat tagasi, tasub volframiini psüühikahäirete kontekstis kindlasti tulevikus jätkuvalt uurida.

Mutatsioonid huntingtiini geenis viivad Huntingtoni tõve väljakujunemisele. Ka selle sündroomi puhul lisaks neuroloogilistele sümptomitele täheldatud kõrgendatud suitsiidiriski (Cummings, 1995). Huntingtiini täpne funktsioon ei ole teada, kuid arvatakse, et ka see valk on sarnaselt volframiinile seotud rakusisese kaltsiumidünaamikaga ning osaleb endoplasmaatilise retiikulumi stressi väljakujunemises (Panov et al., 2002; Reijonen et al., 2008). Võib arvata, et huntingtiin mängib rolli ka kolesterooli metabolismis, kuivõrd *in vitro* uuringud on näidanud, et mutantsetet huntingtiini ekspresseerivates rakkudes on vähem kolesterooli sünteesiga seotud ensüümide mRNA-d (Markianos et al., 2008). Madalat kolesteroolitaset on varemgi seostatud suitsiidiriskiga (Sarchiapone et al., 2000; Sarchiapone et al., 2001; Guillem et al., 2002; Kim et al., 2002), seega on ka huntingtiin edaspidiseks uurimistööks väärt sihtmärk.

TBC1D1 (tre-2/USP6, BUB2, cdc16 *domain family member* 1) kodeerib üht valku TBC1 Rab-GTPaaside perekonnast. Kuigi võrdlemisi vähe uuritud, on siiski teada, et ilmselt on tegemist proteiinkinaas B (Akt) ja 5'AMP poolt aktiveeritud proteiinkinaasi (AMPK) substraadiga (Sakamoto and Holman, 2008). Rab-perekonna valguna on tema funktsiooniks transportvesiikulite suunamine.

On näidatud, et müotsüüdis reguleerib *TBC1D1* glükoosi transporteri eksotsütoosi vastusena insuliini toimele (Roach et al., 2007). Seega oleks huvitav tulevikus välja selgitada, kas ta osaleb ka virgatsainetega seotud transportvesiikulite liikluse reguleerimises.

Kolmas artikkel vaatles variaabelsust limbilise süsteemiga seotud membraanivalgu (*LSAMP*) geenis. Kuna tegemist on väga pika geeniga, ei õnnestunud kogu variaabelsust antud lookuses üheainsa SNPlex markerikomplektiga kirjeldada. Seega valisime välja kaks lõiku: geeni alguse ja lõpu ning vastavalt sellele eelnevad ning järgnevad alad, kus võivad tõenäoliselt paikneda geeni transkriptoorset aktiivsust reguleerivad elemendid.

LSAMP valimine kandidaatgeeniks on põhjendatud asjaoluga, et suitsiidiohvrite ajus on täheldatud mitmeid strukturaalseid muutusi, kusjuures enamik neist lokaliseerub limbilisse süsteemi, ajustruktuuride kompleksi, mis on seotud emotsioonide reguleerimise, otsuste langetamise ja oma tegevuse tulemuste ettenägemisega (Monkul et al., 2007). LSAMP on adhesioonimolekul, mille ülesandeks on närvisüsteemi varastel arenguetappidel juhtida limbilise süsteemi neuronite jätkeid, nii et nendest moodustuksid funktsionaalsed närviringed. Seega on tõenäoline, et puudulikult toimiv LSAMP võib häirida piisavate ühenduste moodustumist, pärssides sellega ajukoorest lähtuva pidurdava modulatsiooni jõudmist madalamatesse limbilistesse struktuuridesse ning soodustades impulsiivse käitumise ja kontrollimatute emotsioonide esilepääsu ning kõrgendatud haavatavust stressi suhtes.

Uuringud närilistel on näidanud, et hiired, kellel puudub *Lsamp* geen, reageerivad keskkondlikule stressile käitumusliku pidurdamatusega (Catania et al., 2008) ning et kõrge ärevusetasemega rottidel on *Lsamp* geeni ekspressioon mandelkehas ja aju veejuhaümbrises hallaines tõusnud (Nelovkov et al., 2003; Nelovkov et al., 2006). Philipsi ja kolleegide veel avaldamata andmetel esineb *Lsamp* geenita hiirtel häireid sotsiaalses interaktsioonis, täpsemalt puurikaaslaste vurrude pügamises, mis hiirte puhul väljendab isenditevahelise hierarhiat.

Meie tulemused näitasid statistilist olulist seost ($p<0.05$) nelja *LSAMP* SNP ja suitsiidi vahel. Kuivõrd tegemist on introni piirkonna polümorfismidega, pole olemasoleva info põhjal võimalik väita, kas ja kuidas need mõjutavad geeniekspressiooni või LSAMP valgu funktsionaalsust. Igal juhul annab leitud seos aluse edaspidisteks täpsustavateks uuringuteks.

Kokkuvõtteks võib öelda, et antud töö tulemusena sai ümber lükatud hüpotees selle kohta, et *TPH2* genotüüp on suitsiidiga seotud, kuid vastu võetud hüpoteesid selle kohta, et suitsiid on seotud variaabelsusega *LSAMP*, *HTT*, *WFS1*, *EVC1* ja *TBC1D1* geenides, mis annab tulevikus alust nende geenide funktsiooni lähemaks uurimiseks.

ACKNOWLEDGEMENTS

This investigation was conducted at the Institute of Physiology and Institute of General and Molecular Pathology of the University of Tartu; Psychiatry Clinic of Tartu University Hospital; and the Estonian Forensic Examination Bureau. It was supported by Estonian Science Foundation grant nos. 3271, 5234, 7053 and 7479; a targeted finance grant SF0180125s08 from the Estonian Ministry of Education and Research; and the European Regional Development Fund.

My sincerest gratitude to everyone who contributed to this work:
- Professor **Eero Vasar** for support, trust, and patience.
- Associate Professor **Aavo Lang** for multitasking as a supervisor, colleague, and friend.
- Senior Research Fellow **Gunnar Tasa** for sharing his laboratory and spending stacks of promotional notepaper answering my never ending questions about DNA.
- Professor **Sulev Kõks** for always finding a moment to help and advice.
- Doctor **Eduard Maron**, Professor **Veiko Vasar**, Doctor **Jakov Shlik**, and Doctor **Innar Tõru** for collaboration in collecting clinical data as well as blood samples from volunteers.
- Professor **Marika Väli** for coordinating the collection of suicide samples.
- Technicians **Anne Tiidla, Egle Toover, and Piret Pärlist** for perfectly performing the most time-consuming and underappreciated part of the job - DNA extraction.
- Helpful colleagues **Kati Koido**, **Ranno Rätsep,** and **Külli Kingo** for sharing the knowledge, tools, reagents, and awe for SNPLex machine.
- Inhabitants of room **3090** for providing a cheerful workplace.
- Inhabitants of room **3089** for providing an island of peace and quiet.
- **Aasa** and **Olev Must** for always being next to me, in both parental as well as academic issues (being born into science, one really cannot tell the difference). All of my **four-legged furries** for boosting my endorphin levels and keeping my spirits up.

ORIGINAL PUBLICATIONS

CURRICULUM VITAE (IN ENGLISH)

Anne Must

Date and place of birth: May 23, 1977 Tartu, Estonia
Citizenship: Estonian
Address: Institute of Physiology, University of Tartu, Ravila 19, Tartu 50411, Estonia
Phone: +372 737 4333
E-mail: anne.must@ut.ee

Education

Since 2002	University of Tartu, Faculty of Medicine, PhD student of Neuroscience
1999–2002	University of Tartu, Faculty of Social Sciences, Department of Psychology, MSc (psychology)
1995–1999	University of Tartu, Faculty of Social Sciences, Department of Psychology, BSc (psychology)
1984–1995	Tartu Secondary School No 10

Professional employment

Since 2003	University of Tartu, Institute of Physiology, teaching assistant (0.5)

Scientific work

The research has been focused on investigating possible associations between genetic variability and male completed suicide in Estonian population. Three publications as a first author and three as co-author.

Publications in peer-reviewed journals

1. **Must A,** Tasa G, Lang A, Vasar E, Kõks S, Maron E, Väli M (2009) Variation in tryptophan hydroxylase-2 gene is not associated to male completed suicide in Estonian population. Neuroscience Letters 453: 112–114. Epub 2009 Feb 10.
2. **Must A**, Kõks S, Vasar E, Tasa G, Lang A, Maron E, Väli M (2009). Common variations in 4p locus are related to male completed suicide. Neuromolecular Medicine 11(1): 13–9. Epub 2008 Dec 25.

3. Maron E, Tõru I, Tasa G, **Must A**, Toover E, Lang A, Vasar V, Shlik J (2008) Association testing of panic disorder candidate genes using CCK-4 challenge in healthy volunteers. Neuroscience Letters 446(2–3): 88–92.
4. **Must A**, Tasa G, Lang A, Vasar E, Kõks S, Maron E, Väli M (2008) Association of limbic system-associated membrane protein (LSAMP) to male completed suicide. BMC Medical Genetics 9: 34.
5. Maron E, Tõru I, **Must A**, Tasa G, Toover E, Vasar V, Lang A, Shlik J (2007) Association study of tryptophan hydroxylase 2 gene polymorphisms in panic disorder. Neuroscience Letters 411(3): 180–4.
6. Maron E, Lang A, Tasa G, Liivlaid L, Tõru I, **Must A**, Vasar V, Shlik J (2005) Associations between serotonin-related gene polymorphisms and panic disorder. International Journal of Neuropsychopharmacology 8(2): 261–6.

CURRICULUM VITAE (IN ESTONIAN)

Anne Must

Sünniaeg ja -koht:	23. mai 1977, Tartu
Kodakondsus:	Eesti
Aadress:	TÜ füsioloogia instituut, Ravila 19, Tartu 50411
Telefon:	737 4333
E-mail:	anne.must@ut.ee

Haridus

Alates 2002	Tartu Ülikool, arstiteaduskond, neuroteaduste doktorantuur
1999–2002	Tartu Ülikool, sotsiaalteaduskond, psühholoogia osakond, *MSc* (psühholoogia)
1995–1999	Tartu Ülikool, sotsiaalteaduskond, psühholoogia osakond, *BSc* (psühholoogia)
1984–1995	Tartu 10. Keskkool

Erialane töökogemus

Alates 2003	Tartu Ülikool, füsioloogia instituut, assistent (0.5 k)

Teaduslik tegevus

Senine teaduslik tegevus on olnud suunatud meeste suitsiidi geneetilise tausta uurimisele Eesti populatsioonis. Ilmunud on kolm teadusartiklit esimese autorina ning kolm kaasautorina.

Artiklid rahvusvaheliselt tunnustatud teadusajakirjades

1. **Must A,** Tasa G, Lang A, Vasar E, Kõks S, Maron E, Väli M (2009) Variation in tryptophan hydroxylase-2 gene is not associated to male completed suicide in Estonian population. Neuroscience Letters 453: 112–114. Epub 2009 Feb 10.
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DISSERTATIONES NEUROSCIENTIAE UNIVERSITATIS TARTUENSIS

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7. **Urho Abramov.** Sex and environmental factors determine the behavioural phenotype of mice lacking CCK_2 receptors: implications for the behavioural studies in transgenic lines. Tartu, 2008.
8. **Hendrik Luuk.** Distribution and behavioral effects of WFS1 protein in the central nervous system. Tartu, 2009.