The association of specific polymorphisms in the serotonergic system with aggressive, impulsive and suicidal behaviour

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Declaration

I, Susan Louw, declare that the dissertation hereby submitted for the Master's degree qualification, *M.Sc* in Behavioural Genetics at the University of the Free State is my own independent effort and had not previously been submitted for a qualification at another University/Faculty.

I, Susan Louw, furthermore waive copyright of the dissertation in favour of the University of the Free State. All royalties with regards to intellectual property that was developed during the course and/or in connection with the study at the University of the Free State will accrue to the University.

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5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine/serotonin
5-HTT	serotonin transporters
5-HT1A	serotonin receptor 1A
5-HT1B	serotonin receptor 1B
5-HT2A	serotonin receptor 2A
α	alpha
β	beta
χ^2	chi square
°C	degrees Celsius
=	equals
<	less than
%	percent
μg	microgram
μl	microliter
μM	micro molar
A	adenine
ADHD	attention deficit hyperactivity disorder
APA	American Psychological Association
ANCOVA	analysis of covariance
BIS-11	Barrat Impulsiveness Scale version 11
BLAST	Basic Local Alignment Search Tool
bp	base pair
BPAQ	Buss Perry Aggression Questionnaire
BDHI	Buss Durkee Hostility Inventory
BSA	bovine serum albumin
C	cytosine
CDC	Centre for Disease Control
CI	confidence interval

cm	centimeter
CNS	central nervous system
COMT	catechol-O-methyltransferases
CSF	cerebrospinal fluid
dATP	dideoxyadenine triphosphate
dCTP	dideoxycytosine triphosphate
DDQ	Delay Discounting Task
dGTP	dideoxyguanine triphosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DSH	deliberate self-harm
DSM-V	Fifth edition of the Diagnostic and Statistical Manual of Mental Disorders
dTTP	dideoxythymine triphosphate
DZ	dizygotic
EDTA	ethylenediaminetetraacetic acid
EtBr	ethidium bromide
Fig.	figure
g	gravitational force
G	Guanine
GABA	gamma-aminobutyric acid
HIV	Human Immunodefiency Virus
HTR1A	5-hydroxytryptamine receptor 1A gene
HTR1B	5-hydroxytryptamine receptor 1B gene
HTR2A	5-hydroxytryptamine receptor 2A gene
HTTLPR	hydroxytryptamine transporter-linked polymorphic region
HVA	homovanilic acid
HWE	Hardy-Weinberg Equilibrium
kbp	kilobase pairs
LD	linkage disequilibrium
M	molar

MAO-A	monoamine oxidase-A
MgCl₂	magnesium chloride
ml	milliliter
mM	millimolar
MSSA	medically serious suicide attempt
MWM	molecular weight marker
MZ	monozygotic
NaCl	sodium cloride
NCBI	National Centre for Biotechnology information
NE	noradrenaline/norepinephrine
ng	nanogram
ng/ml	nanogram per mililiter
ng/ul	nanogram per microliter
nm	nanometer
NIMSS	National Injury Mortality Surveillance System
NMSSA	non-medically serious suicide attempt
NSSI	non-suicidal self-injury
OCD	obsessive compulsive disorder
OR	odds ratio
PCR	polymerase chain reaction
PFC	prefrontal cortex
PSAP	Point Subtraction Aggression Paradigm
r	correlation
RE	restriction endonucleases
RFLP	restriction fragment length polymorphism
RPQ	Reactive-Proactive Aggression Questionnaire
SA	South Africa
SASHS	South African Stress and Health Study
SD	standard deviation
SDS	sodium dodecyl sulphate
SLC6A4	solute carrier family 6 serotonin transporter member 4 gene

SNP	single nucleotide polymorphism
T	thymine
T_a	annealing temperature
TBE	tris(hydroxymethyl)aminomethane borate ethylenediaminetetraacetic acid
TCI	Temperament Character Inventory
TH	tyrosine hydroxylase
Tm	melting temperature
TPH	tryptophan hydroxylase
TPH1	trytopan hydroxylase 1 gene
TPH2	trytophan hydroxylase 2 gene
Tris	2-amino-2-(hydoxymethyl)-1,3-propanediol
USA	United States of America
UV	ultraviolet
V	volts
V/cm	volts per centimeter
VNTR	variable number tandem repeat
WHO	World Health Organisation

Abstract

Suicidal behaviour and the consequences thereof are a major global issue and need to be researched in order to promote a better understanding of this maladaptive behaviour. However, understanding the aetiology of suicidal behaviour is important, but difficult, as it is multifactorial and complex. Although there is a growing body of research pertaining to suicidal behaviour, there is a lack of research on the genetic contribution towards an endophenotype for suicidal behaviour in South Africa.

Due to the complex nature of suicidal behaviour, it was suggested that the use of an endophenotype would contribute to the possible intervention in this maladaptive behaviour. It has been suggested that some people were genetically predisposed to suicidal behaviour, and more importantly, the tendency to act on suicidal thoughts, and that this genetic vulnerability, underlain by the serotonergic system, might possibly be linked to inherited personality traits such as impulsiveness and aggression. Impulsiveness and aggression have therefore been suggested as possible endophenotypes of suicidal behaviour. Variation in the chosen genetic markers of the serotonergic system may modify the endophenotype of impulsivity and aggression, and in turn, influence the phenotype, suicidal behaviour.

The aim of the research was to determine if impulsivity and aggression can act as a potential endophenotype for suicidal behaviour, and therefore, firstly, to determine whether aggression and impulsivity, as personal variables, are associated with suicidal behaviour, and secondly, to investigate the possible association of candidate polymorphisms (*HTR1A* rs6295, *HTR1B* rs6296, *HTR2A* rs6311 and *SLC6A4 HTTLPR)* of the serotonergic system to impulsivity, aggression and attempted suicide. Genes can thus be studied as a contributing factor and not the only factor that influence suicidal behaviour.

A cohort of 25 research participants with a previous suicide attempt were recruited and matched to 25 healthy controls. All participants completed the BIS-11, RPQ, and BPAQ as quantifiable measures. Participants were also genotyped for *HTR1A* (rs6295), *HTR1B* (rs6296), *HTR2A* (rs6311) and *SLC6A4 HTTLPR*. The results for this study indicated that the suicide attempters scored significantly higher than the control group in all the questionnaires for aggression and impulsivity. This led to the conclusion that impulsivity and aggression is positively associated with suicidal behaviour. However, with regards to the molecular genetic analysis, only the *HTR2A* gene variant, rs6311, showed a significant difference between the suicide attempters and controls, with the A allele being more frequent in the suicide attempters ($p = 0.0066$). The suicide attempters were the only group that presented with the X allele for *SLC6A4 HTTLPR* and further studies are needed to replicate this finding. Interesting trends were observed regarding the other genetic variants, but no significant results were obtained. For *HTR1A* rs6295, the homozygous GG genotype conferred the highest risk for impulsive and aggressive behaviours in suicide attempters. The G allele for *HTR2A* rs6311, together with the S allele for *HTTLPR*, also seemed to increase impulsive and aggressive traits. In the case of *HTTLPR*, this finding was valid irrespective of the presence of suicidal behaviour. Overall, the results provide support for the use of behavioural measures of impulsivity and aggression as an endophenotype for suicidal behaviour. Some support was also found for the use of impulsive aggression as a single construct with regards to suicidal behaviour.

The combination of psychology and genetic results are among the first ever reported for suicide attempters in South Africa. Despite the limited size of the study, perhaps due to the sensitivity of the construct under investigation, this study nevertheless adds significant value to the body of research pertaining to the under-studied topic of suicidal behaviour in South Africa. This study can improve phenotyping that will ultimately benefit South-African individuals.

Opsomming

Selfmoordgedrag en die gevolge daarvan is 'n globale probleem en navorsing sal bydra tot beter insig in hierdie wanaangepaste gedrag. Dis is noodsaaklik om die oorsprong van selfmoordgedrag te verstaan, alhoewel dit bemoeilik word deurdat selfmoordgedrag kompleks en multi-faktoriaal is. Ten spyte daarvan dat navorsing rakende selfmoordgedrag toeneem, is daar steeds 'n tekort aan navorsing oor die bydraende genetiese faktore van 'n endofenotipe vir selfmoordgedrag in Suid-Afrika.

Die komplekse natuur van selfmoordgedrag noodsaak die gebruik van 'n endofenotipe om die gedrag te bestudeer. Dit kan moontlik intervensies en voorkoming van selfmoordgedrag vergemaklik. Sommige individue is geneties meer geneig tot selfmoordgedrag, en ook om te reageer op selfmoordneigings. Hierdie genetiese ingesteldheid, onderliggend aan die serotonergiese neurologiese sisteem, kan moontlik verwant wees aan oorerflike persoonlikheidseienskappe soos impulsiwiteit en aggressie. Impulsiwiteit en aggressie is gevolglik bestudeer as 'n moontlike endofenotipe vir selfmoordgedrag. Variasie in die gekose genetiese merkers van die serotonergiese sisteem kan dalk die endofenotipe van impulsiwiteit en aggressie wysig, wat weer die fenotipe, selfmoordgedrag, kan beïnvloed.

Die doel van die navorsing was om vas te stel of impulsiwiteit en aggressie as 'n moontlike endofenotipe vir selfmoordgedrag kan geld: eerstens, om vas te stel of impulsiwiteit en aggressie, as persoonlikheidsveranderlikes, geassosieer kan word met selfmoordgedrag; tweedens, om die moontlike assosiasie tussen die gekose veranderlike gene van die serotonergiese sisteem (*HTR1A* rs6295, *HTR1B* rs6296, *HTR2A* rs6311 en *SLC6A4 HTTLPR)* en impulsiewe, aggressiewe en selfmoordgedrag, te bepaal. Gene word dus as bydraende faktor ondersoek, en nie slegs as die enigste faktor wat bydra tot selfmoordgedrag, nie.

Vyf-en-twintig navorsingsdeelnemers en 'n kontrole-groep van 25 ooreenstemmende individue is gewerf. Alle deelnemers het die BIS-11, RPQ en BPAQ, wat dien as 'n meetbare toetsinstrument, voltooi. Deelnemers is gegenotipeer vir *HTR1A* (rs6295), *HTR1B* (rs6296), *HTR2A* (rs6311), en *SLC6A4* (*HTTLPR*). Die resultate vir hierdie studie toon beduidende hoër tellings vir impulsiwiteit en aggressie in die navorsingsgroep. Dit lei tot die gevolgtrekking dat impulsiwiteit en aggressie positief geassosieer kan word met selfmoordgedrag. In verband met die molekulêre analise, het slegs die uitdrukking van die *HTR2A* geen (rs6311) verskil tussen die navorsings- en kontrole-groep (p = 0.0066). Die A-alleel het meer algemeen voorgekom by dié met selfmoordpogings. 'n Verdere bevinding is dat die X-alleel van *SLC6A4* slegs voorgekom het by die navorsingsgroep. Interessante patrone is gevind vir die genetiese variasie in terme van impulsiwiteit en aggressie, maar geen statisties beduidende resultate het voorgekom nie. Die homosigotiese GG genotipe van *HTR1A* rs6295 het die hoogste risiko vir impulsiwiteit en aggressie by die navorsingsgroep getoon. Die G-alleel vir *HTR2A* rs6311 en die S-alleel van *SLC6A4* *HTTLPR* het ook 'n moontlike bydrae tot impulsiwiteit en aggressie getoon. Vir *HTTLPR* was dit waar, ongeag die voorkoms van selfmoordgedrag. Oor die algemeen bevind hierdie studie dat impulsiwiteit en aggressie as 'n endofenotipe vir selfmoordgedrag kan dien. Die gebruik van impulsiewe aggressie as 'n enkele konstruk wanneer dit in verband met selfmoordgedrag gebruik word, word ook ondersteun. Die genetiese bydrae tot hierdie fenotipe as enkele konstruk, behoort verder ondersoek te word.

Die kombinasie van sielkunde en genetika is van die eerste studies in verband met selfmoordgedrag in Suid-Afrika. Ten spyte van die klein studiegrootte, moontlik as gevolg van die sensitiwiteit van die konstruk wat ondersoek word, dra die studie by tot die liggaam van navorsing oor selfmoordgedrag in Suid-Afrika. Hierdie studie kan lei tot die verbetering van fenotipering, wat uiteindelik Suid-Afrikaners kan bevoordeel.

Sleutelwoorde: Selfmoordgedrag, impulsiwiteit, aggressie, endofenotipe, serotonien, genetiese variasie

Chapter 1 Motivation

1.1 Introduction

Suicidal behaviour has been part of society for many centuries, however, it appears as if the global scale of suicidal behaviour is often underestimated as a universal emergency. Suicide is the tenth leading cause of death in the United States of America (USA) with an average rate of 12.4 per 100 000 of the population (Xu, Kochanek, Murphy, & Arias, 2012). In 2010 the annual economic cost of suicide death was estimated to be more than $44 billion (Xu et al., 2012). The average completed suicide rate in South Africa has been determined at 19 per 100 000 (Schlebusch, 2001). Suicidal behaviour is generally recognised as a very complex phenomenon, with no single cause. It involves intricate interactions between different variables, including psychosocial and biological variables. Statistics regarding suicidal behaviour clearly indicates a need for greater concern and more research, to bring better depth to the understanding of this complex human behaviour.

During the last five decades, great strides have been made in identifying possible contributing factors to suicidal behaviour. With the advancement of technology, researchers have been able to identify underlying genetic mechanisms that might act as a predisposition to suicidal behaviour (Özalp, 2009). The present study will be conducted from a behavioural genetics paradigm. This is to determine if impulsive and aggressive behaviours in suicide attempters might be associated with contributing genetic factors linked to the serotonergic neurotransmitter system. This would verify impulsive and aggressive behaviours as a possible endophenotype for suicidal behaviour, in this case specifically, suicide attempts in a South African population. This research project may contribute to better clarification of the underlying factors of suicidal behaviour. This may lead to more personalised medication to combat the global health problem of suicide and related behaviours.

1.2 Background

Globally more than 1 million deaths per year occur due to suicide, the extreme form of self-directed aggression. This indicates that suicidal behaviour is a major global health problem (Diekstra, 1993; Hawton & Van Heeringen, 2000; Schlebusch, 2000). To date, there has been a lack of systematic data collection regarding the occurrence of attempted suicide in South Africa, and thus accurate statistics are severely lacking. The estimated suicide rates in South Africa in the past differed from 6/100 000 to 19/100 000 (Schlebusch, 2000). The alarming fact is that the number of attempted suicides can be up to 20 times higher than that of completed suicides (Schlebusch, 2000). The cost of suicidal behaviour should not just be determined in view of the financial costs involved, as the personal costs and trauma involved are immeasurable. This clearly indicates a need to address the high rate of suicide attempts and to reduce the possible trauma associated with suicidal behaviour.

Suicide, at its most basic, is defined as taking one's own life intentionally, whereas attempted suicide is where an individual intends to complete the act, but unintentionally fails (Mashego, Peltzer, Williamson, & Setwaba, 2006). The most comprehensive definition of a suicide attempt, which will be used to determine the inclusion criteria for this study, states that the attempt should comply with the following: it must be self-initiated, potentially harmful behaviour; it must be with the intention to die; and it must result in a non-lethal outcome (Apter, 2010). An in depth discussion of the different facets that constitute suicidal behaviour will follow in Chapter 2.

Suicidal behaviour is highly complex and poorly understood. Several possible contributing factors include the following: dysfunctional family dynamics, lack of problem-solving skills, imitation effects, neurobiological and genetic factors, substance abuse, effects of media and information technology, aggression, impulsivity, depression, brain pathology and many others (Schlebusch, 2005; Van Heeringen, Hawton, & Williams, 2000; Wasserman & Wasserman, 2009; Wasserman, 2001). Genetic factors are suggested to play a role in

30 - 50% of reported cases with suicidal behaviour, independent of other psychological stressors or disturbances (McGuffin, Marusic, & Farmer, 2001; Roy, Segal, & Sarchiapone, 1995). Since it is difficult to determine the genes responsible for suicidal behaviour, it was suggested to use an endophenotype approach to study suicidal behaviour (Mann et al., 2009). An endophenotype is described as an internal phenotype that exists in between the genes and disease (Gottesman & Gould, 2003). This approach identifies genes associated with heritable intermediate phenotypes and might facilitate the process to determine genes related to the predisposition to suicidal behaviour. Possible endophenotypes include impulsive-aggressive traits, early-onset major depression and neurocognitive function (Mann et al., 2009). It is suggested that if the endophenotype is present, certain predisposing genetic factors underlying that endophenotype will also be present. Since the endophenotype is capable of predicting suicidal behaviour, it follows that the identified genes possibly associated with the endophenotype, will then be associated with suicidal behaviour.

A possible endophenotype for suicidal behaviour includes impulsive aggressive traits (Mann et al., 2009). As explained by Brent and Mann (2005), several individuals are genetically predisposed to suicidal behaviour and that this genetic vulnerability may be linked to inherited personality traits such as impulsiveness and aggression. Impulsivity is a multi-dimensional construct; it refers primarily to acting without thinking, but more precisely contains three different components; i) not planning carefully, ii) acting without thinking, and iii) not being able to pay attention (Cardinal, 2006; Patton, Stanford, & Barratt, 1995). As with other complex human traits, genetic factors also influence impulsivity. Twin, family and adoption studies have been carried out and heritability of impulsivity is determined at about 45% (Hur & Bouchard, 1997; Pederson, Plomin, McClearn, & Friberg, 1988). Aggression can be defined as an act that leads to harm or injury to self or others (Coccaro et al., 1989). It is a complex behaviour influenced by both environmental and genetic factors. The heritability of aggression has been comprehensively studied and confirmed through twin, adoption and family studies (Bergeman & Seroczynski, 1998; Miles & Carey, 1997). It has been estimated that the heritability for aggression ranges between

44 - 72% (Coccaro et al., 1989). Studies suggested that there are two distinct forms of aggression, typically referred to as reactive (impulsive) and proactive (premeditated) aggression (Baker, Raine, Liu, & Jacobson, 2008; Dodge & Coie, 1987; Geen, 2001). Different genetic factors are thought to underlie these two forms of aggression and are still under investigation (Linnoila et al., 1983; Tuvblad, Raine, Zheng, & Baker, 2009; Virkkunen et al., 1994).

In order to link these specific genes underlying the endophenotype to suicidal behaviour, it is necessary to establish a positive link between the endophenotype and suicidal behaviour. Research has shown that a strong correlation exists between impulsivity, aggression and suicidal behaviour (Apter et al., 1990; Borowsky, Ireland, & Resnick, 2001; Brent et al., 1994; Crumley, 1979; Garrison, McKeown, Valois, & Vincent, 1993; Gut-Fayand et al., 2001; Ivanoff & Jang, 1991; Koller, Preuss, Bottlender, Wenzel, & Soyka, 2002; Pezawas et al., 2002; Shaffer et al., 1996). It was found that impulsive aggressive behaviours (also referred to as reactive aggression) play an important role in the predisposition to suicidal behaviour. Reactive aggression is a type of aggression that leads a person to be prone to reflective anger, especially in stressful situations. This kind of aggression acts as a predisposition to suicidal behaviour. Life-time outward directed aggression was increased in suicide attempters and the opposite is also true (Mann, 1998). Individuals who committed suicide experienced higher degrees of threatened and attempted violence than people who died accidentally (Conner, Duberstein, Conwell, Seidlitz, & Caine, 2001). Adolescent suicide victims had a life-time history of aggression (Brent et al., 1994). These findings have also been replicated in South Africa (Schlebusch, 2005). Schlebusch (2005) found a link between suicidal behaviour and violence, which was associated with aggression and impulsivity, by looking at the methods used to commit suicide. Impulsivity has been found to be higher in suicide attempters across various studies. It also correlates positively with more medically severe suicide attempts. It seems to play its role in suicidal behaviour by reducing inhibitions towards suicidal behaviour and interacting with other risk factors such as depression or hopelessness (McGirr & Turecki, 2007). The serotonergic system was also implicated when hypofunction of the serotonergic

system correlated positively with more lethal suicide attempt methods (Hawton & Van Heeringen, 2000; Wasserman & Wasserman, 2009).

In order to quantify impulsive and aggressive behaviours, the following questionnaires will be used: Reactive-Proactive Aggression Questionnaire (RPQ; Raine et al., 2006), The Barratt Impulsiveness Scale version 11 (BIS-11; Patton et al., 1995) and The Buss Perry Aggression Questionnaire (BPAQ; Buss & Perry, 1992). Each of these has been used in several previous research studies and shows good reliability and validity (Baker et al., 2008; Fossati, Maffei, Acquarini, & Di Ceglie, 2003; Stanford et al., 2009; Valdivia-Peralta, Fonseca-Pedrero, González-Bravo, & Lemos-Giráldez, 2014; Vasconcelos, Malloy-Diniz, & Correa, 2012). In the literature review impulsivity and aggression will both be discussed as separate constructs, each with its own genetic components and link to suicidal behaviour, however, there has been a call to use impulsive aggression as a single construct. Impulsivity has been mostly associated with reactive aggression and the genetic components of reactive aggression seem to differ from proactive aggression. For this reason the RPQ that determines reactive and proactive aggression scores separately will also be included in the study.

Since the genetic basis of both aggressive and impulsive behaviours have been confirmed by several studies (Gottesman, 1963; Lesch & Merschdorf, 2000; Livesley, Jang, & Vernon, 1998; Miles & Carey, 1997; Pederson et al., 1988; Seroczynski, Bergeman, & Coccaro, 1999; Taylor, Loney, Bobadilla, Iacono, & McGue, 2003), it is possible to look at the genes that may play a significant role in these behavioural traits. Research has focused on the serotonergic/5-hydroxytryptamine (5-HT) system, but evidence suggests that other systems such as catecholamines, steroids, neuropeptides and cholesterol might also be involved (Carballo et al., 2009; Crisafulli, Calati, Ronchi, Sidoti, & Angelo, 2010; Ernst, Mechawar, & Turecki, 2009; Mann, 2003). Serotonin has been the focus of many scientific studies which have shown evidence for the role of serotonin in several key behaviours (Best, Nijhout, & Reed, 2010; Chojnacka-Wojcik, 1995; Dumais, Lesage, Alda, et al., 2005; Kamali, Oquendo, & Mann, 2001; Newman, Shapira, & Lerer, 1998; Zanarini, Frankenburg, &

Prachini, 2004). These include impulsive aggressive behaviours, anxiety, affect, appetite, cardiovascular and cognitive function, sleep patterns, regulation of the endocrine system, motor activity, pain, reproductive and sensory functions. The serotonergic system consists of different serotonin receptors, serotonin transporters (5-HTT) and serotonin metabolising enzymes (Murphy et al., 1998) and dysregulation from any of these may lead to behavioural changes. The conclusion of results from various studies indicate that lower serotonergic activity is related to aggression, impulsivity and suicidal behaviour (Kamali et al., 2001; Lindberg, Belfrage, Bertilsson, Evendon, & Asberg, 2000; Linnoila et al., 1983; Mann et al., 2009; Spreux-Varoquaux et al., 2001; Stanley et al., 2000; Van Heeringen, 2003; Virkkunen et al., 1994). Since these systems are controlled by genes, molecular genetic studies have attempted to determine the specific genes that underlie these systems. Several genes associated with impulsivity and aggression through these molecular genetic studies include *HTR1A* (serotonin receptor 1A gene)*, HTR1B* (serotonin receptor 1B gene)*, HTR2A* (serotonin receptor 2A gene)*, TPH1* (tryptophan hydroxylase 1 gene)*, TPH2* (tryptophan hydroxylase 2 gene)*, SLC6A4* (solute carrier family 6 serotonin transporter member 4 gene) and *MAO-A* (monoamine oxidase-A gene) (Arango et al., 1990; Arora & Meltzer, 1989; Brunner, Nelen, Breakefield, Ropers, & Van Oost, 1993; Courtet et al., 2004; Du et al., 1999; Guo, Ou, Roettger, & Shih, 2008; Juhasz et al., 2010; Kim-Cohen et al., 2006; Lesch & Merschdorf, 2000; Nielson et al., 1998; Rogers et al., 2003; Sonuga-Barke et al., 2011; Stoltenberg et al., 2006; Walderhaug, Herman, Magnusson, Morgan, & Landrø, 2010; Winstanley, Theobald, Dalley, Cardinal, & Robbins, 2005).

The endophenotype approach implies that if the genetic basis of the endophenotype, in this case impulsive and aggressive behaviours, is similar to that of the complex behaviour, particularly suicidal behaviour, then the endophenotype can be used to predict the complex behaviour. As the serotonergic system has been suggested in impulsive and aggressive behaviours, it is necessary to determine if there are possible genes within the serotonergic system that can be associated with suicidal behaviour. Özalp (2009) researched numerous genes in relation to suicidal behaviour: *5-HTT*, trytophan hydroxylase (*TPH*), various serotonin receptor genes including *HTR1A*, *HTR1B,* and *HTR2A*, Catechol-O-

methyltransferases (*COMT*), *MAO-A*, and tyrosine hydroxylase (*TH*). As can be seen, some of these genes show an overlap with the genes identified to play a role in impulsive aggression traits.

As it is outside the scope of this study to research all of the genes previously mentioned, the main focus of this study will fall on the following genes within the serotonergic system:

(a) *HTR1A,* which has been linked to anxiety and obsessive-compulsive disorder (OCD), stress sensitivity, depression and aggression (Oliver, Lorenz, & Kornegay, 1997).

(b) *HTR1B*, which has been associated with antisocial personality disorder and intermittent explosive disorder (Lappalainen, 1998). Later studies also found correlations to alcoholism, suicidal behaviour and OCD (Huang et al., 2003; Sanders, Duan, & Gejman, 2002).

(c) *HTR2A*, which has been correlated to schizophrenia, suicidal behaviour, problematic impulse control and aggression (Abdolmaleky, Faraone, Glatt, & Tsuang, 2004; Bjork et al., 2002; Khait et al., 2005).

(d) The transporter *SLC6A4*, which has been associated with mental instability (Hariri et al., 2002; Lesch et al., 1996) and stress sensitivity (Chaouloff, Berton, & Mormede, 1999). It has been linked to anxiety (Lesch et al., 1996), depression, neuroticism, affective disorders and suicidal behaviour (Du, Bakish, & Hrdina, 2000; Greenberg et al., 2000; Lesch et al., 1996).

Variations in the above mentioned genes will be determined in this research study through the use of molecular genetics techniques. Studies have shown a connection between suicidal behaviour and the underlying genetic mechanisms of behavioural traits such as anger and impulsivity. This all leads to the clarification of the tendency towards suicidal behaviour (Özalp, 2009). The hypothesis is that the endophenotypes, in this case,

impulsivity and aggression, will correlate positively with suicidal behaviour. Following on that assumption, it is also predicted that the specific genes that associate with impulsive and aggressive behaviours, will show a positive association with suicidal behaviour.

1.3 Research statement

Suicidal behaviour is largely recognised as a very complex phenomenon with no single cause. It involves intricate interactions between different variables, including psychosocial, environmental, biological and genetic variables (Schlebusch, 2000; Van Heeringen et al., 2000). It is clearly difficult to predict the behaviour and therefore it complicates the prevention of suicide. Most research in South Africa focused on environmental factors and how to reduce the impact of these environmental factors that might contribute to suicidal behaviour. However, in order to address environmental factors it is necessary to determine the underlying causes that affect each individual. The question arises whether it can be proven if aggression and impulsivity can be linked to attempted suicide in a South African population and whether genetic factors might play a role. Research on the genetics of suicidal behaviour in South Africa is limited. There is clearly a necessity for further research in South Africa in order to address this gap in the literature. This will further better understanding and might lead to a reduction of suicidal behaviour.

1.4 Aim of this study

The serotonergic system has been linked to several behavioural disorders including depression, anxiety, suicidal behaviour and aggression. However, no conclusive associations have been made. In light of the above discussion, the aim of this study is firstly to determine whether aggression and impulsivity, as personal variables, do in fact play a role in suicidal behaviour, and secondly, to investigate the possible association of candidate polymorphisms within selected genes to attempted suicide. Genes can thus be studied as a contributing factor and not the only factor that influence suicidal behaviour.

1.5 Objectives of this study

In order to realise the above mentioned aims, the project will consist of the following:

(a)   standardised questionnaires will be completed by all participants, which will allow the psychological constructs of impulsivity and aggression to be quantified in order to draw correlations between these and suicide attempts. The main purpose of this assessment is to have a stable measure whereby behaviour can be quantified;

(b)   molecular analysis will be carried out to determine the polymorphisms present in each participant's deoxyribonucleic acid (DNA). This will be used to associate specific polymorphisms within the previously mentioned neurochemical pathways in order to better understand the manifestation of suicidal behaviour.

1.5.1 Theoretical objectives

The theoretical objectives are there to provide a sound basis for the study and to provide the intellectual background needed to channel the study, in order to solve the problem that was posed. It will also guide the researcher in the process of determining which specific methods can be utilised that will be appropriate for the study (Ellis & Levy, 2009). The theoretical objectives will be comprehensively discussed in Chapter 2 of this dissertation, these include:

(a)   to review the existent literature on the possible influence of an endophenotype on suicidal behaviour;

(b)   to summarise and review possible polymorphisms in genes related to the serotonergic system that may contribute to suicidal behaviour.

1.5.2 Empirical objectives

The empirical objectives are based on empirical data. It will thus be founded on the results of experiments (Leedy & Ormrod, 2005). The empirical objectives of this study (which will be discussed in Chapter 3 and 4) are to determine in a South African population of suicide attempters, whether:

(a) a correlation exists between:
　　a.1. aggression and impulsivity
　　a.2. aggression and suicide attempts
　　a.3. impulsivity and suicide attempts
(b) a stronger positive correlation can be found between reactive aggression than proactive aggression and suicide attempts;
(c) specific polymorphisms within selected genes can be associated with:
　　c.1. aggression and impulsivity
　　c.2. suicide attempts

1.6 Research outline of this study

This research project consists of several components to meet the objectives. The theoretical objectives will be addressed in Chapter 2, in the form of a literature review. This entails a comprehensive overview of the existing literature with regards to the environmental factors (mainly aggressive and impulsive behaviours), and genetic factors influencing the serotonergic system, that could contribute to suicidal behaviour.

The empirical objectives will entail quantitative analysis of the constructs of impulsivity, aggression and reactive aggression. These constructs were identified as a possible endophenotype underlying suicidal behaviour as described in the introduction. In order to quantify these traits, different questionnaires will be used as identified in section 1.2. Subsequent quantitative molecular analysis of the DNA obtained from the participants

will allow the researcher to determine the genetic variability of the specific genes in question. Statistical analysis will be done on the data collected to determine if any correlations can be drawn between data sets. This whole process of the materials and the methods will be discussed in Chapter 3.

Chapter 4 will consist of a comprehensive overview of the results obtained using the methods as described in Chapter 3. This will allow the researcher to determine if impulsivity and aggression are correlated with suicidal behaviour and the specific genes under investigation can be associated with the specific endophenotype for suicidal behaviour.

These results and inferences drawn from the results will be discussed in depth in Chapter 5. The current study will also be compared to available research studies based on the constructs of impulsivity, aggression and suicidal behaviour, as well as previous molecular genetic research.

A conclusion will be drawn and shortcomings and future possibilities of the research discussed in Chapter 6. References will follow in Chapter 7.

Chapter 2 Literature Review

2.1 Background

Suicidal behaviour has been part of society for centuries. It is a complex phenomenon with multiple factors contributing to the behaviour. Worldwide, more than one million deaths per year can be attributed to suicide. This indicates that suicidal behaviour is a major health problem (Schlebusch, 2000) and one of the ten top most common causes of death in the western world (Gvion & Apter, 2012; Xu et al., 2012). With the alarming statistics regarding suicide, it is important that studies be undertaken to clarify the behaviour, which in turn, could lead to better prevention strategies. It is a great challenge for health professionals to prevent suicide, since so many factors can play a role in this kind of behaviour (Cassels, Paterson, Dowding, & Morrison, 2005). The cost of suicide is multi-factorial; public resources are often used for health care and psychiatric help, while other costs include mental, physical and emotional stress of friends and family (Gvion & Apter, 2012). This study was conducted within the parameters of a positivist research paradigm. This paradigm is of the assertion that reality can be observed objectively and empirically and then explained through logical analysis. It is based on conducting experiments in a controlled environment (Kaboub, 2008). However, using just the positivist paradigm may lack validity in a reality where a multitude of different factors interact. Critical multiplism is based on the belief that no singular approach can be used to lead to a valid understanding of complex phenomenon and that a multitude of research questions and designs should be used (Kaboub, 2008).

To the best of the author's knowledge, only very few molecular genetic studies on suicidal behaviour, within a South African context, have been published in peer-reviewed articles. Published results mainly include samples from European ancestry (Dalvie et al., 2015). Genetic research in Africa should be promoted as indigenous population can be a valuable resource (Campbell & Tishkoff, 2008). However, research on African-specific

functional variants is scarce. This literature review will highlight some of the most important factors from a behavioural genetics viewpoint thought to play a role in suicidal behaviour, in order to contribute to the theoretical and empirical research on the association between genetic factors and suicidal behaviour. The theoretical literature refers mainly to the previous studies that provided the intellectual background for this particular study (Ellis & Levy, 2009). These theoretical literature was based on scientific experiments and formed the basis of this review (Leedy & Ormrod, 2005). The research project combined quantitative designs and molecular genetics to contribute to a holistic view of human behaviour. The main aim was twofold: a) to determine if a possible correlation existed between impulsivity, aggression and suicidal behaviour. Correlation in this study is defined as the magnitude of a possible relationship between variables, and does not infer a causal relationship; b) to determine if a possible association existed between certain serotonergic genes and suicidal behaviour. Genetic association studies determine the putative association that exists between a gene and a specific trait amongst unrelated individuals. Individuals with a particular allele of the specific gene in question, have a different expression of the trait than the individuals with different alleles (Plomin & Rutter, 1998). The information can be used to test individuals who run the risk of committing suicide. This allows interventions to take place early in the lives of these individuals and family trauma can also be prevented. Psychiatric treatment and medication can be adjusted to suit each individual and a reduction of healthcare costs may be obtained.

2.2 Suicide

2.2.1 Burden of suicide

An increase in suicide rates of up to 60% in the last 45 years has been observed. The estimated fatality rate of suicide is one million individuals per annum with an attempted suicide rate of over ten million individuals per annum (World Health Organization (WHO), 2010). This converted to approximately one suicide every 40 seconds and an attempt every

3 seconds (WHO, 2011). It was also predicted that, by the year 2020, there might be as many as 1.53 million suicides per annum worldwide, and more alarmingly, up to 20 times more attempted suicides. This roughly translated into a suicide attempt every one to two seconds, and a fatality from suicide every 20 seconds (Nock, Borges, Bromet, Cha, et al., 2008; WHO, 2007). A global suicide rate of 16 deaths per 100 000 individuals and a South African suicide rate of 17.2 deaths per 100 000 individuals during the 1990s was determined by the WHO (1999), which is higher than the global average in that decade. The latest South African suicide rate was determined at up to 19 per 100 000 individuals (Schlebusch, 2005).

The National Injury Mortality Surveillance System (NIMSS) was established in 1999 to provide comprehensive information on the fatalities in South Africa due to external causes. The information collected during 2008 in seven provinces found death by non-natural causes in 31 177 cases, which constituted between 39 - 52% of all fatalities in the country (NIMSS, 2008). Out of the 31 177 cases, 10% was due to suicide (n = 3 125), which constituted the fourth leading cause of non-natural deaths. Almost 70% (n = 2 164) of these suicides occurred in the age group of 15 – 44 years old. The youths aged 15 – 29, were the most represented group with 35.9% (n = 1 122) of the suicides, followed by adults aged 30 – 44 (33.3%; n = 1 042). The mean age of the victims was 35 (± 14.4 years). Figure 2.1 visually represents the ages of the suicide victims for 2008. The gender ratio was 4:1, with more males committing suicides. Even though it is widely reported that more males successfully commit suicide than females, the attempted suicide rate is higher for females (Callanan & Davis, 2012; Canetto & Sakinofsky, 1998; Joe, Stein, Seedat, Herman, & Williams, 2008; Tsirigotis, Gruszczynski, & Tsirigotis, 2011). The most common method of committing suicide was hanging (46.2%), followed by poisoning (17.0%). The race distribution of suicidal deaths across South Africa, as shown by data collected by NIMSS during 1999-2000, was as follows: 47.4% South African Black, 33.9% South African Whites, 14.9% Coloured and 3.8% other race (as cited in Meehan & Broom, 2007). These results differ slightly from a WHO report (1999) which determined the ethnic distribution of the fatal suicides in South Africa at 43.3% Black, 38.4% White, 15.9% Coloured South Africans and 2% other. Although it seems that most of the total suicide cases were committed by

Black South Africans, the suicide rate is comparatively lower in the total Black population if taken against the demographics of South Africa. According to the South African Census 2011, the total population was estimated at 51 770 560 with 79.2% Black, 8.9% Coloured, 2.5 Indian/Asian, and 8.9% White individuals (Statistics South Africa, 2011). If this is taken into account, the highest incidence of suicide was in the White population group. The Free State province had a total population of 2 745 590 in 2011 with 87.6% Black, 3.1% Coloured, 0.4% Indian/Asian, and 8.7% White race distribution; median age of 26; with 94 males for every 100 females (Statistics South Africa, 2011). In the Bloemfontein and Southern Free State region suicide rates were estimated at 10.9/100 000 of the population per annum. In this region 82.1% of the cases (n = 469) were male, 51.8% fell in the 21 – 40 years age range, and 72.1% of the suicide cases were Black, 26.0% White, 1.1% Coloured and 0.6% Indian individuals (Stark et al., 2010). Although the absolute number for suicide is highest in the Black population, the proportional suicide rate in South Africa is highest in the White population.
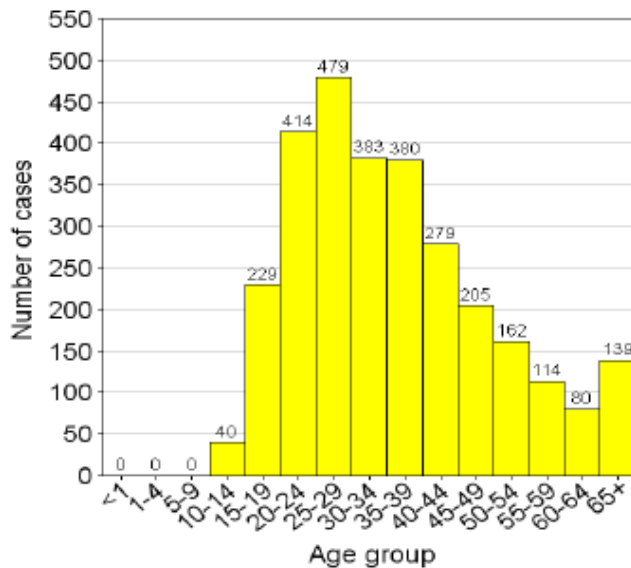
Figure 2.1 Suicide by age (n = 2 904) as presented by the National Injury and Mortality Surveillance System. From the 3 125 cases of suicide analysed in the study, 221 ages were unknown (NIMSS, 2008).

There has not been much systematic data collection regarding the occurrence of attempted suicide in South Africa (Schlebusch, 2005). Most of the data available regarding

the occurrence of suicide attempts were collected through clinical and community samples (Pillay, Wassenaar, & Kramers, 2001; Schlebusch & Bosch, 2000). These findings indicated that between 137 860 – 160 000 South Africans attempted to commit suicide each year. There were approximately 13 333 suicide attempts every week or more than 18 attempts every hour (Schlebusch, 2000, 2005). Schlebush (2000) determined that the completed suicide rates in South Africa ranged between 6/100 000 to 19/100 000, depending on which data was used. Further, attempted suicide rates were up to 20 times higher than that of completed suicides, thereby identifying attempted suicide as a relevant risk factor for completed suicide (Gunnell & Lewis, 2005; Schlebusch, 2000).

Nationally representative data of prevalence and risk factors of non-fatal suicidal behaviour in South Africa was presented by Joe, Stein, Seedat, Herman, and Williams (2008) as determined by the South African Stress and Health Study (SASHS) (Williams et al., 2004). This was done as part of a WHO initiative (WHO, 2004). The study was comparable to the total South African population for age, gender and province, as found in the 2001 South African census. The findings of the survey (n = 4 351) indicated life-time prevalence of 9.1% for suicide ideation, 3.8% for suicidal plans, and 2.9% for suicide attempts amongst South Africans. Female respondents with suicide attempts (3.8%) were found to be twice as many as male respondents with suicide attempts (1.8%). These percentages were for the representative population of the South African cohort as used in the study by Joe et al. (2008).

Racial differences were also observed. The Coloured individuals (7.1%) represented the highest levels of attempted suicide in the above mentioned representative South African population, which was almost thrice that of the White (2.4%) and Black (2.4%) groups. The highest rate of unplanned suicide attempts was also observed under the Coloured population (Joe et al., 2008).

A general hospital-based study done in South Africa found the peak age for suicide attempts was between 20 and 29 years (Bosch, McGill, & Noor Mahomed, 1995). This age range was confirmed in a study by Deonarain and Pillay (2000), which determined a mean age of 25 years for attempted suicides in a general hospital sample in South Africa. Joe et al. (2008) found the median age for suicide ideation and planning to be in the late twenties. However, for the onset of suicide attempts, early thirties was the median age. Suicide attempts were highest in the 20 – 34 years' age group, with unplanned attempts the most common in the 10 - 20 years' age group. In South Africa the highest risk for a suicide attempt was associated with being female, age 18 - 34, and having low or medium education levels (Joe et al., 2008).

2.2.2 Definition of suicide

One of the leaders in the field of suicidology was a sociologist, Emile Durkheim. He categorised different types of suicide according to the social factors that led to the suicide. Altruistic suicide was where an individual experiences too much integration into society and loses own individuality. Soldiers dying in war and suicide bombers fell into this category. Egoistic suicide was where a loss of social support plays the biggest part in the reason to commit suicide and was common amongst the elderly. They lacked an experience of integration and a sense of belonging. Anomic suicides occurred after a significant stressor that disrupts the life of the person. This type was common in teenage suicide. Fatalistic suicides were the result of losing control over one's own life and destiny. Individuals in oppressive countries and prisoners were some examples of groups to commit fatalistic suicides (Durkheim, 1951). Another definition came from Sigmund Freud who defined suicide as an unconscious hostility directed inwards or towards the self (Freud, 1957). This definition by Freud has generally been accepted; suicide is a form of self-directed aggression, including the intention of physical harm in the form of taking one's own life (Mann, 2003; Mashego et al., 2006).

As the research regarding suicide expanded, different definitions evolved to adequately try and describe this complex concept. Currently, suicidal behaviour is the umbrella term used to describe all related behaviours and distinguishes between several subsets of behaviour, such us (a) suicide/lethal or completed suicide – which occurs when an individual successfully took his/her own life (Nock, Borges, Bromet, Alonso, et al., 2008; Shneidman, 1985); (b) suicide attempts/non-fatal suicide, where the individual had the intention to take his/her own life, but failed in the attempt. Suicide attempts can be grouped into near-fatal medically serious suicide attempt (MSSA) and non-medically serious suicide attempt (NMSSA) (Levi et al., 2008); (c) parasuicide/deliberate self-harm (DSH)/non-suicidal self-injury (NSSI), where such individuals make impulsive suicidal gestures or deliberately harm themselves without the intention to die, the act was carried out primarily to draw attention (Apter, 2010; Mashego et al., 2006).

The variety in distinctions in the definition of suicidal behaviour highlights the multidimensional nature of suicidal behaviour (Silverman, Berman, Sanddal, O'Carroll, & Joiner, 2007). The most comprehensive definition of suicide attempt states that the attempt should comply with the following: it must be self-initiated, potentially harmful behaviour, with the intention to die, and a non-lethal outcome (Apter, 2010). This was the formal definition used in this study and was the directive of the inclusion criteria for the research group. The fifth edition of the Diagnostic and Statistical Manual of Mental Disorder (DSM-V) has included the possibility of the development of suicidal behaviour as a new recognised disorder (American Psychiatric Association (APA), 2013).

2.3 Suicidal behaviour from a behavioural viewpoint

2.3.1 Factors influencing suicidal behaviour

The causes of suicidal behaviour are complex and not clearly understood. Past conceptions highlighted the role of social and psychological factors in the occurrence of

suicide. The key psychosocial risk and protective factors that can be used for interventions are listed in Panel 1. The discussion of each of these factors was outside the scope of this study. For a full review of these refer to O'Connor and Nock (2014). Nowadays biological factors are also considered as a contribution to suicide. The amount of research related to suicidal behaviour indicates the complexity of the behaviour and the need to understand it. A multitude of factors that have been associated with suicidal behaviour include the following: aggressive, impulsive, hopeless or pessimistic traits (Pezawas et al., 2002); substance abuse and alcoholism (Murphy, Wetzel, Robins, & McEvoy, 1992; Murphy & Wetzel, 1990; Rich, Fowler, Fogarty, & Young, 1988; Roy, Lamparski, DeJong, Moore, & Linnoila, 1990; Roy & Linnoila, 1986; Roy, Lamparski, DeJong, Adinoff, et al., 1990); a history of physical or sexual abuse during childhood (Brodsky, Malone, Ellis, Dulit, & Mann, 1997); a history of head injury or neurological disorders (Brent, 1986; Breslau, Davis, & Andreski, 1991; Breslau, 1992; Farrer, 1986; Schoenfeld et al., 1984); and cigarette smoking (Breslau, Kilbey, & Andreski, 1991). Further factors, as cited by Brezo, Paris, and Turecki (2006), include genetics, relational networks, family dynamics, family and personal history of psychopathology, trauma, abuse and stress, family and personal history of suicidal behaviour, and

personality factors. Disorders, diagnosed according to the DSM-V, that often shows comorbidity with suicidal behaviour include Axis I disorders such as schizophrenia, mood, anxiety, and substance-related disorders and Axis II Cluster B disorders (Brent et al., 1994;

Panel 1: Key psychological risk and protective factors for suicidal ideation and suicidal behaviour as taken from O'Connor and Nock (2014).

Personality and Individual Differences

- Hopelessness
- Impulsivity
- Perfectionism
- Neuroticism & extroversion
- Optimism
- Resilience

Cognitive Factors

- Cognitive rigidity
- Rumination
- Thought suppression
- Autobiographical memory bias
- Belongingness
- Burdensomeness
- Fearlessness about injury and death
- Pain insensitivity
- Problem solving and coping
- Agitation
- Implicit associations
- Attentional biases
- Future thinking
- Goal adjustment
- Reasons for living
- Defeat and entrapment

Social Factors

- Social transmission
- Modelling
- Contagion
- Assortative homophily
- Exposure to deaths by suicide of others
- Social isolation

Negative Live Events

- Childhood adversities
- Traumatic life events during adulthood
- Physical illness
- Other personal stressors
- Psychophysiological stress

Mann & Currier, 2009; Wender et al., 1986). Axis I disorders include all the disorders that are not due to mental retardation or personality. Axis II includes all the personality disorders and mental retardation. These are divided into three clusters: Cluster A refers to the personality disorders characterised by odd and eccentric behaviours; Cluster B presents with dramatic, emotional and erratic behaviour, characterised by impulse control problems and emotional dysregulation; and Cluster C experiences maladaptive fear and anxiety (APA, 2013).

Since so many different factors may independently contribute to the risk for suicide, several models have been proposed to explain the occurrence of suicidal behaviour. The origins of psychological theories with regards to suicidal behaviour can be traced back to Freud and research into this complex behaviour has grown extensively over the last 25 years (Ellis & Rutherford, 2008). These theories are important to provide a theoretical and clinical framework to understand the interaction of a multitude of factors that might increase the risk to suicidal behaviour. In addition to this, these theories can lead to interventions for suicidal behaviour (O'Connor & Nock, 2014). Contemporary models include a biological influence and are mostly diathesis-stress models which are based on the assumption that a stressor activates a pre-existing vulnerability or diathesis which culminates in negative behaviour. As an example of this, the clinical model of suicide was developed by Mann, Waternaux, Haas, and Malone (1999) and will be discussed in further detail. Modern-day researchers have highlighted the necessity for a comprehensive, multidisciplinary approach to suicidal behaviour. The possible contributions of individual, trait-like vulnerability underlain by genetics, in addition to environmental and individual stressors needs to be realised (Mann et al., 1999; Riskind, Long, Williams, & White, 2000).

2.3.2 Clinical model of suicidal behaviour

One prominent example of a stress-diathesis model is the clinical model of suicidal behaviour as developed by Mann et al. (1999). They felt studies with regards to suicidal

behaviour restricted to one domain of risk factors, such as social, psychiatric, psychological or familial were too narrow to take into account the importance of the different factors and the interrelationship between them. The aim was to create a model that used multivariate techniques across different diagnostic categories to create a general model to predict and explain suicidal behaviour. The study analysed 347 patients with different Axis I and II diagnoses and examined personality traits of impulsivity and aggression, recent life events, and a set of clinical and demographic variables. These were then compared between suicide attempters and non-attempters. They created the model of suicidal behaviour (see Figure 2.2) that included the significant factors associated with suicidal behaviour.
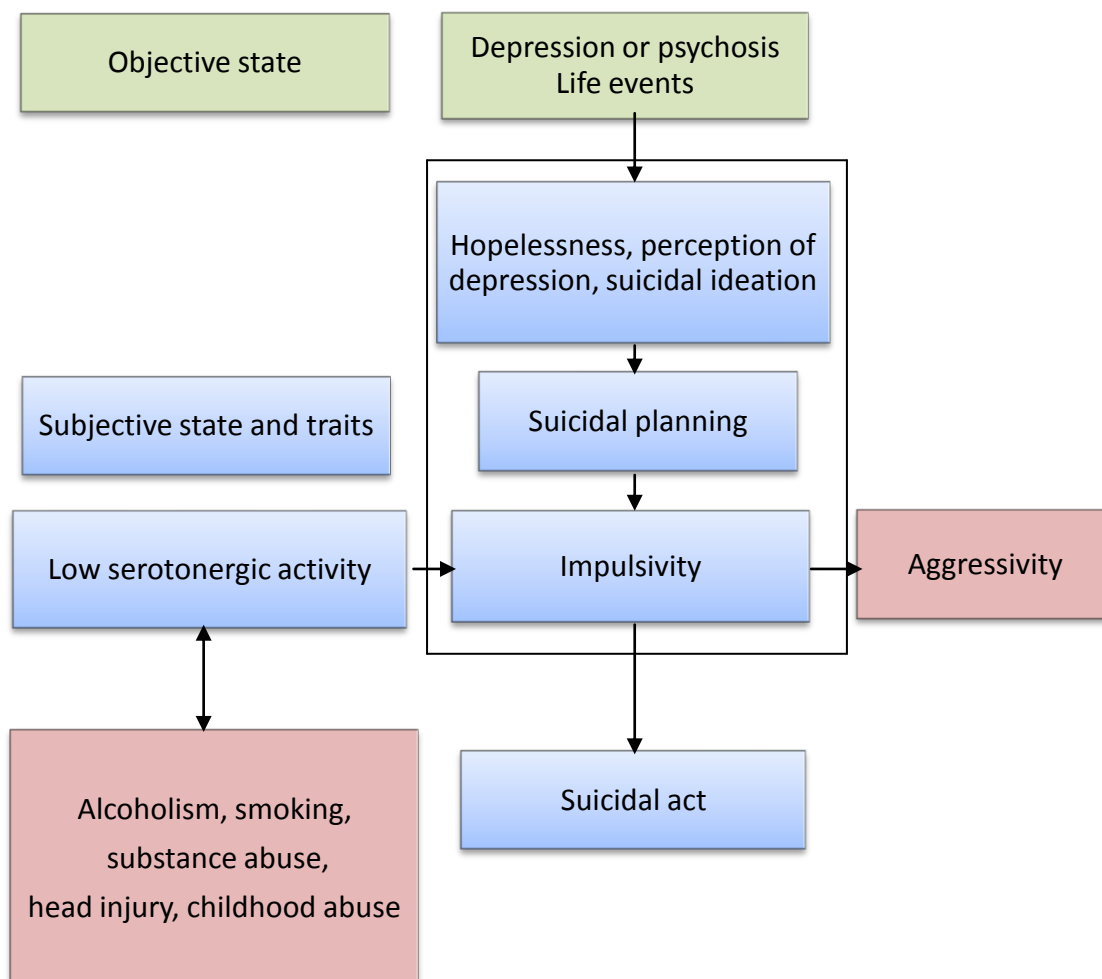
Figure 2.2 A clinical model of suicidal behaviour as proposed by Mann et al. (1999).

The traits of aggression and impulsivity were significantly associated with the suicide attempters, regardless of the psychiatric diagnosis. This higher impulsivity or impaired decision making might lead to a higher propensity to act on aggressive or suicidal thoughts. Although the objective severity of the diagnosis, as measured by clinicians, did not distinguish the suicide attempters, as was the case with life events, the subjective perception of the patient of their own depression did. The subjective experience of depression, suicidal ideation, hopelessness and fewer reasons for living were significantly associated with the suicide attempters. The negative life events experienced did not significantly differ between the suicide attempters and non-attempters and did not explain the difference in the subjective experience of having fewer reasons to live. The difference might be due to the diathesis of the suicide attempters. This diathesis, in turn, might be attributed to the difference in the traits, such as aggression and impulsivity, which were influenced by genetic factors. They also found an association for suicidal behaviour and head injuries, a history of child abuse, alcoholism, substance abuse and smoking, which in turn were also associated with impulsivity and aggression. A common factor that linked these factors together was identified as the serotonergic system. Low serotonergic activity was proposed to modulate the genetic and developmental effects on suicide, impulsivity, aggression and alcoholism, which in turn might contribute to a higher propensity to commit suicidal behaviour, independent of a psychological disorder.

2.4 Suicide from a genetics viewpoint

In the previous section, Mann et al. (1999) postulated that a common, underlying genetic basis might explain the association between suicidal behaviour and impulsive and aggressive behaviours and might form part of the diathesis of suicidal behaviour. The genetic link to suicide has been established through several studies (Arango, Huang, Underwood, & Mann, 2003; Murphy & Wetzel, 1982; Özalp, 2009; Roy, 1983; Van Heeringen, 2003; Wasserman, Geijer, Sokolowski, Rozanov, & Wasserman, 2006). Genetic factors were suggested to play a role in 30 - 50% of reported cases of suicidal behaviour, independent of other psychological stressors or disturbances (McGuffin et al., 2001; Roy et

al., 1995). Family, twin and adoption studies suggested that there is a very strong link between genetic factors and the predisposition to suicidal behaviour.

2.4.1 Family genetic studies

Family studies are predominantly used to determine the degree of risk of relatives developing some disorder or illness, which can be attributed to genetics (Jang, 2005). With regards to suicidal behaviour, the rate of suicidal behaviour in the relatives of the proband with suicidal behaviour is compared to the rate in relatives of the proband with no suicidal behaviour (Brent & Mann, 2005).

A family study, consisting of 243 patients with a family history of suicide, found that 48.6% (n = 118) patients of the total cohort had attempted suicide, 56.4% (n = 137) had a mood disorder and 34.6% (n = 84) was diagnosed with affective disorder. These results were compared with a control group with no history of suicide in the family (n = 5 602). The conclusion was that a family history of suicide would significantly increase the risk for attempted suicide (Roy, 1983). Several other studies have determined that family history of suicide almost doubled the risk for suicide. This was the case even after controlling for any family psychiatric illness, which was also a strong predictor of suicide and indicated that the genetic basis for suicidal behaviour was independent of the heritability of major depression (Qin, Agerbo, & Mortensen, 2003; Runeson & Asberg, 2003).

It was important to establish if the genetic risk for suicidal behaviour was independent of the genetic risk for psychiatric disorders, as these often show comorbidity in individuals (Ernst et al., 2009). One of the first family studies to determine this, was conducted in the Old Order Amish community in Pennsylvania, USA. A review of records kept from 1880 - 1980 allowed for the identification of 26 suicides of which 19 (73%) occurred in the same four families, who also presented with mood disorders. However, in other families with

similar clinical characteristics and overlapping features of mood disorders, no suicides occurred. This was indicative of a genetic basis to suicidal behaviour that was independent of the genetic basis of psychopathology (Egeland & Sussex, 1985). Research on the suicide risk of parent-child transmission found a six times higher risk for the children of suicide attempters to attempt suicide versus those whose parents did not attempt suicide. The children who attempted suicide were found to have a mood disorder in 82% of the cases. If the parent and child also share a history of sexual abuse and high impulsive aggression, the risk for suicide in the children of suicide attempters was even higher (Brent et al., 2002).

In a Swedish population study consisting of 11.4 million individuals, the suicide rate of relatives of 83 951 suicide victims was compared with those relatives of a matched control group. They found statistically significant results for a higher risk for suicidal behaviour amongst first-degree relatives of those individuals who committed suicide. They determined an odds ratio for suicidal behaviour in monozygotic twins to be 12.3 and in dizygotic twins 2.3 (Tidemalm, Runeson, Waern, Frisell, & Carlstro, 2011). All of these studies showed that suicidal behaviour aggregates in families.

2.4.2 Twin studies

Twin studies are important in determining whether genetics affects certain behaviours. Monozygotic (MZ) twins are siblings with identical genotypes, whereas dizygotic (DZ) twins share only half of their genes. In order to determine if the genetic influence is significant, the MZ twins would present with a higher concordance rate, or higher similarity for a certain behaviour than the DZ twins (Plomin, De Fries, McClearn, & Rutter, 1997). The results from twin studies have consistently indicated that the concordance rate for MZ twins are higher than for DZ twins for suicidal behaviour (Ernst et al., 2009).

Initial twin studies reviewed by Roy (1992) found that the average concordance rate for fatal suicide was significantly higher for MZ (13.2%) than DZ (0.7%) twins. Roy, Segal, and Sarchiapone (1995) also analysed completed and attempted suicide in twins and found the concordance rate for attempted suicide to be 38%. This was higher than the concordance rate for completed suicides of 13.2% in the previous study in 1992. Roy and Segal (2001) conducted another two twin studies and, together with the review study from 1992, found the average concordance rate for suicide between al six studies to be 18.5% for the MZ twins and 0.7% for the DZ twins.

One of the largest published twin studies found the risk of a serious suicide attempt to be 17 times greater in MZ twins with a serious suicide attempt than the risk in the total sample. The study recruited 5 995 twins in Australia to evaluate different suicidal behaviours. The concordance for serious suicide attempt in MZ twins was 23.1% and 0% for DZ twins. Even after controlling for other risk factors, the familial risk for suicidal behaviour was still 3.8 times higher for the MZ co-twin to make an attempt, than for the DZ co-twin. The heritability for serious suicide attempt was as high as 55% (Statham et al., 1998). Another twin study with 3 401 female twins in Missouri, USA, found similar results. The additive genetic effects were calculated at 48% and concordance for MZ and DZ twins were 25% and 12.8% respectively for suicide attempts. The heritability for an attempt was 38% (Glowinski et al., 2001). These results were higher than in the previous study, but still with a significant difference between MZ and DZ twins. With a study of 6 744 adult male twins in Missouri, USA, the results were similar. However, the heritability for suicide attempts was calculated at 30%, which is lower than in previous studies, but still proves a genetic transmission (Fu et al., 2002).

2.4.3 Adoption studies

Adoption studies have two sets of factors, namely the biological and adoptive parents, that can be compared to possibly account for differences in behaviour in the child. If

behaviour is more similar between the child and the biological parents, this can possibly be attributed to genetic factors (Plomin et al., 1997). Adoption studies have also confirmed a genetic link to suicide (Ernst et al., 2009). Schulsinger, Kety, Rosenthal, and Wender (1979) studied 57 adoptees who had committed suicide and a matched control sample of adoptees. The biological relatives of those adoptees who committed suicide had 12/269 suicides versus 2/269 of the biological relatives of the control group with no suicides. This was a six times higher rate of suicide in the biological relatives of the adopted children who committed suicide. This, together with the absence of suicide in the adoptive parents of children with suicide, supported the role of a genetic effect on suicidal behaviour. No suicides occurred amongst the adoptive relatives of either group. A subsequent study determined the frequency rate of suicide among the relatives of suicidal adoptees (n = 71) were 15 times higher than those of the control group. The findings also indicated a significantly higher frequency of suicides amongst those biological relatives of adoptees who were more liable to act on an emotional stressor with an impulsive suicide attempts. This led to the conclusion that the genetic predisposition to suicidal behaviour might possibly be linked to impulse control (Wender et al., 1986). Reviewing the existing family, twin and adoption studies, Brent and Mann (2005) suggested that some people were genetically predisposed to suicidal behaviour, and more importantly, the tendency to act on suicidal thoughts, and that this genetic vulnerability might possibly be linked to inherited personality traits such as impulsiveness and aggression. Impulsiveness and aggression have therefore been suggested as possible endophenotypes of suicidal behaviour.

2.5 Endophenotypes of suicidal behaviour

Since suicidal behaviour is a complex behaviour, it is difficult to determine the specific genes that might contribute to this maladaptive behaviour. As a consequence, the endophenotype approach might be a possible solution to studying suicidal behaviour (Carballo et al., 2009). An endophenotype is described as an internal phenotype between a gene and disease. This approach identifies genes associated with heritable intermediate phenotypes and might make it easier to determine genes related to the predisposition to

complex behaviour, such as suicidal behaviour. The endophenotype exists out of more basic constructs, which allows for easier measurement than that of the complex behaviour (Gottesman & Gould, 2003). Accordingly, personality traits, which have a genetic basis, may be endophenotypes for the underlying genetic basis of suicidal behaviour (Baud, 2005). Traits are characteristic, behaviour-motivating dispositions that determine how individuals relate to and cope with their realities (Cloninger, 1994; Krueger, Caspi, & Moffitt, 2000; McCrae & Costa, 1997). Individual personality traits are formed through interactions between genes and the environment and are mostly stable through the life-span (Brezo, Paris, & Turecki, 2006; Furnham, 1981; Harkness & Lilienfield, 1997). These personality traits are different from mood or emotional states. States are temporary moods or states of mind (Allport & Odbert, 1936). Some overlap between state and trait dimensions can occur. Suggested endophenotypes for suicidal behaviour include impulsive and aggressive traits, early-onset major depression, neurocognitive function, and cortisol response to psychosocial stress (Mann et al., 2009).

In the current study, the focus fell on impulsive and aggressive behaviours as a possible endophenotype for suicidal behaviour. According to the clinical model of suicidal behaviour (Mann et al., 1999), the role of aggression and impulsivity were highlighted as one of the strongest influences on suicidal behaviour (see section 2.3.2). As early as 1986, it was reported that the familial link between impulsive aggression and suicidal behaviour was transmitted independently of mood disorders. This adoption study of mood disorders in Denmark found a significant association ($p < 0.0001$) in the relatives with Cluster B personality disorders. These included diagnoses of affective reaction and were linked to impulsivity. This association was stronger than in the Axis I mood disorders such as bipolar disorder (BPD) and depression (see section 2.3.1) (Wender et al., 1986).

Impulsivity and aggression form a strong basis for an endophenotype of suicidal behaviour, because amongst suicidal behaviour, impulsivity and aggressive behaviours are often comorbid, and are often expressed since childhood. These two traits are mostly

stable through the lifespan and heritable; and the genes contributing to these personality traits might also confer a vulnerability for suicidal behaviour (Turecki, 2005),

2.5.1 Impulsivity

2.5.1.1 Definition

Impulsivity is a complex human trait and as such there are some difficulties with defining this construct. Impulsivity was defined as "a predisposition towards rapid, unplanned reactions to internal or external stimuli without regard to the negative consequences of those reactions to the impulsive individual or to others" (p.1784, Moeller, Barratt, Dougherty, Schmitz, & Swann, 2001). Impulsivity mainly refers to acting without thinking or forethought, and with no consideration of the possible consequences (Cardinal, 2006; Evenden, 1999). It is the difficulty to inhibit thoughts and actions, and the inability to delay any rewards (Congdon & Canli, 2006). Impulsive behaviours can be conceptualised on a continuum and also have a state dimension, which is an emotional response dependent on a specific moment (Turecki, 2005). High impulsivity can become maladaptive, and may often have undesirable outcomes (Evenden, 1999). It appears that there is a lack of a filtering process that should take place to regulate behaviour (Bechara, Damasio, Tranel, & Damasio, 1997). It can play a role in several types of psychopathology which include attention deficit hyperactivity disorder (ADHD), addiction and substance abuse and personality disorders (Congdon & Canli, 2006). Impulse control disorders are common and have a prevalence of 8.9% in the general population (Kessler, Chiu, Demler, Merikangas, & Walters, 2005).

Evidence for a genetic component for impulsivity has been confirmed by twin and family studies (Livesley et al., 1998; Taylor et al., 2003). Pederson, Plomin, McClearn, and Friberg (1988) found a heritability estimate of 0.45 and Seroczynski, Bergeman, and Coccaro (1999) confirmed this result when they determined a heritability estimate of 0.44 during a

twin study. Across various studies, heritability was estimated at between 30 - 45%, depending on the methods used in the study (Eaves & Eysenck, 1975; Scarr, Webber, Weinberg, & Wittig, 1981).

2.5.1.2 Impulsivity and suicidal behaviour

Various prospective and retrospective studies have found an association between impulsivity and suicidal behaviour (Crumley, 1979; Gut-Fayand et al., 2001; Koller et al., 2002; Pezawas et al., 2002; Wender et al., 1986). The WHO World Mental Health Survey Initiative conducted a project which consisted of interviews of 84 850 adults in 17 different countries regarding socio-demographic and psychiatric risk factors for suicidal behaviour (WHO, 2008). This study found that the strongest predictors of suicide tended to be impulse control disorders in low- and middle-income countries. South Africa was classified under the low- and middle-income group (World Bank, 2003). Impulsivity has also been positively associated with MSSA and a history of previous suicide attempts in a group of bipolar patients (n = 48). Approximately one quarter of MSSA had been carried out without premeditation. Impulsive suicide attempters had lower expectations of dying, but the method of the suicide attempt was more violent (Swann et al., 2005). This is consistent with the disassociation that exists between actions and intentions in impulsive individuals (Simon et al., 2001). Serious suicide attempts are connected to the planning of the attempts, which would suggest less impulsivity. However, when impulsivity was measured as a personality trait as opposed to a state of mind, it was associated with more severe suicide attempts (Beautrais, Joyce, & Mulder, 1999). This might seem conflicting and thus it is important to distinguish between impulsivity as a trait or state dimension.

Impulsivity creates a predisposition to suicidal behaviour by unconsciously creating a faulty filtering process and impulsively reacting on suicidal ideation or such emotions as anger. These interact with other risk factors such as depression or hopelessness (Mann et

al., 2009; Swann et al., 2005). This faulty processing prohibits an individual from thinking before acting, and failing to determine the consequences of his or her actions.

2.5.2 Aggression

2.5.2.1 Definition

Aggression is a complex trait that can show a large spectrum of behaviours (Lesch & Merschdorf, 2000). The most basic definition of aggression is that it includes behaviours that are intended to harm or hurt others or the self, however, this does not fully explain this complex behaviour (Ligthart, Bartels, Hoekstra, Hudziak, & Boomsma, 2005; Weinshenker & Siegel, 2002). The role of the victim in aggressive behaviour must also be considered. The victim has to make an effort to avoid the harm, and the aggressor has to be aware of the effort of harm avoidance from the victim. This can be explained by the definition provided by Geen (2001) which states the following: "Aggression is the delivery of an aversive stimulus from one person to another, with intent to harm and with an expectation of causing such harm, when the other person is motivated to escape or avoid the stimulus." (Geen, 2001, p.168).

The subtypes of aggression that shows the most distinction, are those of the impulsive-reactive-hostile-affective subtype versus the controlled-proactive-instrumental-predatory subtype (Vitiello & Stoff, 1997). These are generally referred to as reactive and proactive aggression, respectively. Proactive aggression is usually without anger and is used to obtain something such as power or goods. It is, therefore, mostly offensive and premeditated and stems from a lack of emotional sensitivity. Conversely, reactive aggression stems from excessive emotional sensitivity. It typically occurs in response to a threat and is more defensive and volatile (Blair, Peschardt, Budhani, Mitchell, & Pine, 2006; Brendgen, Vitaro, Boivin, Dionne, & Perusse, 2006; Crick & Dodge, 1996). Even though these two main subtypes of aggression display many overlapping features, they are fairly

distinct in some areas, one which includes impulse control (Struber, Luck, & Roth, 2008). Proactive aggression demonstrates a control of impulsivity, whereas reactive aggression exhibits an impulsive element. Reactive aggression is therefore also referred to as impulsive aggression. Although aggression and impulsivity are two different psychological constructs, there is a call to use impulsive aggression as a single construct when it is discussed in relation to suicidal behaviour.

Aggression as a trait is relatively enduring through the life-span and is to a large extent heritable. Individual differences in aggression are most likely to be a result of the interaction of genetic variation and environmental factors (Lesch & Merschdorf, 2000). Twin, adoption and family studies have all shown consistent findings to suggest a genetic basis for aggression. Miles and Carey (1997) conducted a meta-analysis of 24 genetically informative studies on aggression from 1963 – 1990. Even though the first of these studies, using 68 pairs of adolescent twins, reported correlations of 0.57 and 0.18 for aggression in MZ and DZ twins respectively (Gottesman, 1963), not all studies had similar findings. The different findings for heritability of aggression ranged from 44 - 72%. Miles and Carey (1997) concluded that the overall genetic effect might be responsible for up to 50% of individual differences in aggression.

2.5.2.2 Aggression and suicidal behaviour

Evidence that linked aggressive behaviour to suicidal behaviour has been confirmed by multiple studies (Borowsky et al., 2001; Brent et al., 1994; Garrison et al., 1993; Ivanoff & Jang, 1991; Shaffer et al., 1996). Suicidal behaviour can be described as a form of internally directed aggression and a statistically significant association have been found for the existence of both internal and externally directed aggression within the same individual (Roy-Byrne & Upadhyaya, 2002). Almost 30% of violent individuals perform self-destructive acts and a past history of externally directed violence can be found in 10 - 20% of suicidal individuals (Mann, 1994). A higher degree of threatened and attempted violence was

present in the lives of those who committed suicide than those who died accidentally (Conner et al., 2001). Adolescent suicide victims had a life-time history of aggression (Brent et al., 1994). Brent, Bridge, Johnson, and Connolly (1996) found the rate of suicidal behaviour increased in the first-degree relatives of the research group. This was true even after controlling for psychiatric disorders. Their results also indicated a possible association between the heritability of aggression and suicidal behaviour in a study using 58 adolescents who committed suicide and 55 community controls. Anger-related personality traits could also play a role in the characteristics of the suicide attempt by affecting the intent and choice of violent method used (Giegling et al., 2009).

2.5.3 Impulsive aggression and suicidal behaviour

Impulsive aggression has been defined as a tendency to respond with hostility or aggression to a provocation or frustration (Wender et al., 1986). Impulsivity is strongly associated with aggression (Apter et al., 1990). This has been found to be particularly true for the reactive subtype of aggression which is characterised by angry and impulsive responses when the individual perceives the situation to be stressful (Coccaro, Bergeman, Kavoussi, & Seroczynski, 1997; Seroczynski et al., 1999). An explanation for this can be that people will act easier on their angry impulses when emotional stress causes less impulse control and self-regulation. Seroczynski et al. (1999) studied the contribution of both genetic and environmental factors on the behavioural (phenotypic) relationship between impulsivity and aggression in 182 and 181 pairs of MZ and DZ male twins, respectively. They concluded that the genetic and environmental influences leading to impulsivity were common to those that would lead to reactive aggression. Reactive or impulsive aggression has however been described inconsistently as follows: a single trait-like dimension (Coccaro et al., 1989; Siever & Davis, 1991); a subconstruct of impulsive behaviours (Seroczynski et al., 1999); a subconstruct of aggressive behaviours (Barratt, Monahan, & Steadman, 1994; Barratt & Slaughter, 1998); or the amalgamation of two separate traits (Depue & Lezenwerger, 2001). It is clear that depending on which definition was used, different conclusion might have been drawn based on the definition of the terms. Critchfield, Levy,

and Clarkin (2004) called for the use of impulsive aggression as a single trait-like dimension based on the assumption that reduced central serotonergic functioning in mood and/or personality disorder patients is associated with the dysregulation of impulsivity, which subsequently enhances the likelihood of aggressive behaviours in the presence of specific environmental triggers. Mann et al. (2009) also called for the use of impulsive aggression as a single trait-like construct when referring to suicidal behaviours.

Impulsive aggressive traits are amongst the most studied personality traits with regards to suicidal behaviour and are under some genetic control (Baud, 2005; Brent et al., 2004; Seroczynski et al., 1999). One hypothesis was that the genetic contribution for suicide that results in a high familial transmission, might be mediated by impulsive-aggressive behaviours (Coccaro, Silverman, Klar, Horvath, & Siever, 1994; Mattes & Fink, 1990; Stewart & Leone, 1978). This was confirmed by Brent et al. (1996), who studied the relationship between psychiatric disorders and a life-time history of aggression and suicidal behaviour in a family and family history study, using the relatives of 58 adolescent suicide probands and 55 controls. They found that suicide attempters with high aggression had significantly higher familial loading for suicidal behaviour than less aggressive suicide attempters. The relatives of a subgroup of fatal suicides had a high rate of personality disorders characterised by impulsive-aggressive behaviours (Brent et al., 1996). A family history of suicidal behaviour, together with life-time impulsive aggressive behaviours, were associated with a younger age of onset and recurrent attempts in offspring of suicide attempters (Brent et al., 2003). A study with suicidal and non-suicidal psychiatric patients found that anger-related traits interacted with high impulsivity to create a higher risk for suicide (Horesh et al., 1997). Several other studies have confirmed the association of impulsive aggression and suicidal behaviour (Brodsky et al., 2001; Mann et al., 1999; McGirr & Turecki, 2007; Melhem et al., 2007; Placidi et al., 2001; Turecki, 2005). Another hypothesis was proposed to clarify the role of aggression in suicide risk and suggested that impulsivity was a catalyst for aggression and suicidal behaviours. Poor impulse control was confirmed to be significantly related to suicidal behaviour even after controlling for psychiatric disorders (Conner,

Meldrum, Wieczorek, Duberstein, & Welte, 2004; Sanislow, Grilo, Fehon, Axelrod, & McGlashan, 2003).

There are converging lines of evidence to suggest that the neurobiological basis underlying the association between impulsivity, aggression and suicidal behaviour is similar (Joiner, Brown, & Wingate, 2005; Mann & Currier, 2009). This can be seen as evidence for impulsive and aggressive behaviours as an endophenotype for suicidal behaviour. The current study used the definition of impulsive aggression as a subtype of aggression and therefore used the term reactive aggression.

2.6 Neurotransmitter pathways and behaviour

The association between impulsivity, aggression and suicidal behaviour might be linked to predisposing, biological factors which can include an imbalance in different neurotransmitter pathways (Seo, Patrick, & Kennealy, 2008). Neurotransmitters are responsible for a myriad of processes including behaviour. Behaviour is the result of an interaction between the environment and the brain and is mediated through a complex messenger system rooted in the central nervous system (CNS). Specialised cells named neurons act as intermediaries between the brain and environment and can influence the resulting behaviour (Anholt & MacKay, 2010).

The neuron relays an electrical impulse via an action potential. Each stimulus has a specific rate and pattern of electrical impulse and these are essential to relay the correct information (Jacobs, 1991). The action potential changes along the neuron which allows the impulse to be relayed to the axon terminal of the presynaptic neuron. This axon terminal is separated from the postsynaptic neuron through the synaptic cleft. Two neurons are never in contact with each other; communication takes place through neurotransmitters which diffuse through the synaptic cleft. A specific neurotransmitter is released according to a

specific stimulus and allows the impulse to be carried over to the postsynaptic neuron. Neurotransmitters are stored in vesicles in the axon terminal. Once a specific stimulus reaches the axon terminal, the relevant neurotransmitter is released into the synaptic cleft where it binds to a respective receptor in a lock-and-key fashion (Sherwood, 2007). Neurotransmitters can be either excitatory or inhibitory or both. Once the function of the neurotransmitter is completed it has to be removed from the synaptic cleft. This can be done through reuptake of a transporter, or by metabolism. If there is any malfunction in this process, or in any of the receptors, transporters or metabolising enzymes, the pathway will be disrupted. Signal transduction to and from the brain might be impaired (Crossman & Neary, 2005). Changes in the genes that regulate these pathways also have an effect. Neurobiological studies have been conducted to determine the involvement of different neurotransmitter systems in suicidal behaviour. These systems included those of serotonin (5-HT), dopamine (DA), noradrenalin (NE) and gamma-aminobutyric acid (GABA) (Fig. 2.3) (Carballo et al., 2009; Crisafulli et al., 2010; Ernst et al., 2009; Mann, 2003).

Deficient serotonergic activity has been suggested as a possible neurochemical basis for the predisposition to impulsive and aggressive behaviours, with these behaviours, in turn, being a strong predictor of suicidal behaviour. This association has been proven by numerous studies (Dumais, Lesage, Alda, et al., 2005; Kamali et al., 2001; Newman et al., 1998; Zanarini et al., 2004). High lethality suicide attempts have been significantly associated with a low level of serotonin (Malone, Corbitt, Li, & Mann, 1996). The theory has been proposed that impulsive and aggressive behaviours may stem from a lack of inhibitory capabilities, such as the inability to control aggressive impulses and negative emotions, which is under the control of the ventral prefrontal cortex (PFC). This might be due to the hypofunction of serotonergic activity in this part of the brain. This might lead to self-directed impulsive aggression or suicidal behaviour when an individual experienced a major life stressor. A similar neurobiological mechanism of deficient serotonergic functioning, underlying both impulsive, aggressive and suicidal behaviours, might result in a predisposition for the mentioned behaviours (Kamali et al., 2001; Mann et al., 1996; Oquendo et al., 2003).

The noradrenergic system has been found to play a role in the regulation of stress response and has been studied in relation to suicidal behaviour (Crisafulli et al., 2010). High concentrations of NE have been positively associated with high levels of aggression in post-mortem studies of suicides (Carballo, Akamnonu, & Oquendo, 2008). A higher amount of TH and α- and β-adrenergic receptor dysfunctions have been indicated in most studies of suicide subjects versus controls (Heim & Nemeroff, 2001; Ordway, Widdowson, Smith, & Halaris, 1994; Pandey & Dwivedi, 2007). However, less noadrenic neurons were found in the brains of depressed and suicidal patients, but with greater β-adrenergic and less α-adrenergic receptor binding. The overall effect of these changes led to noradrenergic hyperactivity which might have stemmed from the depletion of the NE neurons in suicide victims (Arango, Ernsberger, Sved, & Mann, 1993). Some studies also indicated lower CSF (cerebrospinal fluid) 3-methoxy-4-hydroxphenylglycol (MHPG), which is a metabolite of NE, in various suicidal behaviour study groups (Agren & Niklasson, 1986; Virkkunen, De Jong, Bartko, & Linnoila, 1989).

There are not many studies regarding the role of the dopaminergic system in suicidal behaviour, however, a relationship was observed between the dopaminergic system and depressive disorders and alcoholism (Dailly, Chenu, Renard, & Bourin, 2004). The main finding was for an association between higher concentrations of DA and aggression (Rujesco, Giegling, Gietl, Hartmann, & Moller, 2003). This could lead to an association with violent suicide attempts or completed suicides (Carballo et al., 2008; Rujesco et al., 2003). No consensus has yet been reached concerning the levels of dopamine metabolite homovanilic acid (HVA) as a possible predictor of suicidal behaviour (Placidi et al., 2001; Roy, De Jong, & Linnoila, 1989; Sher et al., 2006; Traskman-Bendz et al., 1992). Even though some other neurotransmitter systems, including GABA and cholinergic systems, have been considered, most evidence were found for the role of serotonin in impulsive and aggressive and suicidal behaviour (Crisafulli et al., 2010). For the purposes of this study, the serotonergic neurotransmitter system will be discussed in more detail, as the common underlying neurobiological basis for the association between impulsivity, aggression and suicidal behaviour.
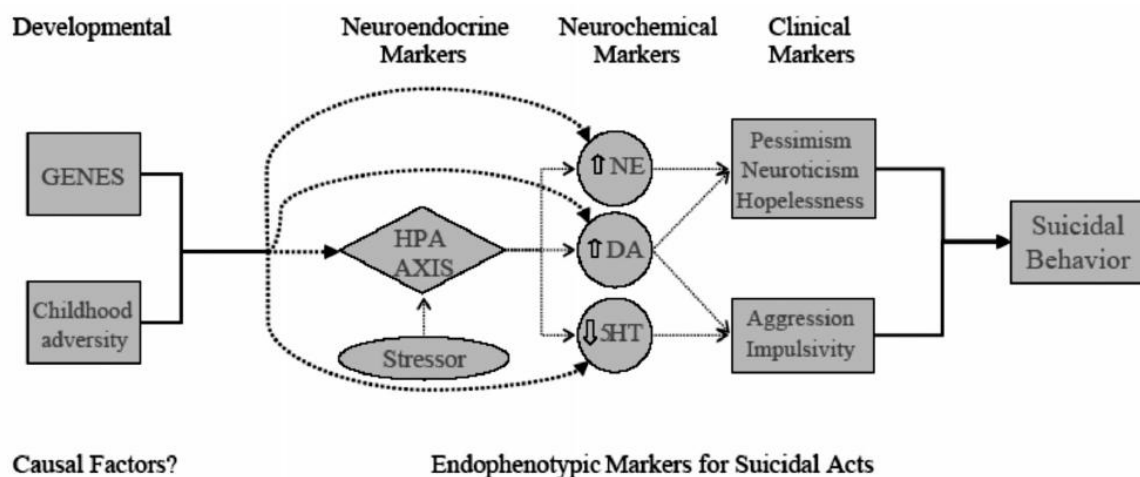
Figure 2.3 Possible endophenotypic markers for suicidal behaviour that includes serotonin (5-HT) as well as aggression and impulsivity (Carballo et al., 2009).

2.7 Serotonin and suicidal behaviour

2.7.1 Serotonergic pathway

Serotonin is a neurotransmitter found in neurons, mast cells, enterochromaffin cells and platelets. Serotonin influences around 500 000 targets, including other neurons, glands and muscle cells. The brain contains only 1 - 2% of all the serotonin in the body, but the branching of the serotonergic system is the most expansive of the neurotransmitter systems in the brain (Jacobs, 1991; Lesch & Merschdorf, 2000). Since the blood-barrier prevents the movement of 5-HT into the brain, all serotonin has to be produced in the CNS. This is achieved by the absorption of tryptophan from the blood, which is mostly ingested as part of the diet. Tryptophan is used to synthesise serotonin in the serotonergic terminals through the working of the enzyme TPH which is encoded by the gene, *TPH*. Serotonin is then stored in vesicles until neuronal activation takes place which allows the vesicles to fuse with the neuronal membrane. Serotonin neurons have a very distinct firing pattern of a relatively slow and constant discharge rate of three spikes per second during an awake state. The spiking rate increases during emotional arousal to around four or five spikes per second causing an increase in serotonin release. Serotonin is released into the synaptic cleft where it is free to bind to various 5-HT receptors, which are encoded by the *5-HT* receptor

genes (*HTR1A, HTR2A, HTR1B, etc.)* on both pre- and postsynaptic neurons. Serotonergic neurotransmission is effectively triggered when binding of serotonin with the receptors takes place. These receptors function through a negative feedback mechanism where the released serotonin binds to the autoreceptors on the releasing cell and acts as an inhibitor of further activity (Jacobs, 1991).The serotonin transporter (5-HTT), encoded by *5-HTT*, is responsible for the modulation of the concentration of serotonin in the synaptic cleft and will activate the reuptake of serotonin into the presynaptic neuron. There the serotonin either return to the vesicles, or it is metabolised by MAO to 5-hydroxyindoleacetic acid (5-HIAA), which is an inactive metabolite (Best et al., 2010; Cooper & Bloom, 1996; Delgado et al., 1990).

This whole process of serotonergic neurotransmission is regulated by numerous proteins which include the serotonin receptors such as serotonin receptor 1A (5-HT1A), serotonin receptor 1B (5-HT1B), and serotonin receptor 2A (5-HT2A), etc. and serotonin transporters (5-HTT), as well as serotonin synthesising (TPH) and metabolising (MAO) enzymes (Lesch, 2005). Evidence suggested that alterations in brain serotonin synthesis and receptor binding might alter neurotransmitter functioning (Lanzenberger et al., 2010; Stokes, 1995). Each of these 5-HT-related proteins is encoded by respective *5-HT*-related genes. Polymorphisms of some of these genes might affect the functioning of the serotonergic system in various ways and will be discussed further in section 2.7.3. Figure 2.4 visually represents the serotonergic neurotransmitter pathway.
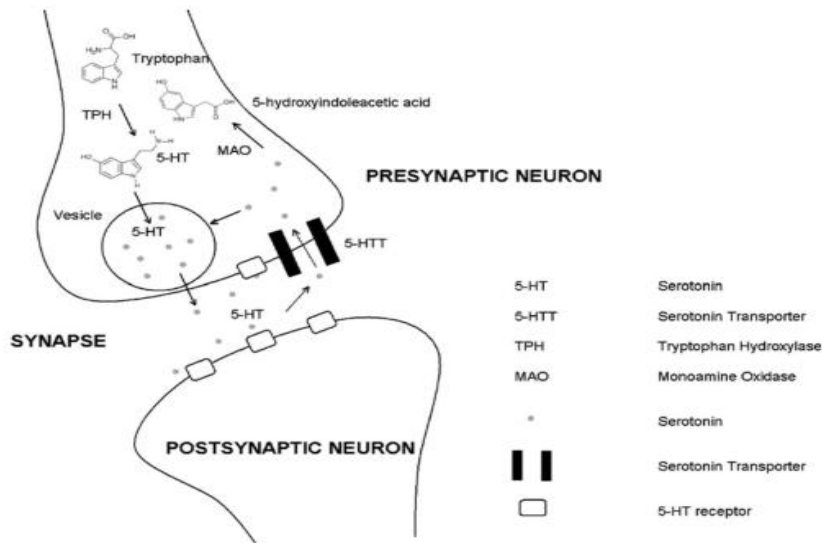
Figure 2.4 A schematic view of serotonergic neurotransmission (Watanabe *et al.*, 2011).

2.7.2 Serotonin and behaviour

Serotonin has been the focus of many scientific studies which have shown evidence for the role of serotonin in key behaviours. Almost all serotonin-related activity is initiated from neurons which are located in the raphe nuclei. These neuron clusters are located in the brainstem, which is the most primitive part of the brain and it is therefore understandable that serotonin is involved in many key psychological and behavioural aspects of functioning (Jacobs, 1991). Serotonin has been found to be an essential modulator of brain development (Azmitia & Whitaker-Azmitia, 1997), and emotional behaviour, including anxiety, aggression and impulsivity (Crawford, Craige, & Beck, 2010; Huang, 2010; Westenberg, Murphy, & Den Boer, 1996). Other behaviours influenced by serotonin include complex brain functions such as cognition, motor activity and sensory processing. Appetite, cardiovascular function, regulation of body temperature, experiencing of pain, sleep patterns, regulation of the endocrine system and reproductive functioning are all in some way affected by serotonin (Best et al., 2010; Chojnacka-Wojcik, 1995; Haleem, 2011; Kamali et al., 2001; Lanzenberger et al., 2007; Westenberg et al., 1996). As well as acting as a neurotransmitter, serotonin can act as a neuromodulator, which will have an impact on other neurotransmitter systems (Robinson, Hermans, Seipel, & Wightman, 2008).

This is one the reasons why serotonin is involved in the regulation of so many diverse functions (Lanzenberger et al., 2010; Lesch & Merschdorf, 2000; Stokes, 1995).

Serotonergic activity is partly under genetic control, thus environmental factors still have a significant influence on the regulation of serotonin (Higley et al., 1993). This interplay between genetic and environmental factors can cause a wide range of serotonin-mediated behaviours and might range from minor personality characteristics to major psychiatric disorders (Staner & Mendlewicz, 1998). The heritability of the serotonergic system has led to the proposal that the genetic predisposition underlying the risk for suicidal behaviour, depression, aggressive behaviour, alcoholism and substance abuse are mediated by serotonergic functioning (Lesch et al., 1996; Mann, 1998). Several studies have shown support for this assumption, for example, suicidal behaviour and variation in serotonergic functioning have been associated with polymorphisms in the *TPH* gene (Mann et al., 1997). In addition, *HTR1A*, *HTR2A, HTR1B* and *SLC6A4* have also shown to be candidate genes implicated in certain serotonergic abnormalities identified in individuals with major depression or a history of suicidal behaviour (Mann, Brent, & Arango, 2001).

Serotonergic functioning can be determined through different measures, including CSF 5-HIAA studies, brain imaging and pharmacochallenge studies (Turecki, 2005). The most replicated findings for the influence of the serotonergic system in suicidal behaviour comes from CSF 5-HIAA studies. With CSF 5-HIAA studies, the level of 5-HIAA, a metabolite of serotonin, in the CSF was measured to determine if serotonergic functioning was increased or decreased in certain study groups. Lower CSF 5-HIAA levels indicated lower serotonergic functioning. Although some limitations have been found with the use of this method, it has shed some light onto the relationship between behaviour and brain chemistry (Underwood & Mann, 2003).

Several CSF studies have been carried out regarding suicide and serotonin. Lowered levels of 5-HIAA have consistently been found in individuals with suicidal behaviour and was

also found to be indicative of further attempts (Asberg, 1997; Cooper, Kelly, & King, 1992; Lester, 1995). Researchers also looked into personality variables regarding suicidal behaviour and levels of 5-HIAA. CSF studies in impulsivity and aggression yielded similar results as with suicidal behaviour. When comparing 27 impulsive with nine non-impulsive violent offenders, the impulsive sample had lower CSF 5-HIAA-levels (Linnoila et al., 1983). A study conducted on 58 violent alcoholic offenders and 21 healthy controls found lower levels of CSF 5-HIAA in the irritable and aggressive alcoholics (Virkkunen et al., 1994). A study was conducted on drug-free convicted murderers and normal controls (n = 70) (Lindberg et al., 2000). No difference was found in concentration of CSF 5-HIAA. However, when looking into suicide attempts of the murderers, it was found that a third of the convicted murderers had tried to commit suicide and significantly lower levels op CSF 5-HIAA were recorded for those individuals. Those with impulse control disorders also recorded lower levels. Findings suggested that lower CSF 5-HIAA levels might be more common to impulsive rather than premeditated aggression (Linnoila et al., 1983; Virkkunen et al., 1994). It was suggested that impulsiveness might be the main factor, rather than aggression, that played a role in lower levels of 5-HT functioning (Virkkunen et al., 1994). However, the researchers suggested that impulsive aggression as a combined construct might be more closely associated with lower CSF 5-HIAA functioning (Lidberg, Tuck, Asberg, Scalia-Tomba, & Bertilsson, 1985; Linnoila et al., 1983).

Brain imaging studies of autopsies of suicide victims have been performed to analyse the brain and determine possible serotonin transporter and receptor binding abnormalities (Joiner et al., 2005). A high concentration of 5-HT2A, 5-HT1A and 5-HTT sites are present in the PFC (Arango et al., 1990). The overall finding for post mortem brain studies pointed towards less 5-HT transporter binding, particularly in the ventral areas (Arango, Underwood, Gubbi, & Mann, 1995; Mann et al., 1996, 2000; Tiihonen et al., 1997), increased presynaptic 5-HT1A binding in the neuronal clusters called the raphe nuclei (Stockmeier et al., 1998), and the upregulation of postsynaptic serotonergic receptors, especially 5-HT1A and 5-HT2A (Arango, Underwood, et al., 1995; Hrdina, Demeter, Vu, Sotonyi, & Palkovits, 1993; Turecki et al., 1999). These all led to reduced serotonergic input

in the brain. This brain region has also been implicated in impulse and aggression regulation (Davidson, Jackson, & Kalin, 2000). Most evidence for impulsive aggression showed that lowered serotonergic activity was mediated through 5-HT2A receptors, but other serotonergic agents might also play a role. For a complete review on post-mortem brain studies of suicide victims, see Furczyk, Schutová, Michel, Thome, and Büttner (2013).

Pharmacochallenge studies selectively alter the functioning of serotonin by changing the synthesis, release, reuptake or metabolism thereof through the use of selective chemical agents. As an example of a pharmacochallenge study, fenfluramine challenge studies used fenfluramine to manipulate the amount of serotonin available for use. Fenfluramine caused serotonin to be released from pre-synaptic vesicles in neurons, inhibited its reuptake, and also stimulated the 5-HT receptors on the post-synaptic neuron (Carlton & Rowland, 1989). This resulted in serotonergic activation which caused an increase in prolactin and also cortisol (Quattrone et al., 1983). The prolactin and cortisol responses were then measured to determine the levels of serotonergic activity. Higher concentration of fenfluramine led to an increase in serotonin levels, which in turn led to higher levels of prolactin or cortisol (Malone et al., 1996). Fenfluramine challenge studies have been used to study the activity of serotonin with regards to suicidal behaviour, impulsivity and aggression. These studies mostly replicated the findings of CSF studies and brain imaging studies (Kamali et al., 2001).

Some inconsistencies have been reported in studies with using these different measures of serotonergic functioning and this emphasised the difficult nature of working with humans and different psychological constructs (Coccaro & Siever, 2002). These inconsistencies might also be due to different methods being employed, small sample sizes, or differences in the definitions of the construct (Varga et al., 2012).

2.7.3 Serotonergic genes

The Human Genome Project made it possible to study the nucleotide configuration of a variety of genes related to disease, illness and behaviour. Studies have shown a connection between suicidal behaviour and the underlying genetic mechanisms of behavioural traits such as anger and impulsivity. In recent years an increasing number of molecular genetic studies have been conducted to determine candidate genes that might have an influence on suicidal behaviour. These helped with the identification of possible genes that play a role in predisposing an individual to suicidal behaviour. This is leading to a more comprehensive understanding of the tendency towards suicidal behaviour (Özalp, 2009).

As previously mentioned, the serotonergic neurotransmitter system has been implicated in suicidal behaviour. As a result, the focus of genetic association studies has fallen on serotonergic genetic variants, including some serotonin receptor genes such as *HTR1A, HTR1B* and *HTR2;* serotonin transporter genes including *SLC6A4*; serotonin metabolising genes (*MAO-A, MAO-B*); *TPH*; *COMT*, and *TH.* Table 2.1 included these studies performed on serotonergic genes as reviewed by Savitz, Cupido, and Ramesar (2006).

A review study performed by Özalp (2009) concurred with the above mentioned genes as the most influential candidate genes possibly related to suicide. The inclusion of the serotonin receptor genes, *HTR1A*, *HTR1B* and *HTR2A*, as well as the serotonin transporter gene, *SLC6A4*, in this study, were based on evidence from published literature and the premise that serotonergic dysfunction causing aggression, impulsivity or anxiety may predispose towards suicidal behaviour.

Table 2.1 Genetic variants implicated in suicidal behaviour (Savitz et al., 2006).

Gene	OMIM	Locus	Association evidence
Serotonin transporter	182138	17q11.2	7 + and 7 - studies, 2 + meta-analysis
Tryptophan hydroxylase	191060	11p15-14	5 + and 7 - studies, 2 + meta-analysis and 1 - meta-analysis
Monoamine oxidase A	309850	Xp11.23	3 + and 2 - studies
Serotonin receptor 1A (5-HT1A)	109760	5q11.2-q13	1 + and 2 - studies
Serotonin receptor 1B (5-HT1B)	182131	6q13	1 + and 5 - studies
Serotonin receptor 2A (5-HT2A)	182135	13q14.2	3 + and 9 - studies, 1 - meta-analysis
Catechol-O-methyltransferase (COMT)	116790	22q11.21	3 + and 2 - studies
Dopamine receptor 2 (DRD2)	126450	11q23	1 + study and 1 - study
Dopamine receptor 4 (DRD4)	126452	11p15.5	2 - studies

+ and – indicate positive and negative studies

2.7.3.1 HTR1A receptor gene

HTR1A is located on chromosome 5 (5q11.2-q13) and consists of only one exon of 1.27 kilobase pairs (kbp) with several single nucleotide polymorphisms (SNPs) (Angles, Ocaña, Medellín, & Tovilla-Zárate, 2012; Videtic et al., 2009). The gene encodes a G protein-coupled receptor located at pre- and post-synaptic neurons. The 5-HT1A receptor mediates serotonin release and uptake through a negative feedback mechanism (Czesak et al., 2012; Yoon & Kim, 2009). This receptor plays a pivotal role in mood regulation including anxiety, stress and aggression (Bhagwagar, Rabiner, Sargent, Grasby, & Cowen, 2004; Drevets et al., 199AD; Neumeister et al., 2004; Parsey et al., 2002; Pederson et al., 1988; Sargent et al., 2000; Tauscher et al., 2000), and has been implicated as a possible role player in suicidal behaviour (Ohtani, Shindo, & Yoshioka, 2004; Samadi Rad et al., 2012; Serretti et al., 2009; Serretti, Mandelli, et al., 2007; Videtic et al., 2009; Wasserman et al., 2006).

The most studied SNP to date is the functional C1019G variant (rs6295). The presence of the G allele has been reported to lead to a decrease in serotonergic neurotransmission (Lemonde et al., 2003). *HTR1A* rs6295 has been implicated in various disorders including depression, aggression, impulsivity and anxiety (Kishi et al., 2009; Lesch, Zeng, Reif, &

Gutknecht, 2003; Parsey et al., 2002; Strobel et al., 2003) and suicidal behaviour (Hsiung et al., 2003; Pitchot et al., 2005; Serretti et al., 2009; Wasserman et al., 2006).

2.7.3.2 HTR1B receptor gene

HTR1B in humans consists of an exon of 1.179 kbp in length located on chromosome 6q13 (Hamblin, Metcalf, McGuffin, & Karpells, 1992). It functions in the regulation of serotonin neurotransmitters firing as hetero- or autoreceptors at the nerve terminals of pre- and post-synaptic neurons (Maura, Thellung, Andrioli, Ruelle, & Raiteri, 1993). *HTR1B* plays a role in the control of impulsive and aggressive behaviours, brain neuropsychiatric functions, movement, feeding and regulation of body temperature (Barnes & Sharp, 1999; Mochizuki, Yuyama, Tsujita, Komaki, & Sagai, 1992). *HTR1B* has been linked to suicidal behaviour, alcoholism, aggression and cocaine use (Cao, Larocque, & Li, 2013; Huang, Grailhe, Arango, Hen, & Mann, 1999; Lowther, Katona, Crompton, & Horton, 1997).

Six common SNPs, including a coding SNP and a dinucleotide deletion, have been reported (Huang et al., 1999; Ohara et al., 1996; Sanders et al., 2001). Functional polymorphisms in this gene may be related to psychopathology, which include suicide, aggression, major depression, alcoholism or substance abuse (Arango, Khait, & Mann, 1995; Hamblin et al., 1992; Huang et al., 1999). A synonymous SNP, rs6296, included in this study, was identified in the coding region of *HTR1B* (Sidenberg et al., 1993). It has been associated with anti-social behaviour in alcoholics, a history of suicide attempts in patients with personality disorders, major depression and substance abuse (Fehr et al., 2000; Hawi et al., 2002; Huang et al., 2003; Lappalainen et al., 1998; New et al., 2001).

2.7.3.3 HTR2A receptor gene

HTR2A is located on chromosome 13 (13q14.1-q21) with two introns and three exons spanning 63 kbp (Chen, Yang, Grimsby, & Shih, 1992). *HTR2A* is responsible for the encoding of post-synaptic heteroreceptors primarily found in the PFC. This serotonergic receptor plays a role in vasoconstriction and dilation, hormone signalling, the functioning of the smooth muscles in the peripheral nervous system, and inflammatory processes (Cohen, Fuller, & Wiley, 1981; McLennan & Taylor, 1984; Nau, Yu, & Martin, 2013; Van de Kar et al., 2001).

The *HTR2A* gene was identified for this research study as a possible influence on suicidal behaviour after an association between abnormalities in the 5-HT2A receptors and schizophrenia, dysfunctional impulse control, aggressive behaviour, suicidal behaviour and psychological disorders was observed (Abdolmaleky et al., 2004; Arias et al., 2001; Bjork et al., 2002; Correa et al., 2002; Correa, Nicolata, Teixeira, De Marco, & Romano-Silva, 2006; Du, Bakish, Lapierre, Ravindran, & Hrdina, 2000; Khait et al., 2005; Wrzosek et al., 2012). This gene contains 230 SNPs (Norton & Owen, 2005; Yoon & Kim, 2009) which might be responsible for variation in the expression of the 5-HT2A receptors in the brain (Underwood, Mann, Huang, & Arango, 2008). The two principle SNPs studied in relation to suicidal behaviour were A1438G (rs6311) and T102C (rs6313) (González-Castro, Tovilla-Zárate, Juárez-Rojop, García, Velázquez-Sánchez, et al., 2013; Saiz et al., 2008). Several studies have found the above mentioned SNPs to be in linkage disequilibrium (LD) (Arranz et al., 2000; Bray, Buckland, Hall, Owen, & O'Donovan, 2004; Kouzmenko et al., 1999; Martinez-Barrondo et al., 2005; Saiz et al., 2008; Spurlock et al., 1998). The SNPs, rs6311, has been associated with aggression, depression and anxiety disorder (Myers, Airey, Manier, Shelton, & Sanders-Bush, 2007), and rs313 with schizophrenia, depression and alcoholism (Correa et al., 2006; Khait et al., 2005; Wrzosek et al., 2012), respectively. This study focused on rs6311 as there was less existing literature on this specific SNP, and due to the association of this SNP with aggression.

2.7.3.4 SLC6A4 transporter gene

The *SLC6A4* transporter gene is located on chromosome 17q11.2 and spans 39.5 kbp with 14 exons (NCBI, n.d.). *SLC6A4* encodes the serotonin transporter, 5-HTT. The binding site of 5-HTT is located on serotonin nerve terminals and platelets and is responsible for the concentration of serotonin found in the synaptic cleft between two neurons. It transports serotonin from the synaptic cleft back into the pre-synaptic terminal for reuse (Tao-Cheng & Zhou, 1999). It affects human behaviour by regulating emotional aspects (Meyer-Lindenberg, 2009) and the dysregulation of this serotonergic transporter has been implicated in various disorders, including, but not limited to, mood disorders, schizophrenia, substance abuse, eating and neurodegenerative disorders (Joyce et al., 1993; Koenen et al., 2011; M. Stanley, Virgilio, & Gershon, 1982; Watanabe et al., 2011).

A variable number of tandem repeats (VNTR) in this gene, named *HTTLPR*, has been intensively studied. This 44 base pair (bp) deletion in the 5' promoter region gives rise to short (S) and long (L) alleles at 5-HTTLPR (5-hydrocytryptamite transporter-linked polymorphic region) (Lesch et al., 1996). An extra-long (X) variant was also found, but mostly in African-American populations. This allele is 81 bp longer than the L allele (Gelernter, Kranzler, & Cubells, 1997). The S allele was implicated in lower gene expression and reduced transcriptional activity with reduced serotonin uptake (Lesch et al., 1996) and has been implicated in suicidal behaviour and was therefore included in this study (Courtet et al., 2004; Joiner et al., 2005; Mann et al., 2000). Figure 2.5 visually depicts the chosen SNPs in the serotonergic pathway and possible maladaptive behaviour associated with genetic variation. The genes encode for the serotonergic receptors and transporters. Receptor and transporter abnormalities may possibly be associated with maladaptive behaviours.
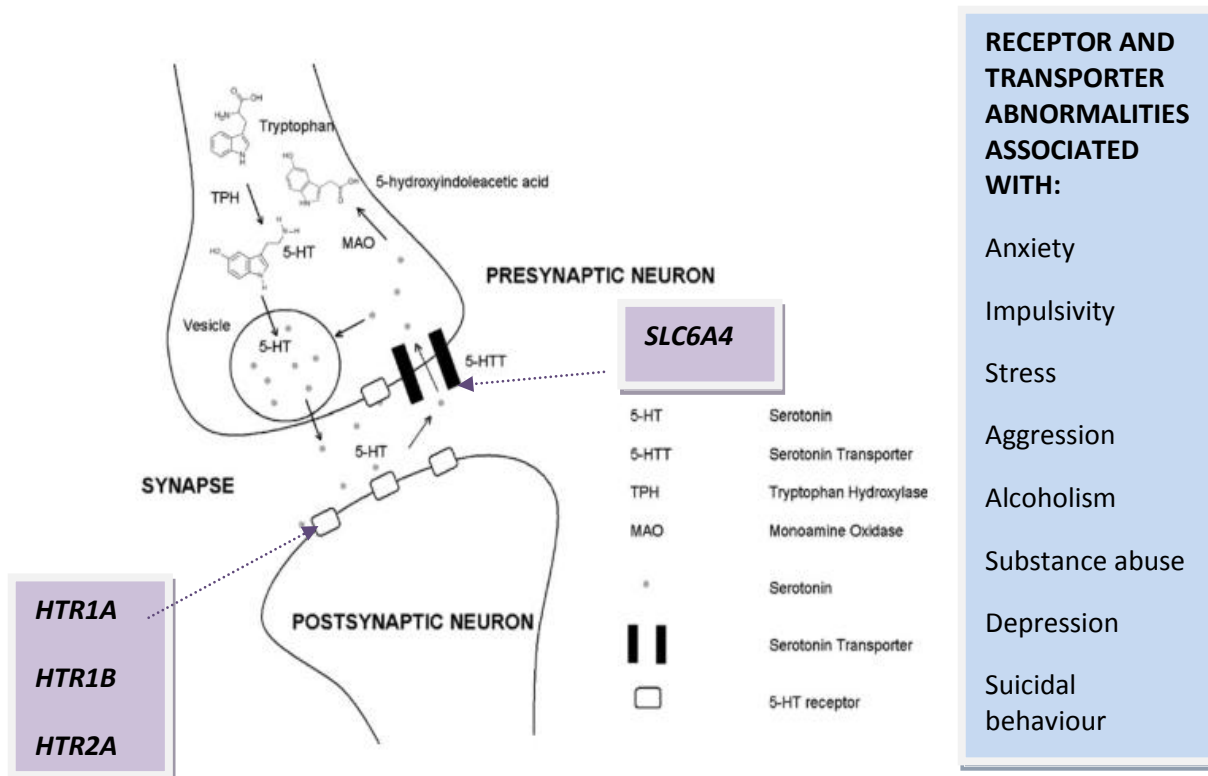
Figure 2.5 Chosen genetic variants in the serotonergic pathway and resultant maladaptive behaviours, adapted from Watanabe et al. (2011).

2.8 Remarks

Suicidal behaviour is overall recognised as a very complex phenomenon with no single cause. It involves intricate interactions between different variables, including psychosocial, biological and genetic variables (Van Heeringen et al., 2000). The clinical model of suicide as developed by Mann et al. (1999) showed impulsive and aggressive behaviours as a diathesis for suicidal behaviour. There was also a call to use impulsive and aggressive behaviours as an endophenotype for suicidal behaviour. An endophenotype approach is based on the assumption that the use of endophenotypes might give rise to less complicated identification of the genetic basis of complex disease phenotypes. Serotonergic dysfunction has been associated with impulsivity and aggression, as well as in suicidal behaviour. The possibility exists that, as impulsivity and aggression have been significantly associated with suicidal behaviour in various studies, the dysfunction in the serotonergic system underpins all of these behaviours. Genetic variations in the serotonergic system have therefore been

identified. The previously mentioned genes show promise as potential candidate genes in suicidal behaviour, even though the literature shows inconsistent findings. As far as the author is aware, none of these have yet been investigated in a South African study group of individuals presenting with suicidal behaviour. Research is still lacking in South Africa and this study can enhance the understanding of molecular genetics underlying this behaviour. The possibility of interventions by either targeting impulsive and aggressive behaviours, or targeting the genes through psychopharmacology, may lead to a possible reduction in suicidal behaviour.

Chapter 3 Materials and Methods

3.1 Research design and methodology

In order to bring better depth and understanding to a complex human behaviour such as suicidal behaviour, the possible interplay between nature (genetics) and nurture (environmental factors) was assessed in this study using different quantitative approaches. Quantitative genetics can be useful in indicating both genetic and environmental factors that might contribute to the variance seen in different populations (Plomin, Owen, & McGuffin, 1994). This approach was used to determine genetic factors as well as environmental factors that might contribute to suicidal behaviour. Questionnaires and DNA samples were analysed using quantitative measures and conclusions were based on statistical analysis. This study is therefore a fusion between psychology and genetics through a case-control study.

3.2 Cohort

The cohort (n = 50) comprised individuals who had previously attempted suicide (n = 25) as well as matched controls (n = 25). Suicide attempters were recruited with the help of professional psychologists and psychiatrists at Bloemcare Private Clinic and Optima Psychiatric Hospital in Bloemfontein, Free State, South Africa; whereby information leaflets (Appendix A) were distributed to patients who met the inclusion criteria, who then contacted either the researcher directly or indicated their participation via the psychologist or psychiatrist. The reason for choosing these facilities included the high intake of patients; the availability of psychologists or psychiatrists on the premises that can facilitate the proper identification of possible research participants; the opportunity to include different ethnic groups as representative of SA; the proximity of these facilities to ensure timely collection of the DNA samples; and the willingness of these to participate in the research study.

Random sampling was not possible due to the difficult nature of the construct under investigation and therefore, convenience sampling was used. This type of non-probability sampling allowed the researcher the access to the highest possible number of participants and available data in a certain time frame. This sampling technique was deemed appropriate for this study. The control group were recruited from the Bloemfontein area and were matched to the research group according to gender, race and age (± 5 years); with no previous suicidal behaviour, excluding unspecific thoughts about suicide. Each participant completed a questionnaire in English and provided a 1 - 2 ml saliva sample.

The suicide attempters (n = 25) comprised three (12%) males and 22 (88%) females with ages ranging between 18 – 51 years with the mean age and standard deviation for males 31.67 ± 16.74 years old and females 31.86 ± 10.07 years old, as calculated using R version 3.2.1 (also see Figure 3.1). The majority of suicide attempters (75%) were between 18 and 40 years old (Figure 3.1). The ethnic distribution consisted of 18 (72%) White, three (12%) Coloured and four (16%) Black individuals. Ethnicity in this regard was based on the terms defined in the South African census (Statistics South Africa, 2011). Individuals in the control group were matched to the suicide individuals in terms of gender, ethnicity and age (± 5 years). Information regarding the education level of all participants was obtained, but not used in the statistical analysis.
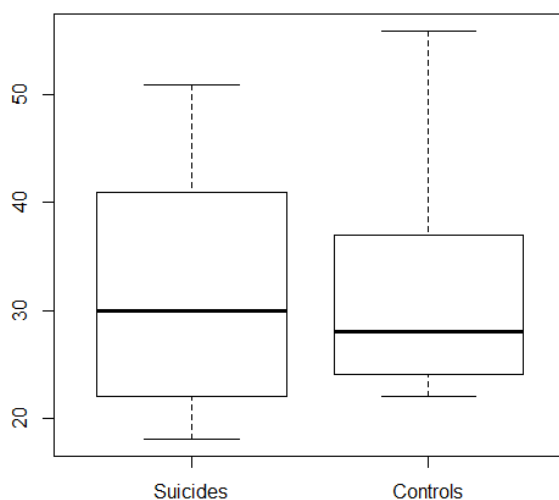
Figure 3.1 A box plot of the observed range and mean ages of the suicide attempters and the control group.

3.3 Ethical considerations

The research study was approved by the Ethics Committee of the Natural and Agricultural Sciences Department of the University of the Free State (number UFS-HSD2014/0024) (Appendix B). Permission was obtained from various registered psychologists and psychiatrists at Bloemcare and Optima to approach and involve eligible participants (Appendix C). To eliminate the possibility of a parasuicide attempt, the psychiatrists and psychologists had to ensure that the patients met the inclusion criteria for a suicide attempt as defined in the Chapter 2, section 2.2.2. The psychologists and psychiatrists, who agreed to partake in the study, were informed by the researcher on how to explain the procedure of DNA collection to the potential participant to eliminate any DNA contamination.

All participants were provided with accurate information with regards to the study in the form of an information sheet (Appendix D) and written informed consent was obtained from each volunteer (Appendix D). Possible participants under the age of 18 were excluded from the study. Each participant was assigned a unique sample identifier to ensure confidentiality, and participants were informed that they could withdraw from the study at any time. All information was handled confidentially.

The study comprised quantitative and molecular analysis with the suicide attempters and control group participating in both. This included the quantitative analysis of traits and molecular analysis of genotypes. Results from both quantitative and molecular analysis were interpreted separately as well as together to derive the final conclusions.

3.4 Quantitative analysis

Even though a significant part of this study is concerned with human behaviour, which is qualitative in nature, the aim was not to determine the underlying motives of the behaviour. Instead quantitative analysis was applied where the behavioural traits in question were expressed in terms of quantities (Dochtermann & Roff, 2010). Quantitative analytical techniques were employed to draw certain inferences from the captured data regarding possible relationships between the variables.

3.4.1 Questionnaires

The participants received a questionnaire pack where all questionnaires were combined into one battery of self-report tests (Appendix E). The questionnaire used in the analysis consisted of four sections as follows:

(a) Section A: Demographic information

(b) Section B: The Barrat Impulsiveness scale Version 11 (BIS-11) with subscales non-planning, motor impulsiveness and attentional impulsiveness (Patton et al., 1995).

(c) Section C: The Reactive Proactive Aggression Questionnaire (RPQ) with subscales proactive and reactive aggression (Raine et al., 2006).

(d) Section D: Buss-Perry Aggression Questionnaire (BPAQ) with subscales physical aggression, verbal aggression, anger and hostility (Buss & Perry, 1992).

Section A comprised demographic information (information on characteristics of participants including age, gender, ethnicity, qualification level and home language) and differed in one respect between the suicide attempters and control group. The control group were asked if they had any previous plans to commit suicide. Participants were excluded from the study if they had any concrete plans or serious suicidal ideation. Participants with some general thoughts regarding suicide were included. The rest of the

self-report battery comprised standardised questionnaire for impulsivity and aggression, as mentioned above (Appendix E).

The measuring instruments were chosen after a thorough review of the available data in the current literature, based on the theoretical constructs as described in Chapter 2, sections 2.5.1.1, 2.5.2.1, and 2.5.3, and for their psychometric properties. It was decided that self-report measures would be a good fit for this research study. Self-report instruments are used to capture data based on individuals' knowledge and experience of themselves and their world (Meyer et al., 2001). These measurements are usually based on existent theory and are therefore, able to assess the predictions made in the literature. The measurement is limited by the individual's ability to make correct judgements of self and the motivation to answer the measurement honestly, known as self-report bias (Aklin, Lejuez, Zvolensky, Kahler, & Gwadz, 2005; Evenden, 1999; Keilp, Sackeim, & Mann, 2005; Meyer et al., 2001). To allow the respondent to be more open and honest it has been found that it is useful to use anonymous self-report tests to investigate socially undesirable or stigmatised behaviour (Meyer et al., 2001). Another disadvantage is that even though these measurements were typically designed to measure stable traits, they might still be subject to mood congruent response (Campbell-Sills, Liverant, & Brown, 2004). However, the chosen measures have demonstrated good internal and test-retest reliability, as well as good construct and predictive validity across various adult and adolescent populations in both non-clinical and clinical samples (Buss & Perry, 1992; Cloninger, Svrakic, & Przybeck, 1993; Fossati, Borroni, Eisenberg, & Maffei, 2010; Patton et al., 1995; Raine et al., 2006; Stanford et al., 2009). Validity refers to the degree to which the questionnaire measures what it intends to measure (De Vaus, 2002). Reliability in turn refers to ability of the test to provide the same results upon repeated use by the same individual (De Vaus, 2002). The self-report measures in the following section were therefore deemed appropriate for this study.

3.4.1.1 Barratt Impulsiveness Scale Version 11 (BIS-11) (Appendix E)

The BIS-11 is the current version available of the questionnaire developed by Patton, Stanford, and Barratt (1995). It is a self-report questionnaire with 30 items answered on a 4-point scale (1 – rarely, 2 – occasionally, 3 – often, 4 – always). Items were scored as 1, 2, 3 or 4, with the 4 indicating higher impulsiveness. Some questions were reverse scored, as they were worded in such a way to indicate non-impulsiveness. The questionnaire also differentiated between three factors or subscales of impulsivity. To obtain the total score, all items were summed. A higher level of impulsiveness was indicated by the higher summed score.

Table 3.1 Factor structure and scoring of the Barratt Impulsiveness Scale (BIS-11)

2nd Order Factors	1st Order Factors	Number of items	Items contributing to each subscale
Attentional	Attention	5	5, 9*, 11, 20*, 28
	Cognitive instability	3	6, 24, 26
Motor	Motor	7	2, 3, 4, 17, 19, 22, 25
	Perseverance	4	16, 21, 23, 30*
Non-planning	Self-control	6	1*, 7*, 8*, 12*, 13*, 14
	Cognitive Complexity	5	10*, 15*, 18, 27, 29

*reverse scored items

The properties of the BIS-11 were determined by Patton et al. (1995) in three different study groups. The questionnaire showed good internal consistency with high Cronbach's alphas (α) across the three groups consisting of 412 college undergraduates (α = 0.82), 248 psychiatric inpatients divided into substance-abuse patients (α = 0.79) and general psychiatric patients (α = 0.83), and 73 male prison inmates (α = 0.80). No significant gender differences were found within the groups. However, a difference was noted between the different groups with the patient groups scoring significantly higher than the undergraduate group ($p < .0001$). Stanford et al. (2009) also demonstrated good internal consistency (α =

0.83) and good test-retest reliability (rho = 0.83) in a review study (n = 1577). However, not all cultures fit the three-factor model proposed by the BIS-11 (Vasconcelos et al., 2012).

3.4.1.2 The Reactive Proactive Aggression Questionnaire (RPQ) (Appendix E)

The RPQ is a 23-item self-report questionnaire originally designed to measure aggression in children and adolescents. The questionnaire consisted of two sub constructs, namely reactive and proactive aggression. Reactive aggression was measured by items 1, 3, 5, 7, 8, 11, 13, 14, 16, 19 and 22. Proactive aggression was measured by items 2, 4, 6, 9, 10, 12, 15, 17, 18, 20, 21 and 23. Statements were scored as 0 (never), 1 (sometimes) or 2 (often). Each subscale was summed to determine proactive or reactive aggression scores. Total aggression was determined by adding the two subscales.

During the development of the questionnaire by Raine et al., (2006), the RPQ showed good internal reliability with both subscales and total scale showing Cronbach's alpha values of higher than 0.83 in a population of 334 adolescent boys. The study also found good reliability and validity measures. Reactive aggression was more prevalent than proactive aggression with proactive scores considerably lower than the scores on reactive aggression. Further support for the two-factor structure was shown by Baker, Raine, Liu, and Jacobson (2008) in a longitudinal twin study (n = 874) and by Fossati, Borroni, Eisenberg, and Maffei (2010) in an Italian population (n = 3666).

3.4.1.3 The Buss-Perry Aggression Questionnaire (BPAQ) (Appendix E)

The BPAQ is a frequently used self-report measure of aggression based on Buss and Durkee Hostility inventory (BDHI) (Buss & Durkee, 1957). The questionnaire consisted of 27 items scored on a Likert scale ranging from 1 – 7 (1 – extremely unlike me; 7 – extremely like me). The BPAQ measured four subscales: physical aggression (items 1 - 9), verbal aggression

(items 10 - 14), anger (items 15 - 21) and hostility (items 22 - 29). Items 7 and 18 were reverse scored. The total aggression score was obtained by adding the total of the subscales together.

The BPAQ showed good consistency and reliability in various studies. In the original validation study (n = 1253), internal consistency for al subscales and total score ranged from 0.72 – 0.89. Test-retest reliability for the total score was a correlation of 0.80 over a nine-week period. The four-factor model showed a good fit through confirmatory analysis across various studies using different cultures (Bernstein & Gesn, 1997; Buss & Perry, 1992; Fossati et al., 2003; Tsorbatzoudis, 2006; Von Collani & Werner, 2005). Abd-el-Fattah (2007) found no gender bias in the BPAQ (n = 510). Appropriate psychometric properties were found in a population of Chilean students (n = 346) with regards to internal consistency ($\alpha = 0.89$), test-retest reliability, convergent and discriminant validity (Valdivia-Peralta et al., 2014). A newer version has been developed by Buss and Warren (2000) and includes a subscale for indirect aggression. However, the validated version of Buss and Perry (1992) was deemed sufficient for this study.

3.4.2 Statistical analysis

The data were analysed using the SAS software package (version 9.22, SAS procedures FREQ, MEANS, CORR, GLM, NPAR1WAY, UNIVARIATE and MIXED) (SAS, 2009). Questionnaire data from 25 matched pairs (50 respondents) were available for analysis. The matched pairs comprised one suicide attempter (SUI) and one control (CON) subject, matched for age, sex and race. Demographic information was collected in section A. Section B referred to BIS-11, section C to RPQ, and section D to BPAQ. Each questionnaire scale (section B, C, and D) and subscale (Non-planning - NP, Motor impulsiveness - MP, Attentional impulsiveness - A, Proactive Aggression - P, Reactive aggression - R, Physical aggression - PA, Verbal aggression - VA, Anger - AA, and Hostility - H) were abbreviated by the researcher for statistical purposes. The primary objective of the analysis was to

determine whether the two groups of subjects, suicide attempters and control, differed with regard to average responses and domain averages of the three questionnaire sections.

For each question in the questionnaire, frequencies of the Likert categories were presented by group. Similarly, descriptive statistics (mean, standard deviation (SD) and median) were presented by group for the scale and subscale averages, as well as for the age of the respondents. Internal consistency of the questionnaire was assessed by calculating the Cronbach alpha coefficient for each subscale of the questionnaire, as well as for total scale of each questionnaire. The scale and subscale averages were analysed using a mixed model fitting group (suicide versus control group) as fixed effect, and matched pair as random effect. The correlation between the section and domain averages was investigated with a simple analysis of covariance (ANCOVA) model. The matched pairs were omitted from the analysis as it did not statistically impact on the results. Partial Pearson correlation coefficients were calculated to determine if the correlation between the scale and subscale averages differed between the suicide attempters and control group. The difference between reactive and proactive aggression (R − P) was calculated for each subject. Thereafter, the two groups of respondents were compared with regard to the reactive and proactive difference using the non-parametric Wilcoxon test. The two groups did not differ significantly, and the median P − R difference was calculated for the pooled data, using the signed rank test. All tests were conducted at a 95% confidence interval.

3.5 Molecular genetic analysis

The field of molecular genetics has created the possibility to identify specific genes that might influence the susceptibility to problematic behaviour (Plomin & Rutter, 1998). Linkage and association are two well-known strategies for determining possible genes influencing behaviour (Plomin et al., 1994). This study was based on association and two types of DNA markers were used to test for possible associations. The first method involved the use of DNA markers with a single nucleotide base pair change, more commonly referred

to as SNPs, and the second utilised DNA markers that involved sequences that repeat, known as VNTRs. Three basic steps of molecular genetics as described in Plomin and Rutter (1998) were followed: (a) obtaining the samples with biological material containing DNA; (b) extraction of the DNA; and (c) genotyping the DNA.

3.5.1 DNA extraction and quantification

Saliva samples of 1 – 2 ml were collected in 10 ml sterile tubes with the corresponding unique identifier as used in the questionnaire packet. Each sample was mixed with an equal volume of lysis buffer containing 50 mM Tris (pH 8.0), 50 mM EDTA, 50 mM sucrose, 100 mM NaCl and 1% SDS. A high salting out method of DNA extraction was followed as described in the standard in-house protocol based on the method described by Quinque, Kittler, Kayser, Stoneking, and Nasidze (2006) with the following slight modifications; (a) only 20 µl proteinase K (Roche, Germany) was added to the saliva buffer mixture, (b) the first centrifugation step was adapted to 7 200 g for 20 minutes (min) and 700 µl isopropanol added to the mixture, (c) an extra wash step was included before the pellets were air dried and subsequently dissolved in 100 µl of TE (10 mM Tris, 0.1 mM EDTA). Quantification of the DNA was performed with the NanoDrop™ Lite Spectrophotometer (Thermo Scientific) to ascertain the concentration of the respective samples. DNA samples were subsequently diluted to a working solution of 50 ng/µl. Several samples weren't diluted, as the DNA concentration of the isolated DNA was lower than 50 ng/µl. Isolated DNA was stored at -20°C.

3.5.2 Gene and SNP selection

Candidate genes and respective variants were identified through the current literature regarding suicidal behaviour. The genes *HTR1A*, *HTR1B*, *HTR2A* and *SLC6A4* were chosen and four variants within these genes were identified (Table 3.2). These included three SNPs;

rs2695 for *HTR1A*, rs2696 for *HTR1B*, and rs6311 for *HTR2A*, and one VNTR for *SLC6A4* known as *HTTLPR*.

Table 3.2 Genes and SNPs selected for this study.

Gene	Chromosomal location	Variant type	Variant ID	Alleles	Study positively associated with the SNP and suicidal behaviour
HTR1A	5q11.2-q13	SNP	rs6295	G/C	Lemonde et al. (2003)
HTR1B	6q13	SNP	rs6296	G/C	New et al. (2001)
HTR2A	13q14.1-q21	SNP	rs6311	G/A	Li, Duan, and Hi (2006)
SLC6A4	17q12	VNTR	*HTTLPR*	X/L/S	Anguelova, Benkelfat, and Turecki (2003)

3.5.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) was performed to amplify the target regions of all the selected genes. This resulted in the exponential accumulation of the target DNA which allowed for visualisation (Mullis & Faloona, 1987; Saiki et al., 1988). *HTR2A*, *HTR1B and HTR2A* were separately amplified in 20 µl total volume reactions that contained forward and reverse primers (0.5 µM for each *HTR1A* primer or 0.75 µM for each *HTR1B* and *HTR2A* primers (Table 3.3)), 3 µl DreamTaq™ Master Mix (2X DreamTaq buffer, 4mM magnesium chloride {MgCl$_2$} and 0.4mM of each dATP, dCTP, dTTP, and dGTP) (Thermo Scientific), and 200 ng of DNA. *SLC6A4* (5-HTTLPR region) was amplified in a total volume of 23 µl using 0.35 µM of each primer (Table 3.2), 10 µl of KAPATaq™ Hotstart (5X U/µl Wild-type Taq with HotStart antibody, 5X KAPA Taq HotStart Buffer {Mg^{2+} free} and MgCl$_2$ {25 mM}) (KAPA Biosystems), 200 ng of DNA, and 1.25 µl DMSO. Typical cycling conditions for each gene were followed on the G-Storm GSi Thermal Cycler (Gene Technologies Ltd) according to optimised in-house protocols (Appendix F).

Primer sequences used were all previously published, but were verified in terms of properties and specificity using bioinformatic tools. Oligo Calculator version 3.1

(http://mcb.berkeley.edu/labs/krantz/tools/oligocalc.html) was used to assess melting temperature and GC content; OligoAnalyzer version 3.1 (http://eu.idtdna.com/calc/analyzer) was used to assess predicted primer dimer formation and Basic Local Alignment Search Tool (BLAST) release 2.2.25 (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to assess the specificity of primers to their target regions and to determine expected amplicon length. This information was used to help optimise the PCR thermal cycling conditions within the laboratory as described in Appendix F.

Table 3.3 Forward and reverse primers and their respective properties.

Gene	Primer Sequence	T_a (°C)	Tm(°C)	Length (bp)	GC (%)
HTR1A	Forward: 5'-CTGAGGGAGTAAGGCTGGAC-3'	59.50	59.17	20	60
(rs2695)	Reverse: 5'-GAAGAAGACCGAGTGTGTCTAC-3'		58.18	22	50
HTR1B	Forward: 5'-GAAACAGACGCCCAACAGGAC-3'	61.00	61.74	21	57
(rs2696)	Reverse: 5'-CCAGAAACCGC GAAAGAAGAT-3'		58.92	21	48
HTR2A	Forward: 5'- AAGCTGCAAGGTAGCAACAGC -3'	60.00	61.75	21	52
(rs6311)	Reverse: 5'- AACCAACTTATTTCCTACCAC -3'		53.41	21	38
SLC6A4	Forward: 5'-GGCGTTGCCGCTCTGAATGC-3'	61.00	65.23	20	60
(HTTLPR)	Reverse: 5'- GAGGGACTGAGCTGGACAACCAC -3'		64.85	23	61

HTR1A (rs6295) (Strobel et al., 2003), *HTR1B* (rs6296) (Lappalainen, 1998), *HTR2A* (rs6311) (Nakamura et al., 1999), and *SLC6A4* (HTTLPR) (Heils et al., 1996).

3.5.2 Genotyping

3.5.3.1 Gel electrophoresis

After PCR amplification, all *HTR1A*, *HTR1B* and *HTR2A* PCR products were visualised on a 1% agarose gel to confirm successful amplification of target fragments. Products underwent electrophoresis at 100 V/cm for 30 min. Since the fragments that were amplified in *SLC6A4* contained VNTRs, the amplicons within a sample contained different sized alleles. They therefore underwent electrophoresis on a 3% agarose gel for 90 min at

100 V/cm. The 3% agarose gel allowed better visualisation, as the DNA fragments separated better according to size differences, which were relatively small between the different alleles. The agarose gels were prepared using Seakem® LE Agarose (Lonza) and Tris, acetic acid, EDTA (TAE) buffer. Bromophenol blue was added to allow visualisation of the DNA while it was loaded into the agarose gel and to allow for the visual tracking of the DNA as it migrated through the gel. GelRed™ intercalated with double stranded DNA to allow for visualisation under ultraviolet (UV) light. A 50 bp molecular weight marker (MWM) (O'RangeRuler, Thermo Scientific) was used to estimate the sizes of the DNA fragments.

3.5.3.2 Restriction enzyme digestion

Restriction enzyme digestion is a technique based on digestion of DNA with restriction endonucleases (RE) to determine the variation in a DNA sequence, and can therefore be used for genotyping samples. REs were used to recognise if a specific base pair was present and consequently digest the DNA fragment into smaller fragments at that site. If the base pair was not present, the DNA was not digested at that site and the fragment remained intact. The fragments were then detected by means of gel electrophoresis as described in the previous section. This allowed for the identification of specific alleles according to different DNA fragment lengths present in a sample. This technique is target specific and uses a unique target DNA and restriction enzyme combination (Thiers, Jaffredo, Tuveri, Chodan, & Bréchot, 1997).

Restriction enzyme digestion was used to detect specific polymorphisms in *HTR1A*, *HTR1B* and *HTR2A* (Table 3.4). Primers for *HTR1A* were specifically modified to contain a A1016T polymorphism, three positions from the C1019G SNP, which allowed the restriction endonuclease, *BseGI*, to digest the DNA sequence if the G allele was present (Strobel et al., 2003). Digested fragments of 146 bp and 17 bp were indicative of the presence of the variant G allele in the *HTR1A* SNP (rs6295). The 163 bp undigested fragment represented the ancestral C allele. Due to digestion, three genotypes could be ascertained: participants

homozygous for the C allele were indicated by only one 163 bp fragment; participants homozygous for the G allele showed two fragments of 146 bp and 17 bp; and heterozygous individuals had three different fragments of 163 bp, 146 bp and 17 bp (Strobel et al., 2003). The undigested amplicon for *HTR1B* was 548 bp. Similarly, by using *HincII* restriction endonuclease, for *HTR1B* SNP (rs6296) digested fragments of 452 bp and 96 bp depicted the ancestral G allele, whereas the C allele was represented by fragments of 310 bp, 142 bp and 96 bp. Four fragments were visible for heterozygous individuals (452 bp, 310 bp, 142 bp and 96 bp). The restriction endonuclease, *MspI,* was used for digestion of *HTR2A*. The *HTR2A* SNP (rs6311) would be digested if the ancestral G allele was present, as indicated by digested fragments of 224 bp and 244 bp. If no digestion took place, a 468 bp fragment was indicative of the presence of the variant A allele.

In the promoter region of the *SLC6A4* gene, there is a VNTR called *HTTLPR*. A 44 bp deletion in the 5' promoter region between the sixth and eighth repeat gives rise to short (S) and long (L) alleles. *HTTLPR* PCR amplification resulted in DNA fragments of either 529 bp (long allele) or 486 bp (short allele). The rare extra long allele (X) was also observed.

Table 3.4 Digestion sites of the restriction endonucleases for each SNP.

Gene	Restriction Endonucleases	Restriction Site
HTR1A	*BseGI*	5'- G G A T G N N ⌐ -3' 3' - C C T A C ⌐ N N -5'
HTR1B	*HincII*	5' - G T Y ▼ R A C - 3' 3' - C A R ▲ Y T G - 5'
HTR2A	*MspI*	5' – C ▼ C G G - 3' 3' - G G C ▲ C - 5'

The blue arrow represents the cutting site of the strand, as recognised by the digestive enzyme.

A volume of 10 µl of each PCR product containing 0.075 µM of *HTR1A* primers or 0.1125 µM *HTR1B* and *HTR2A* primers, was digested with 3 µl of the corresponding

restriction enzyme (Table 3.4) and 10x Tango Buffer mix {33 mM Tris-acetate (pH 7.9), 10 mM Magnesium acetate, 66 mM Potassium acetate and 0.1 mg/ml Bovine Serum Albumin (BSA)} (Thermos Scientific) and nuclease free water in a total volume of 31 µl. *HTR1A* digestion was performed at 55 °C for 16 hours, with the restriction enzyme, *BseGI*. *HTR1B* and *HTR2A* amplified fragments were digested with *HincII* and *MspI* restriction enzymes respectively, and both incubated at 37°C for 16 hours.

All digested products were visualised on 2% agarose gel, (prepared as described in section 3.5.3.1) and run at 100 V/cm for 90 min. A 50 bp MWM (O'RangeRuler, Thermo Scientific) was used to size the fragments. The *SLC6A4* VNTRs did not have to undergo any digestion. Each possible allele for each specific genotype has previously been sequenced to verify the genotype when the in-house genotyping assay was established and optimised. The genotype indicated by Sanger sequencing was in 100% accordance with the genotyping result yielded by the genotyping assays (Odendaal, 2013).   Therefore, due to limited resources, sequencing was not carried out in this study to confirm genotypes, as the validated in-house assays were followed in accordance with quality measures.

3.5.4 Statistical analysis

The data were analysed using the SAS software package (version 9.22, SAS procedures FREQ, MEANS and MIXED). Genotyped DNA analysis data from 25 matched pairs (50 respondents) used for the quantitative analysis in section 3.4.2 were available for analysis. A few respondents were not genotyped for all the genes and those unknown genotypes were subsequently omitted from further analysis. The primary objective of the analysis was to determine whether the two groups of subjects differed with regard to genotype frequencies. Furthermore, the association between genotypes and questionnaire responses (domain averages and total averages of the three questionnaire sections) was calculated.

SNPs were tested for Hardy-Weinberg equilibrium (HWE) by using a chi-square (χ^2) test. For each questionnaire domain and section average, descriptive statistics were calculated by genotype. For each chromosome, the genotype frequencies were compared between the two groups using the likelihood ratio chi-square test. Because of the relatively small numbers of respondents in some categories, an exact P-value was calculated. The section averages as well as the domain averages of each section were analysed using a mixed model fitting genotype as fixed effect, and matched pair as random effect. Based on this mixed model, mean response per genotype was calculated, as well as a point estimate and 95% confidence interval for the between genotype differences in mean response, and the P-value associated with the null-hypothesis of zero difference between genotypes.

The results from the quantitative and molecular analysis will subsequently be presented in the next chapter. A discussion of these will follow in Chapter 5.

Chapter 4 Results

4.1 Quantitative analysis

Standardised questionnaires were completed by all participants, which allowed the psychological constructs of impulsivity and aggression to be quantified in order to draw correlations between these constructs and suicide attempts. The main purpose of this assessment was to have a stable measure whereby behaviour could be quantified. Questionnaire data from 25 matched pairs (50 respondents) was available for analysis (Appendix E).

Analysis was conducted to determine if the suicide attempters differed significantly from the control group with regards to impulsive and aggression traits; if a positive correlation existed between impulsivity or aggression and suicide attempts; and if reactive aggression was higher than proactive aggression in suicide attempters.

4.1.1 Cronbach's alpha

Cronbach's alpha coefficients for each questionnaire was calculated to determine the reliability of the measures used (Table 4.1). A reliability coefficient of 0.65 or higher was deemed satisfactory for measurements used to assess groups (Foxcroft & Roodt, 2005; Huysamen, 1996). Therefore, all three questionnaires used, BIS-11, RPQ and BPAQ, had acceptable internal consistency, ranging from $\alpha = 0.676$ for motor impulsiveness to $\alpha = 0.939$ for the BPAQ. BIS-11, RPQ and BPAQ complete questionnaires also showed high internal consistencies of 0.858, 0.877, and 0.939, respectively. As the results confirm the reliability of the measures used, the findings from the analysis could be applied to all questionnaire measures with confidence.

4.1.2 Difference between impulsivity and aggression for the suicide attempters and control group

In order to determine if the suicide attempters and control groups differed with regards to the average response of the scales and subscales used to assess impulsivity and aggression (described in section 4.1), the mean, standard deviation and median values were calculated. The scale and subscale averages were analysed using a mixed model fitting group (suicide group/control group) as fixed effect, and matched pair as random effect. Based on this mixed model, mean response per group was calculated to assess if suicide attempters obtained a higher or lower score with regards to impulsivity and aggression measures than the control group. A point estimate and 95% confidence interval for the difference in mean response between the two groups were calculated to determine if the two groups differed significantly with regards to impulsivity and aggression and the related subscales (using the SAS software package version 9.22, SAS procedures FREQ, MEANS, CORR, GLM, NPAR1WAY, UNIVARIATE and MIXED) (SAS, 2009).

These results are presented in Table 4.1 and consist of the mean response totals, standard deviation and median by group (suicide and control), for each subscale and total scale of BIS-11, BPAQ and RPQ, as well as the Cronbach's alpha coefficient (discussed in section 4.1.1) and the p-value associated with the difference in the mean response between suicide and control group at a 95% confidence interval.

Table 4.1 Summary of mean scores, standard deviation, median values, and Cronbach's alpha for BIS-11, BPAQ and PRQ, and statistical analysis.

Scale and subscales	Suicide group (n = 25)			Control group (n = 25)			Cronbach's alpha	p-value
	Mean	SD	Median	Mean	SD	Median	α	
Barrat Impulsiveness Scale version 11								
Non-planning	27.64	6.30	28.00	23.04	5.44	23.00	0.761	**0.0030***
Motor impulsiveness	25.16	5.40	24.00	21.48	5.00	20.00	0.676	**0.0155***
Attentional impulsiveness	21.28	3.96	21.00	17.00	5.59	18.00	0.700	**0.0081***
BIS-11	74.08	12.58	72.00	61.52	13.76	61.00	0.858	**0.0015***
Reactive Proactive Aggression Questionnaire								
Proactive	4.44	4.40	2.00	1.36	1.98	1.00	0.740	**0.0035***
Reactive	11.00	5.45	10.00	7.52	4.43	7.00	0.863	**0.0084***
RPQ	15.44	8.65	14.00	8.88	5.93	8.00	0.877	**0.0029***
Buss Perry Aggression Questionnaire								
Physical aggression	38.28	13.16	37.00	49.28	9.26	50.00	0.828	**0.0013***
Verbal aggression	19.72	9.32	19.00	22.32	6.66	25.00	0.866	0.2364
Anger	25.52	10.84	22.00	32.96	9.19	34.00	0.818	**0.0113***
Hostility	24.72	11.25	22.00	38.72	11.09	41.00	0.871	**<0.0001***
BPAQ	108.24	39.91	98.00	143.28	29.37	145.00	0.939	**0.0009***

*Significant p-values. Degrees of freedom = 48. P-value associated with 95% confidence interval to assess the difference in mean values between suicide and control group.

Observation of the mean and median values of the questionnaires for the suicide and control groups indicated significantly higher scores for the suicide attempters compared to the control group. When interpreting the results from the statistical analysis of the BPAQ, reverse scoring was used; a lower score indicated higher aggression. For BIS-11, RPQ and BPAQ the mean values for the suicide attempters were 74.08, 15.44 and 108.24 respectively; and for the control group 61.52, 8.88 and 143.28 respectively. The suicide attempters therefore presented with higher impulsivity, higher reactive-proactive aggression and higher trait aggression. The p-values ranged from 0.0001 for hostility to 0.2364 for verbal aggression. The most significant difference between the mean scores for the suicide and control group was found for hostility in the BPAQ ($p < 0.0001$). This indicated that hostility was significantly higher in the suicide attempters than in the control group. Verbal aggression was the only subscale that did not differ significantly between suicide attempters and control group, even though the mean score for the suicide group (mean = 19.72) was lower than that of the control group (mean = 22.32). Therefore, the

control group obtained a higher score, which indicated lower verbal aggression, than the suicide attempters, which was consistent with the other results, even though it did not reach statistical significance.

4.1.3 Correlations between impulsivity and aggression scales

Partial Pearson correlations (adjusting for group) were calculated between the total scales and subscales of the questionnaires (presented for the suicide attempters and control group) to determine if impulsivity and aggression were correlated with each other, as found in previous studies with regards to these traits (see Chapter 2, section 2.5.3). Correlation was defined as the magnitude of a possible relationship between variables, and does not infer a causal relationship. The correlation coefficient ranged from 1 for a positive association, to -1 for a negative association, with 0 indicating no relationship.

The Partial Pearson correlations between BIS-11, RPQ, and BPAQ scales and subscales representing the suicide attempters and control group (n = 25) are shown in Table 4.2. The majority of correlations were significant at the 95% confidence interval. All significant correlations are marked with an asterisk and presented in boldface. The negative correlations were due to the reverse scoring of the items of the BPAQ.

Table 4.2 Observed partial Pearson correlations between BIS-11 impulsivity and RPQ and BPAQ aggression measures in the suicide and control group (n=25). The results for the control group are presented in blue.

Partial Pearson Correlation Coefficients (r)
p-values significant at 95% confidence interval

	Non-planning	Motor impulsiveness	Attentional impulsiveness	BIS-11	Proactive aggression	Reactive aggression	RPQ	Physical aggression	Verbal aggression	Anger	Hostility	BPAQ
Non-planning	1.0000	0.53071 **0.0063***	0.58619 **0.0021***	0.91357 **<.0001***	0.41025 **0.0417***	0.38104 0.0602	0.4487 **0.0245***	-0.3188 0.1207	-0.22172 0.2868	-0.3147 0.1255	-0.49898 **0.0111***	-0.38301 0.0588
	1.0000	0.71567 **<.0001***	0.6053 **0.0013***	0.90135 **<.0001***	0.47888 **0.0154***	0.27409 0.1849	0.36406 0.0736	-0.2689 0.1938	-0.12112 0.5641	-0.4423 **0.0268***	-0.59975 **0.0015***	-0.47711 **0.0159***
Motor impulsiveness		1.0000	0.19256 0.3564	0.75575 **<.0001***	0.60068 **0.0015***	0.2069 0.3210	0.43587 **0.0294***	-0.295 0.1523	-0.20703 0.3207	-0.35311 0.0834	-0.28458 0.1680	-0.32179 0.1167
		1.0000	0.49631 **0.0116***	0.84796 **<.0001***	0.30215 0.1421	0.22354 0.2828	0.26746 0.1962	-0.32505 0.1129	-0.18755 0.3693	-0.46102 **0.0204***	-0.44454 **0.0260***	-0.45715 **0.0216***
Attentional impulsiveness			1.0000	0.69147 **0.0001***	0.37032 0.0684	0.52111 **0.0076***	0.51663 **0.0082***	-0.55896 **0.0037***	-0.20992 0.3139	-0.3816 0.0598	-0.61474 **0.0011***	-0.51035 **0.0091***
			1.0000	0.82587 **<.0001***	0.29037 0.1591	0.27616 0.1815	0.3028 0.1412	-0.22532 0.2788	-0.13549 0.5184	-0.50772 **0.0096***	-0.58815 **0.0020***	-0.48273 **0.0145***
BIS-11				1.0000	0.58004 **0.0024***	0.44393 **0.0262***	0.57468 **0.0027***	-0.46236 **0.0200***	-0.2661 0.1985	-0.42947 **0.0322***	-0.56587 **0.0032***	-0.49083 **0.0127***
				1.0000	0.4171 **0.0380***	0.30178 0.1426	0.36414 0.0735	-0.31594 0.1239	-0.17108 0.4136	-0.54864 **0.0045***	-0.63759 **0.0006***	-0.55085 **0.0045***
Proactive aggression					1.0000	0.53751 **0.0056***	0.84724 **<.0001***	-0.42398 **0.0347***	-0.30603 0.1368	-0.35011 0.0862	-0.36872 0.0697	-0.41035 **0.0416***
					1.0000	0.66827 **0.0003***	0.8318 **<.0001***	-0.30616 0.1366	-0.46515 **0.0191**	-0.31114 0.1300	-0.10357 0.6222	-0.33847 0.0979
Reactive aggression						1.0000	0.90335 **<.0001***	-0.7816 **<.0001***	-0.47297 **0.0170***	-0.62079 **0.0009***	-0.71177 **<.0001***	-0.73753 **<.0001***
						1.0000	0.9688 **<.0001***	-0.42545 **0.0340***	-0.44143 **0.0272***	-0.61813 **0.0010***	-0.14207 0.4981	-0.48132 **0.0149***
RPQ							1.0000	-0.70802 **<.0001***	-0.45361 **0.0228***	-0.56915 **0.0030***	-0.63592 **0.0006***	-0.67333 **0.0002***
							1.0000	-0.41945 **0.0369***	-0.48435 **0.0141***	-0.56488 **0.0033***	-0.14051 0.5029	-0.4719 **0.0172***
Physical aggression								1.0000	0.64041 **0.0006***	0.67669 **0.0002***	0.76225 **<.0001***	0.87806 **<.0001***
								1.0000	0.68914 **0.0001***	0.67319 **0.0002***	0.45475 **0.0224***	0.85397 **<.0001***
Verbal aggression									1.0000	0.80162 **<.0001***	0.72775 **<.0001***	0.86761 **<.0001***
									1.0000	0.54511 **0.0048***	0.26883 0.1938	0.7161 **<.0001***
Anger										1.0000	0.81152 **<.0001***	0.91079 **<.0001***
										1.0000	0.61606 **0.0010***	0.88142 **<.0001***
Hostility											1.0000	0.9237 **<.0001***
											1.0000	0.77437 **<.0001***
BPAQ												1.0000
												1.0000

BIS-11: Barratt Impulsiveness Scale; RPQ: Reactive Proactive Questionnaire; BPAQ: Buss Perry Aggression Questionnaire; r: partial Pearson correlation coefficient

*Significant p-values at 95% CI associated with the correlation between scales and subscales

Trait measures of the total scales of impulsivity (BIS-11) and aggression (RPQ and BPAQ) yielded correlations of $r = 0.57468$ ($p = 0.0027$) for BIS-11 impulsivity with RPQ aggression; BIS-11 with BPAQ aggression, $r = -0.49083$ ($p < 0.0127$); and RPQ aggression with BPAQ aggression, $r = -0.6333$ ($p < 0.0002$) for the suicide group. All of these correlations were significant. In the control group these correlations were $r = 0.6414$ ($p = 0.0735$), $r = -0.55085$ ($p = 0.0043$), and $r = -0.47190$ ($p = 0.0172$) respectively. The strongest correlations between different measures observed for the suicide group was physical aggression with reactive aggression ($r = -0.78160$; $p < 0.0001$) and total aggression for BPAQ and reactive aggression ($r = -0.73753$, $p < 0.0001$). For the control group they were hostility with total aggression for BPAQ with $r = -0.63759$ ($p = 0.0006$) and reactive aggression and anger with $r = -0.61813$ ($p = 0.0010$).

In order to illustrate the different correlations between impulsivity and aggression for the suicide and control group, ANCOVA plots graphically depicting the relationship between the correlations were produced in Fig. 4.1 (see Table 4.2 for equivalent particle correlation coefficients). For each of these plots, the suicide group had a higher average score obtained for both impulsivity and aggression. All of the pairwise correlations were statistically significant. As can be seen by the distance between the solid blue (suicide attempters) and dotted red (control group) line on the graphs, the instance where suicide group differed the most from control group was with regards to the correlation between impulsivity (Ave_B) and reactive aggression (R) (Figure 4.1b). The negative slopes were indicative of the reverse scoring of the BPAQ. The suicide group had higher average correlations between scales than control group. It can, therefore, be inferred that a positive relationship existed between impulsivity, aggression and suicidal behaviour.

(a)
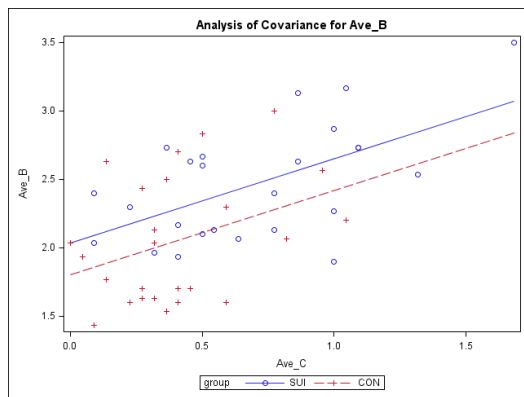
(b)
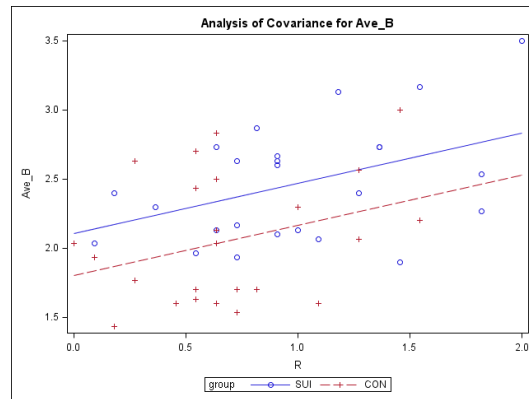
(c)
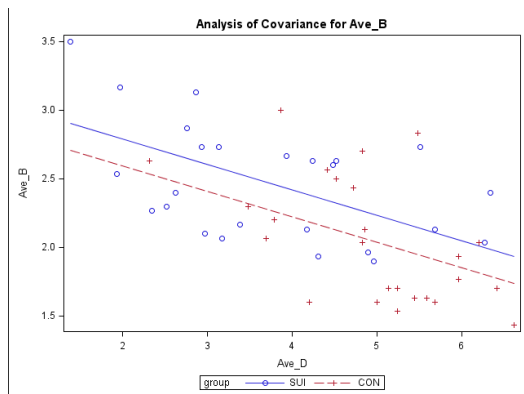
(d)
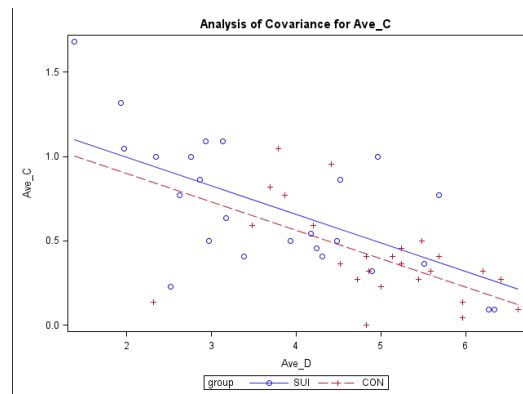
(a) Correlation of impulsivity (Ave_B) with aggression as measured by RPQ (Ave_C); (b) Correlation of impulsivity (Ave_B) with reactive aggression (R); (c) Correlation of impulsivity (Ave_B) with aggression as measured by BPAQ (Ave_D); and (d) correlation of aggression as measured by RPQ (Ave_C) and aggression as measured by BPAQ (Ave_D).

Figure 4.1 ANCOVA plots graphically illustrating the comparison of the relationship between the scales for the suicide and control group.

4.1.4 Reactive aggression in the suicide group

In order to investigate the claim that the type of aggression found in respect to suicidal behaviour is mainly reactive in nature, the difference between reactive and proactive aggression with regards to suicidal behaviour was determined using the non-parametric Wilcoxon test. Since the two groups did not differ significantly regarding the proactive and reactive aggression difference (p = 0.48), a point estimate and distribution-

free 95% confidence interval for the median proactive and reactive aggression difference was calculated for the pooled data and a p-value was calculated using the signed rank test. The median proactive reactive difference was 0.59 at a 95% confidence interval with $p < 0.0001$ (see Appendix G).

4.2 Molecular analysis

For the molecular study, genotyping was performed through restriction enzyme digestion and gel electrophoresis to obtain the genotypes of participants for the serotonergic genes chosen for this study. Statistical analysis was applied to determine if the suicide attempters differed from the control group with regards to genotype and allele frequencies (using the SAS software package version 9.22, SAS procedures FREQ, MEANS, CORR, GLM, NPAR1WAY, UNIVARIATE and MIXED). Furthermore, correlations were obtained between the specific genes under study and the questionnaire scores for the suicidal and control group. This was done in order to determine whether an association existed between certain serotonergic genes and impulsivity, aggression and suicidal behaviour.

4.2.1 Genotyping

4.2.1.1 PCR of target DNA

Following PCR using optimal in-house cycling conditions (as outlined in Appendix F) for DNA isolation, gel electrophoresis was employed for each sample to verify PCR products. A 1% agarose gel was used and GelRed™ allowed for subsequent visualisation under UV light. Samples with no amplification due to degradation were excluded from further processing.

Figure 4.2 is an example of an agarose gel image obtained after a PCR process in order to confirm successful amplification of target fragments, in this particular case, those of

HTR1A. Lane M contains the 50 bp molecular weight marker (MWM) (O'RangeRuler DNA ladder, Fermentas, Thermo Scientific) (Appendix H).
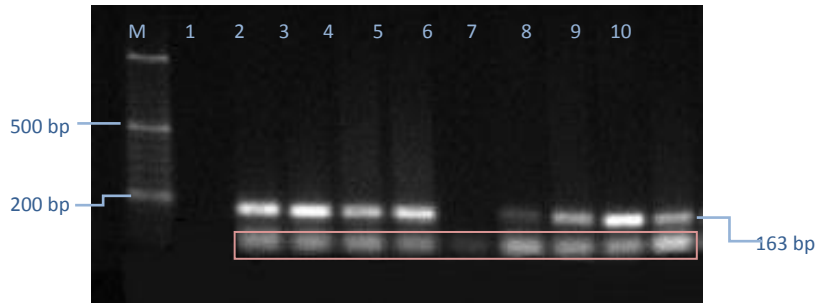
M: 50 bp MWM; 1: Negative water control; 2-10: PCR product of 163 bp for HTR1A; primer dimers can be seen on the bottom as outlined by the box.

Figure 4.2 Representation of the verification of the correct PCR products for *HTR1A* through agarose gel electrophoresis.

Lane 1 contains the negative water control and is clear to indicate no contamination of PCR products. Lanes 2 - 10 contain the PCR products of 163 bp. Only primer dimers are visible in Lane 6, and no visible PCR product. This sample was therefore excluded from downstream analysis. Primer dimers are indicated in the outlined box at the bottom of the agarose gel.

4.2.1.2 Genotyping through the use of restriction enzyme digestion

The PCR products for *HTR1A*, *HTR1B* and *HTR2A* were subsequently digested by *BseGI*, *HincII*, and *MspI*, respectively, as described in Chapter 3, section 3.5.2.2. Digested products were visualised through gel electrophoresis on a 3% agarose gel (run at 100 V/min for 90 min), using GelRed™ to allow for visualisation under UV light. As previously described in Chapter 3, section 3.5.2.2, each respective digestion by the restriction endonucleases would result in different sized DNA fragments, which would allow for the genotyping at the specific locus.

Figure 4.3 is included as an example of samples which have undergone digestion and 3% agarose gel electrophoresis. In this case, the restriction endonuclease, *MspI*, was used for digestion for the *HTR2A* SNP (rs6311). As previously explained, the *HTR2A* SNP (rs6311G>A) would be digested if the ancestral G allele was present, as indicated by two DNA fragments of 224 bp and 244 bp. If no digestion took place, a single 468 bp fragment would indicate of the presence of the A allele. Lane M in figure 4.3 contained the 50 bp O'RangeRuler DNA ladder used as the MWM, while lane 1 represents the negative water control. The lane is clear which indicates no contamination of the DNA products. Lanes 2 and 5 contain two fragments (224 bp and 244 bp) which represent the homozygous GG genotype; lanes 6 and 7 show only one DNA fragment each (468 bp), which represent the homozygous AA genotype; and lanes 3 and 4 have three bands present (224 bp, 244 bp and 468 bp) and, therefore, represent the heterozygous AG genotype. Primer dimers can be faintly detected at the bottom of the agarose gel and are outlined in the box. Each sample was genotyped according to this principle (see Chapter 3, section 3.5.3.2). For *SLC6A4* no digestion took place, as the VNTR size could be visualised though agarose gel electrophoresis with a 3% gel run at 100V/min for 90 min. The same principle was subsequently applied for genotyping *SLC6A4* as described in this section (see Appendix I for a representation of *HTR1A*, *HTR1B*, and *SLC6A4*).
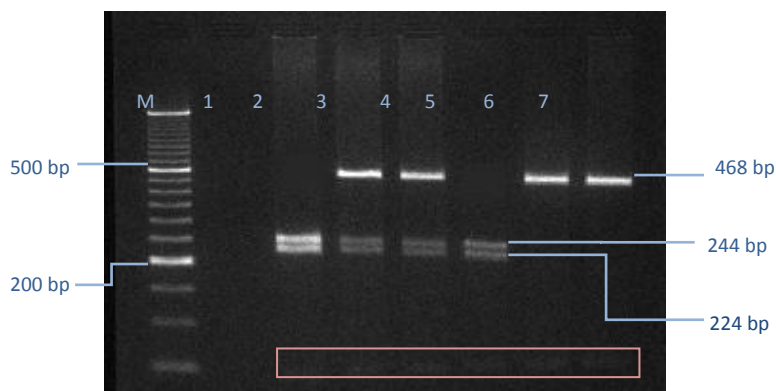
M: 50 bp MWM; 1: Negative water control; 2 - 7: Digested PCR product. Lanes 3 and 4 contain three bands (468, 244, and 224 bp sizes) which indicate that the individuals are heterozygous GA at the locus. Lanes 2 and 5 contain two bands (244 and 224 bp sizes), depicting a homozygous GG genotype at the locus. Lanes 6 and 7 contain one undigested fragment (468 bp), representing a homozygous AA genotype at the locus. Primer dimers faintly visible at the bottom as indicated by the box.

Figure 4.3 *HTR2A* DNA fragments that underwent *MspI* digestion.

4.2.2 Statistical analysis

4.2.2.1 Allele and genotype frequencies

Gel images were analysed and based on the bands present in the gel, the genotypes of participants were deduced (see Appendix J for a breakdown of each participant with their genotypes for each marker analysed in this study). All SNPs were in HWE for the research group as $\chi^2 < 3.841$ (Appendix K). The genotype frequencies for all DNA markers were calculated and are presented in Table 4.3. Alongside the genotype frequencies, the allele frequencies are presented, as calculated for each SNP. In order to determine if the two groups differed with regards to the genotype frequencies, the likelihood ratio chi-square (χ^2) was determined through SAS software package version 9.22 with the respective degrees of freedom (df) and an exact P-value (p) associated with a 95% confidence interval. Allele frequencies were compared between the two groups using a Mantel-Haenszel chi-square test and the exact P-value at a 95% confidence interval. The exact P-value was used to account for the relatively small number of respondents in some categories. The results from the statistical analysis are also presented in Table 4.3. Regarding the *HTR1B* SNP, only one subject had the CC genotype; this subject was therefore excluded from this particular analysis since otherwise the sample size for the CC genotype group of the *HTR1B* SNP would have only been one, but the statistical analysis required at least five observations in the group. This was also the case with XL, XS and XX for *SLC6A4*.

Table 4.3 Allele and genotype frequencies presented for each variant and the statistical analysis.

	n	Genotype frequencies (number (%))						Allele frequencies (number (%))		
		HTR1A (rs6295)								
		CC	CG	GG				C	G	
Suicide attempters	25	4(0.16)	17 (0.68)	4(0.16)				25(0.50)	25(0.50)	
Control	24	6(0.25)	15(0.63)	3(0.13)				27(0.56)	21(0.44)	
Total cohort	49	10(0.20)	32(0.65)	7(0.14)				52(0.53)	46(0.47)	
Statistics		χ^2 = 0.651; df = 2; p = 0.7673						χ^2 = 0.546; df = 1; p = 0.4813		
		HTR1B (rs6296)								
		CC	CG	GG				C	G	
Suicide attempters	22	1(0.05)	7(0.32)	14(0.64)				9(0.20)	35(0.80)	
Control	25	0(0.00)	10(0.40)	15(0.60)				10(0.20)	40(0.80)	
Total cohort	47	1(0.02)	17(0.36)	29(0.62)				19(0.20)	75(0.80)	
Statistics		χ^2 = 1.761; df = 2; p = 0.6505						χ^2 = 0.003; df = 1; p = 1.0000		
		HTR2A (rs6311)								
		AA	AG	GG				A	G	
Suicide attempters	21	9(0.43)	12(0.57)	0(0.00)				30(0.71)	12(0.29)	
Control	25	4(0.16)	14(0.56)	7(0.28)				22(0.44)	28(0.56)	
Total cohort	46	13(0.28)	26(0.57)	7(0.15)				52(0.57)	40(0.43)	
Statistics		χ^2 = 11.483; df = 2; **p = 0.0066***						χ^2 = 8.042; df = 1; **p = 0.0053**		
		SLC6A4 (HTTLPR)								
		LL	LS	SS	XL	XS	XX	L	S	X
Suicide attempters	23	7(0.30)	9(0.39)	4(0.17)	1(0.04)	1(0.04)	1(0.04)	24(0.52)	18(0.39)	4(0.09)
Control	25	11(0.44)	8(0.32)	6(0.24)	0(0.00)	0(0.00)	0(0.00)	30(0.60)	20(0.40)	0(0.00)
Total cohort	48	18(0.38)	17(0.35)	10((0.21)	1(0.02)	1(0.02)	1(0.02)	54(0.56)	38(0.40)	4(0.04)
Statistics		χ^2 = 6.456; df = 5; p = 0.7689						χ^2 = 4.2718; df = 5; p = 0.5855		

Significant p-values at 95% CI associated with the difference in frequencies between the suicide group and control group presented in boldface. Likelihood ratio chi-square value (χ^2); degrees of freedom (df); exact p-value (p). Significance of the differences between the respective frequencies for the suicide and control group at a 95% confidence interval is presented in the statistics highlighted row.

No significant differences were found between the genotype frequencies of the suicide attempters and control group for *HTR1A* (χ^2 = 0.6507; DF = 2; p = 0.7673), *HTR1B* (χ^2 = 1.7614; DF = 2; p = 0.6505), and *SLC6A4* (χ^2 = 6.4556; DF = 6; p = 0.7389). However, the *HTR2A* genotype and allele frequencies differed significantly between the two groups (χ^2 = 11.4833; DF = 2, p = 0.0066) at a 95% confidence interval level. The allele frequencies for *HTR2A* rs6311 are presented in Figure 4.4. The AA genotype was more frequent in the suicide group, whereas the GG genotype was present only in the control group.
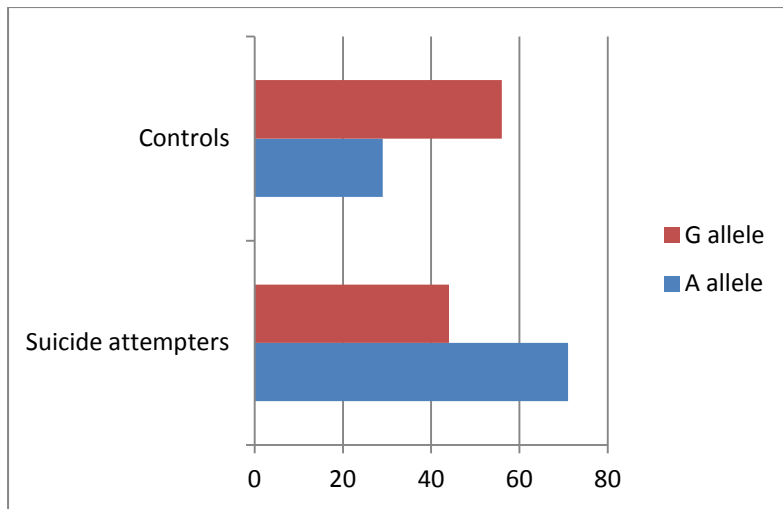
The frequencies showed a significant difference (χ^2 = 11.4833; DF = 2; p = 0.0066) between the suicide attempters and control group.

Figure 4.4 The allelic frequencies as observed in the suicide and control group for *HTR2A* rs6311.

The genotype frequencies for each genotype of the different genes presented in Table 4.3 are visually represented in the following figure (Figure 4.5), together with the genotype frequencies of the respective variants in other populations around the world, based on genotype frequency data from 1000Genomes (2015). The genotype frequencies for the VNTR *HTTLPR* were obtained from various population studies presented by Murphy and Moya (2011). The genotype frequencies for the separate suicide attempters and control group are also included. The genotype frequencies are represented on the y-axis as a percentage and the different population groups are represented on the x-axis.
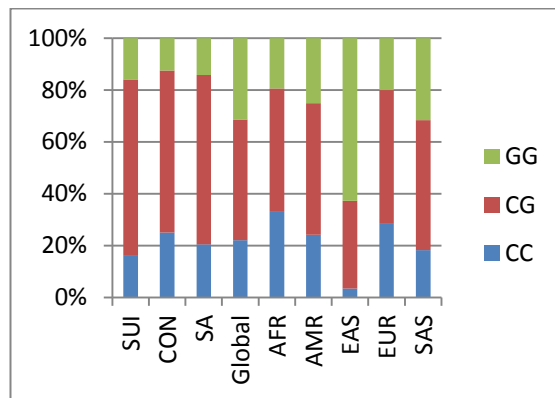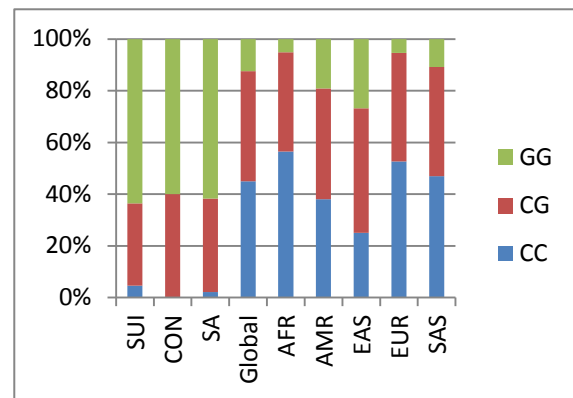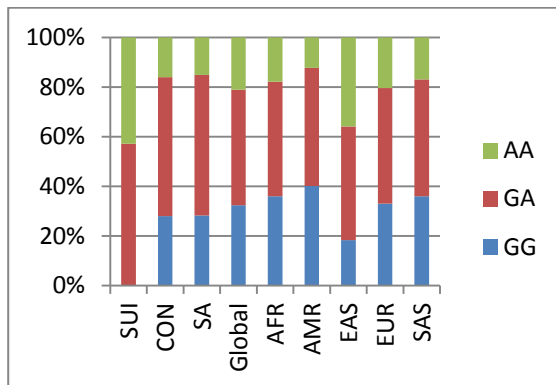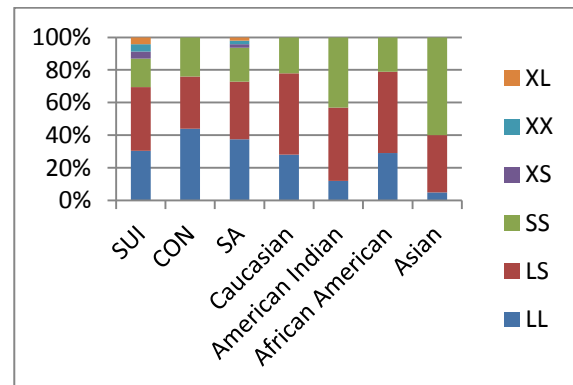
Genotype frequencies for (a) *HTR1A* rs2695; (b) *HTR1B* rs2695; (c) *HTR2A* rs6311; and (d) *SLC6A4 HTTLPR*. SUI – suicide attempters; CON – control group; SA – current South African sample; AFR – Africa; AMR – America; EAS – East Asia; EUR – Europe; SAS – South Asia.

Figure 4.5 Genotypic frequencies of each SNP for the total research group in relation to other population groups globally.

4.2.2.2 Questionnaire responses by genotype

In order to investigate the possible association between impulsivity and aggression and the respective SNPs chosen for this study, the difference in the genotypes for the suicide attempters and control group was investigated by using a one-way ANCOVA model fitting genotype as fixed effect, and for the difference in genotype in the total cohort, a mixed model fitting genotype as fixed effect, and matched pair as random effect, was used. Based on these models, the mean response for each questionnaire was calculated for each genotype, for the total, suicide and control groups. The mean response and standard

deviation for each total scale of the questionnaire (for subscale scores see Appendix L) are shown in Table 4.4, as well the p-value of a 95% confidence interval for the difference in genotypes for the measures of impulsivity and aggression for the suicide and control group. The suicide and control group presented with a few significant results for a difference in the mean response for the questionnaires between the different genotypes.

Mostly no significant differences were found between the genotypes for the suicide and control group and the mean scores for impulsivity and aggression measures with a few exceptions. Comparisons between the CC and GG homozygous genotypes for *HTR1A* showed marginally significant differences in the suicide group for attentional impulsiveness ($p = 0.0243$), trait impulsivity ($p = 0.0240$), hostility ($p = 0.0172$), anger ($p = 0.0451$), and total trait aggression ($p = 0.0472$) (see Appendix L). The suicide group with the AG genotype for *HTR2A* differed from the AA genotype with regards to verbal aggression ($p = 0.0328$). For SLC6A4 in the control group, anger ($p = 0.0481$) and motor impulsivity (0.0498) showed significant differences when comparing the LL and SS genotypes. This was also the case for the total cohort when comparing these two genotypes of *HTTLPR* for motor impulsivity (0.0349), attentional impulsivity (0.0349), and total trait impulsivity (0.0328) (Appendix L). It seemed as if the SS genotype contributed to higher impulsivity, regardless of the presence of suicidal behaviour. Even though overall statistical significant results were not obtained for most of the chosen variable genes, consistent trends in questionnaire mean responses were observed.

Table 4.4 Mean and standard deviation for the BIS-11, RPQ, and BPAQ, stratified for each genotype, with statistical analysis.

			Suicide group				Control group		
		n	BIS-11	RPQ	BPAQ	n	BIS-11	RPQ	BPAQ
					rs6295 (n=49)				
	GG	4	2.82 ±0.50	1.02 ±0.60	2.49 ±0.79	3	2.04 ±0.45	0.30 ±0.07	5.24 ±0.87
Genotypes	GC	17	2.46 ±0.40	0.66 ±0.32	3.86 ±1.25	15	2.04 ±0,45	0.36 ±0.24	4.96 ±1.05
	CC	4	2.15 ±0.10	0.57 ±0.40	4.42 ±1.87	6	2.14 ±0.55	0,58 ±0,36	4.66 ±1.14
	GG vs GC		0.1158	0.0986	0.0707		1.000	0.7231	0.6799
Statistical analysis	GG vs CC		**0.0240**	0.1055	**0.0472**		0.7833	0.1632	0.4428
	CC vs GC		0.1620	0.6762	0.4458		0.6876	0.1148	0.5567
					rs6296 (n=46*)				
Genotypes	GG	14	2.38 ±0.37	0.57 ±0.30	3.92 ±1.32	15	2.04 ±43	0.33 ±0.17	5.09 ±0.76
	GC	7	2.63 ±0.53	0.83 ±0.55	3.35 ±1.77	10	2.06 ±0,53	0.51 ±0.36	4.72 ±1.32
Statistical analysis	GG vs GC		0.2187	0.1745	0.4133		0.9359	0.0964	0.3789
					rs3611 (n=46)				
	GG	-	-	-	-	7	2,25 ±0,46	0,44 ±0,29	4,79 ±1,45
Genotypes	GA	9	2.36 ±0.42	0.75 ±0.34	3.12 ±0.94	14	1.96 ±0.39	0.41 ±0.26	4.88 ±0.72
	AA	12	2.52 ±0.46	0.64 ±0.46	4.32 ±1.64	4	2.02 ±0.69	0.33 ±0,34	5.40 ±1.19
	GG vs GA		-	-	-		0.1938	0.8426	0.8509
Statistical analysis	GG vs AA		-	-	-		0.4322	0.5528	0.3625
	GA vs AA		0.5354	0.5751	0.0662		0.8358	0.6204	0.3922
					HTTLPR (n=45*)				
	LL	7	2.34 ±0.44	0.80 ±0.19	4.70 ±1.37	11	1.89 ±0.36	0.44 ±0.33	5.05 ±1.01
Genotypes	LS	9	2.44 ±0.28	0.55 ±0.45	3.92 ±1.74	8	2.08 ±0,46	0.40 ±0.22	5.26 ±0.84
	SS	4	2.73 ±0.63	1.05 ±0.56	3.09 ±1.44	6	2.30 ±0.56	0.34 ±0.22	4.31 ±1.11
	LL vs LS		0.6579	0.2371	0.7798		0.3639	0.7345	0.6576
Statistical analysis	LL vs SS		0.6579	0.3410	0.5398		0.0848	0.4812	0.1508
	LS vs SS		0.2507	0.0561	0.3854		0.3788	0.7091	0.0880

P-value associated with a 95% confidence interval for the difference in mean scores between each genotype for the suicide and control groups. Significant p-value associated presented in boldface with an asterisk.
**Regarding the HTR1B chromosome the CC genotype and XL, XS and XX for SLC6A4; these genotypes were excluded from statistical analysis due to low number of observations.

4.3 Trends observed between impulsivity and aggression measures and respective genotypes

A trend is defined as the following: to show a tendency, in other words, to show an observable pattern. If one set of results increase, and the other set also shows an increase, a positive trend can be ascertained and vice versa. If one set of results increases, but the

other decreases, a negative trend is observed. If there is no relationship, there is no trend (http://www.oxforddictionaries.com).   This is similar to a correlation, however, as the results showed no statistical significance for the correlations between different genotypes and questionnaire mean scores (see Table 4.4), only patterns or trends were observed. However, these trends should be interpreted with caution as the statistical analysis did not show significant results, with a few exceptions (Appendix L). These trends presented in Table 4.5 were ascertained from the questionnaire mean response as calculated by SAS software package version 9.22, MEAN procedure, for each genotype for the total cohort, suicide attempters and control group.

Table 4.5 Trends observed for the questionnaire mean response stratified by genotype.

Domains	Total Cohort			Suicide Group			Control Group		
HTR1A	CC	CG	GG	CC	CG	GG	CC	CG	GG
BIS-11	2.14	2.27	2.49	2.15	2.46	2.82	2.14	2.04	2.04
RPQ	0.57	0.52	0.71	0.57	0.66	1.02	0.58	0.36	0.30
BPAQ	4.56	4.38	3.67	4.42	3.86	2.49	4.66	4.96	5.24
*HTR1B**	CC	CG	GG	CC	CG	GG	CC	CG	GG
BIS-11	-	2.29	2.21	-	2.63	2.38	-	2.06	2.04
RPQ	-	0.64	0.45	-	0.83	0.57	-	0.51	0.33
BPAQ	-	4.15	4.53	-	3.35	3.92	-	4.72	5.09
HTR2A	AA	AG	GG	AA	AG	GG	AA	AG	GG
BIS-11	2.25	2.22	2.25	2.36	2.52	-	2.02	1.96	2.25
RPQ	0.62	0.52	0.44	0.75	0.64	-	0.33	0.41	0.44
BPAQ	3.82	4.62	4.79	3.12	4.32	-	5.40	4.88	4.79
*SLC6A4**	LL	LS	SS	LL	LS	SS	LL	LS	SS
BIS-11	2.07	2.27	2.47	2.34	2.44	2.73	1.89	2.08	2.30
RPQ	0.58	0.48	0.62	0.80	0.55	1.05	0.44	0.40	0.34
BPAQ	4.53	4.55	3.82	3.70	3.92	3.09	5.05	5.26	4.31

 * No CC, XX, XL or XS genotype included in the analysis due to the small number of observations.

Using the BIS-11, RPQ and BPAQ scales, there were observed differences in the questionnaire scores between the three genotypes in the suicide attempters.   The questionnaire responses indicated that subjects with the homozygous GG genotype showed higher impulsivity (mean = 2.82) and aggression (mean = 1.02 for RPQ and 2.49 for BPAQ) compared to the homozygous CC genotype (BIS-11 mean score = 2.15; RPQ mean = 0.57;

BPAQ mean = 4.42 with reverse scoring), with the CG genotype presenting with scores halfway between the two homozygous genotypes. For *HTR1B*, only one participant had the CC homozygous genotype in the total cohort and the participant was therefore excluded from the analysis, as the sample size would have been only one for the CC genotype. In the suicide group, the mean values for BIS-11, RPQ and BPAQ showed a trend for the genotypes of CG and GG from high to low as follows: BIS-11 mean scores of 2.63 and 2.38, RPQ mean score of 0.83 and 0.57 and BPAQ mean score of 3.35 and 3.92, with no individual presenting with the CC genotype. A similar trend was observed for *HTR2A* for aggression scores. However, no such trend was observed for impulsivity. For *SLC6A4* only subjects with LL, LS or SS genotypes were included in the statistical analysis as the X allele sample size were too small. The SS genotype scored consistently higher in the mean score across all three scales (BIS-11: 2.73; RPQ: 1.05; and BPAQ: 3.09) in the suicide attempters. Even though no statistical significance was obtained, the trends showed some consistency with previous studies regarding these genes, which will be discussed in the next Chapter 5.

In order to answer the research questions presented in Chapter 1, quantitative as well as molecular data were obtained from 25 suicide attempters and a matched control group. The data was subsequently analysed by SAS software (SAS, 2009) and presented in this chapter. These results pertain to impulsivity, aggression, reactive aggression and suicidal behaviour. These results will be discussed in depth in the following chapter, as well as how it relates to variability in the genes identified in the serotonergic system.

Chapter 5 Discussion

Suicidal behaviour is a global health problem and numerous studies have aimed to determine possible contributing factors in order to address this issue. Mann et al. (2009) have suggested the use of an endophenotype approach in order to reduce the complexity of assessing suicidal behaviour in individuals at risk. With the advances in molecular genetics, it is possible to investigate possible target genes associated with an endophenotype for suicidal behaviour, however, this information is severely lacking in South African populations and this study aimed to address this gap of knowledge.

The main aim of this study was to determine if a possible association exists between impulsivity, aggression and suicidal behaviour in a South African population, and if this association can be due to the same underlying genetic predisposition. Even though a number of conflicting results exists, impulsivity and aggression are the most reproduced personality indices associated with suicidal behaviour. These measures also meet the criteria for an endophenotype as described in Chapter 2, section 2.5. The reason for the use of an endophenotype, is the possibility to discover new and innovative ways to target behaviour that increases the risk for suicidal behaviour. In order to do that, in the current study, the association between impulsivity, aggression and suicidal behaviour was determined, as well as the genetic contribution overlapping between these constructs. This would possibly prove impulsive and aggressive behaviours as endophenotypes for suicidal behaviour and interventions, such as cognitive and behavioural therapies, could be implemented to target these impulsive and aggressive behaviours in order to deter the suicidal behaviour. The implication of target genes involved in metabolism can be used in pharmocogenetics in order to create more personalised pharmacological treatment for maladaptive behaviour.

5.1 Cohort

The research sample size of this study (n = 25) was small. Due to the small sample size, the data obtained might not be representative of a larger population group. The initial aim was to recruit 100 suicide attempters to assure adequate power to detect any effects of genetic variation; however, several difficulties were experienced during the course of the study. According to South African studies, the estimated suicide rate is 10.9/100 000 in the Bloemfontein and Free State region, and suicide attempts are estimated to be 20 times more than the suicide rate (Stark et al., 2010). Despite this high estimation, the researcher was unable to recruit the target amount, and this was due to several reasons.

Suicide and mental disorders are often treated as taboo. Due to the sensitive nature of the construct under investigation, prospective participants were probably hesitant to participate. In addition, stigmatisation still occurs in South Africa with regards to suicidal behaviour and might influence respondents not to come forward, even though their information and DNA samples would have been treated confidentially.

Even though the researcher has a background in psychology, the expertise of psychologists and psychiatrists were needed to distinguish between a true suicide attempt and a parasuicide attempt (as defined in Chapter 2, section 2.2.2). Several psychologists and psychiatrists were approached to assist with the inclusion of eligible participants, however, only a few agreed to participate. The heavy workload experienced by psychiatrists and psychologists might have acted as a deterrent. Several did not want to add an extra stressor to their patients. They felt that their patients put a certain amount of trust in them and that introducing them to a researcher might leave them feeling exploited and vulnerable. This raises valid ethical considerations with regards to research of this nature. Several frameworks and policies exist in South Africa which guides the research process surrounding vulnerable individuals and populations. Individuals with mental impairments are recognised as vulnerable individuals within these guidelines and therefore certain additional

considerations need to be taken into account. One of the most pertinent considerations is issues surrounding autonomy and the validity of consent, where 'diminished autonomy' exists. The Mental Health Care Act No 17 of 2002 specifies that decisions with regards to such patients must consider the patients' best interests, and must protect patients from exploitation (De Vries et al., 2011). In this study, these factors were taken into account and respected; psychologists and psychiatrists informed their patients of this study, but only when they saw it was appropriate and in their patients' best interests. Where harm may have been in caused in bringing up this study, it was rather not brought up in that particular session.

The Act also states though, that exclusion of individuals who are disabled or mentally impaired is not permissible. This is in line with the assertion from the National Bioethics Advisory Commission which recognises that although research on vulnerable groups may be inconvenient to carry out, the exclusion of such groups from research is unethical. The value of the research however, must be declared in the protocol and the risk-benefit standard must be upheld. The value of this research came under the scrutiny of the University's research ethics committee, who deemed it appropriate under such ethical frameworks. In this study, the risks and benefits were both relatively low, yet the research was considered valuable to the vulnerable population. The study strived towards best ethical practise, yet this posed a limitation on the number of participants recruited.

Within this framework, the researcher, together with psychologists and psychiatrists, recruited a total of 25 individuals over a one year period. While the total size is relatively small for traditional molecular studies, it is considered an accomplishment within the sensitive nature of this particular research. Due to the scope and time frame of the Master's project, this cohort was deemed sufficient to proceed with the study, even though it was a smaller cohort than anticipated.

This was the first molecular genetic study with regards to suicidal behaviour that has been carried out at the University of the Free State. Studies on suicidal behaviour in the past have focused on psychological and social factors pertaining to suicidal behaviour in adolescents (Campbell, 2012; Du Plessis, 2012; George, 2009; Kruger, 2010). As such, this study came with a unique set of logistical obstacles.

While the number of suicide attempters in the Mangaung Metropole claimed to be high, the identification of individuals who met the inclusion criteria was particularly challenging. Suicide attempters did not come forward readily and the collection phase took a long period of time. In the future, it would be advisable to set up more collaborations with more psychologists and psychiatrists, to help recruit participants for the study. This could include the Departments of Psychology and Psychiatry at the University of the Free State, as well as the Counselling unit on the campus. Hospitals and emergency rooms may also be able to assist in the identification of suicide attempters. Should these settings be utilised in the future, ethical considerations must first be investigated and addressed in full before recruitment of participants takes place.

5.1.1 Gender and suicidal behaviour

International (Barlow & Durand, 2009; Bridge, Goldstein, & Brent, 2006; Callanan & Davis, 2012; Canetto & Sakinofsky, 1998; Kaess et al., 2011; Tsirigotis et al., 2011), as well as South African studies (Joe et al., 2008; Schlebusch, 2001), reported that females were at a higher risk to attempt suicide. Although convenience sampling was used, comparatively more females (88%) than males (12%) recruited for this study had attempted suicide, which was consistent with the reported trends.

International and national studies have found several interpersonal and sociodemographic factors contributed to the higher incidence of suicide attempts amongst

females. One of the most publicised factors was that females tended to choose lower lethality methods, such as poisoning or drugs, as opposed to males who tended to choose hanging or shooting. Hanging and shooting were more violent methods and decreased the chance of survival, as opposed to poisoning or overdosing on drugs, which were less effective (Callanan & Davis, 2012; Narishige, Kawashima, Otaka, Saito, & Okubo, 2014; Tsirigotis et al., 2011). Other factors included the higher incidence of emotional and behavioural problems experienced by females (Fergusson & Horwood, 2002; Kaess et al., 2011; Thompson, Mazza, Herting, Randell, & Eggert, 2005).

South Africa has some unique characteristics that could play a role in gender differences of suicide attempts, especially with the recent changes in the socio-political situation of the country. The transition from apartheid to democracy in 1994 brought about significant changes in the social and economic climate of the country. These changes differed across regions and affected socio-demographic groups differently. Race defined the social position of individuals during the apartheid era, which was characterised by discrimination (Burrows, Vaez, & Laflamme, 2007; Burrows, 2005). With regards to gender, the transition from traditional African cultural identification to a more Western approach, brought changes in gender roles in some South Africans and could influence suicidal behaviour displayed by some South African woman (Schlebusch, Vawda, & Bosch, 2003). In recent years South Africa experienced a sharp increase in levels of interpersonal violence, which could be a contributing factor in female suicidal behaviour (Burrows, 2005; Matthews et al., 2007).

5.1.2 Race and suicidal behaviour

According to this study, the occurrence of suicide attempt amongst the different racial groups was highest in the White group (72%), followed by the Black population (16%) and Coloured (12%) groups. However, since recruitment was done through convenience sampling, race was not investigated as a factor associated with suicide attempts.

Suicide rates have been found to be higher amongst White individuals, globally. Overall, this is still true, but an increase in the suicide rate of Black individuals has been noted more recently (Barlow & Durand, 2009; Bridge et al., 2006; Krug, Dahlberg, Mercy, Zwi, & Lozano, 2002). Previous research in South Africa was carried out on White cohorts, and has seemed to focus less on other racial groups (Schlebusch et al., 2003; Schlebusch, 2005). Regional studies tended to focus on suicide in race-specific groups as race had been a basis for division in South Africa. Presenting data according to racial groups could have created bias. Issues with the quality of the studies regarding the different races, particularly during the apartheid era, have been raised (Mkize, 1992; Wassenaar, Pillay, Descoins, Goltman, & Naidoo, 2000).

A more recent study reported a significant difference in the rate of suicide attempts across the different races (White, Black, Coloured and Indian). This study investigated suicidal behaviour in a national probability population in South Africa (n = 4 351). The study was representative of the total South African population for age, gender and province, as found in the 2001 South African census. For suicide attempts, Coloured individuals (7.1%) reported almost thrice higher levels than Black (2.4%) and White individuals (2.4%) in that representative South African cohort. Coloured individuals also presented with the highest rates of unplanned suicide attempts (Joe et al., 2008).

Reasons for the high incidence of suicidal behaviour among the Coloured population group has been investigated and reasons for this have been reported to include the high level of stress experienced in the recently transformed political and socio-economic conditions in South Africa (Burrows, Vaez, Butchart, & Laflamme, 2003; Burrows et al., 2007). Together with this transformation, Coloured individuals experienced less than expected advancement in post-apartheid South Africa (Joe et al., 2008). A loss of their individuality and their place in the community within South Africa could have led to a higher incidence of suicide and related behaviours (Laubscher, 2003). Alcohol consumption and risky drinking behaviour were also reported in this population group (Peltzer, Davids, & Njuho, 2011), which in turn could influence suicidal behaviour.

5.1.3 Age and suicidal behaviour

In South Africa the median age for an attempted suicide was found to be in the early thirties (Joe et al., 2008). This corresponded with the current study which observed a mean age of 31 years old for the suicide attempters (males: mean age = 31.67 ± 16.74 years old; females: mean age = 31.86 ± 10.07 years old) (see Figure 3.1). Several studies have supported the finding that the risk for suicidal behaviour is highest amongst younger South Africans (Flisher, Liang, Laubscher, & Lambard, 2004; Matsopoulos, Cassim, & Seedat, 2003; Schlebusch & Bosch, 2000). Suicidal ideation and planning tended to occur in the late twenties, with more unplanned suicide attempts in the younger age group (10 – 20 years) (Joe et al., 2008). Internationally suicide is the second leading cause of death among the 15 – 34 age group as reported by the Centre for Disease Control (CDC) (CDC, n.d.).

Several reasons have been put forward to explain the high risk of suicidal behaviour amongst the 18 – 34-year-old age group. This is an age that is characterised by changes in self-identity, intimate relationships, and changing roles in society from adolescence to adulthood (White & Jackson, 2005). Alcohol and drug abuse were common in this age group. Employment and relationship issues were also prominent as this is the time during which most individuals start their careers and families. They might also experience a high incidence of physical and sexual abuse (Bridge et al., 2006; Manion, Akinyemi, Nooraddini, & Haile, 2012; White & Jackson, 2005). All of these were stressors related to an increase in suicidal behaviour.

A multitude of stressors have to be faced by living in a post-apartheid South Africa for young adults of all races. As well as the stress experienced in relation to the change from traditional to more Western-orientated roles (Burrows, 2005), others factors include the violation of human rights, the high rate of HIV in the country, the low level of education, and the high rate of unemployment (Noor Mahomed & Karim, 2000; Schlebusch & Bosch, 2000, 2002; Schlebusch et al., 2003).

5.2 Quantitative analysis

5.2.1 Impulsivity, aggression and suicidal behaviour

This study examined the association of aggression and impulsivity, as quantified by self-report measures, in a South African sample of suicide attempters and a matched control group. Several studies have reported that trait impulsivity and aggression could mediate the propensity to act on suicidal thoughts and could lead to a possible suicide attempt (Brezo et al., 2006; Klonsky & May, 2010; Mathias et al., 2011; Putnins, 2005; Slap, Goodman, & Huang, 2001). These impulsive and aggressive behaviours have been suggested as an endophenotype for suicidal behaviour (Mann et al., 2009; McCloskey, Look, Chen, Pajoumand, & Berman, 2012). The assumption was made that the endophenotype approach might represent less complex clues to the genetic vulnerability underlying a complex disease phenotype, in this case, suicidal behaviour. This would allow the use of quantitative measures to assess the endophenotype, specifically impulsive and aggressive behaviours in this case, that would be a reflection of genetically influenced stable changes in brain function (Courtet, Gottesman, Jollant, & Gould, 2011; Mann et al., 2009). It was predicted that impulsivity and aggression would show a correlation, and that it would be associated with suicidal behaviour. The endophenotype may then be used to provide a more straight forward intervention strategy (Courtet et al., 2011; Gottesman & Gould, 2003; Kendler & Neale, 2010).

The results of the current study indicated elevated levels of both impulsivity and aggression in the suicide attempters compared with the control group. All subscales and scales of the BIS-11, BPAQ, and RPQ found significant differences in the mean scores between the two groups (Table 4.1). This is an indication that the suicide attempters presented with higher trait impulsivity, higher reactive-proactive aggression and trait aggression. This was consistent with the findings of several other studies. Turecki (2005) established an overall effect of impulsivity in suicidal behaviour. They determined the mean scores in a group of suicide attempters as 69.05 (SD 10.53) and for non-attempters 64.97

(SD 9.80) respectively, using the BIS. This was statistically significant at a 95% confidence interval with p < 0.0001. This is comparable with the mean scores of impulsivity of the current study, with 74.08 (SD 12.58) for the suicide attempters and 61.52 (SD 13.76) for the non-suicidal control group (p = 0.0015). In a psychological autopsy study of suicide completers (n = 310), the BIS mean scores for suicide completers with a violent suicide method were calculated as 69.33 (SD 14.49) compared to those who died from a non-violent method of 64.01 (SD 15.22) (p = 0.0600). A life-time history of aggression was also assessed with the Brown-Goodwin Life-time History of Aggression Questionnaire and a significant difference between the two groups (p = 0.004) was found. All of the subjects were previously diagnosed with a mood or personality disorder (Dumais, Lesage, Alda, et al., 2005). The proposed explanation was that individuals with high impulsivity acted on their suicidal thoughts more readily and would, therefore, attempt suicide more easily (Klonsky & May, 2010; Slap et al., 2001).

The most significant difference between the two groups of the current study was found for the hostility subscale as measured by the BPAQ with the total mean score for the suicide group 108.24 (SD 39.91) and for the control group 143.28 (SD 29.37) (p < 0.0001). Due to the reverse scoring of the BPAQ, this indicated significantly higher hostility in the suicide attempters. The only scale that did not reach a statistically significant difference between the two, was that of verbal aggression with a mean score of 19.72 (SD 69.32) for the suicide group and 22.32 (SD 6.66) for the control group (p = 0.2364).

The definition of aggression referred to the observable behaviours of harm towards another or the self. Hostility and anger have been included as subscales of aggression in the BPAQ. According to Berkowitz (1983), anger and hostility referred to the psychological emotions and cognitions experienced internally as part of aggression, however, it does not always lead to outward aggression. Hostile individuals perceive other individuals as distrustful, the world as a threatening place, and they viewed themselves as unable to cope. They frequently experience negative affect, which may also include unexpressed anger (Felsten, 1996). Both anger and impulsiveness have been associated with hostile aggression

(Ramirez & Andreu, 2006). Hostility showed a significant correlation ($r = 0.49$, $p < 0.0001$) with impulsivity as measured by the BIS-Adolescent version, in a group of adolescent suicide attempters ($n = 40$) (Bridge et al., 2015). These feelings of hostility of the suicide attempters in the current study may have resulted in expressing these emotions in the form of a suicide attempt as they did not know how to cope with real-life threatening situations.

A study researched whether the transmission of suicidal behaviour was mediated by impulsivity and aggression in suicidal and non-suicidal probands ($n = 165$) and their siblings, as well as their offspring ($n = 393$) (Brent et al., 2003). The probands were all positively diagnosed with a mood disorder. The results indicated significantly higher impulsivity as measured by the BIS ($p = 0.0007$) and impulsive aggression as measured by the BDHI ($p = 0.03$) in the suicide attempters group with suicidal siblings, who thus have the highest familial loading for suicidal behaviour. The offspring of the sibling pairs with the suicidal behaviour also presented with higher impulsive aggression ($p = 0.005$). However, aggression as measured by the Brown-Goodwin Life-time History of Aggression did not find a significant difference for both groups with or without suicidal behaviour, and their offspring. Similarly, a study conducted on a Chinese college student population ($n = 5245$) also found significant differences between the suicide attempters, suicide ideators and non-suicidal participants when assessing impulsivity and aggression (Wang et al., 2014).

The relationship between impulsivity and aggression were analysed with regards to suicidal behaviour and strong correlations were found in most subscales for the BIS-11, BPAQ and RPQ (Table 4.2), with mostly stronger correlations in the suicide group (Figure 4.1). An important finding was that the higher a person scored on the impulsivity questionnaire, the higher the score was on the aggression measurements. This finding indicated that the score of the impulsivity measures may act as a predictor for the scores on the aggression measures. When comparing the visual representation of the statistical analysis of the total scores for the scales between the two groups (Figure 4.1), suicide attempters showed higher trait impulsivity, aggression and impulsive aggression as an overall effect. In the suicide attempters the highest correlations were shown between

reactive aggression and both physical aggression (r = -0.7816; p < 0.0001) and trait aggression (r = -0.7375; p < 0.0001). It might be that the reactive aggression can manifest as physical aggression against the self as self-harm or suicidal behaviours, which is consistent with the prediction that reactive aggression is the type of aggression that is salient in suicidal behaviour. Numerous studies have tested this relationship and shown a positive correlation between aggression, impulsivity and suicide attempts (Grunebaum et al., 2006; Horesh et al., 1997; Malone et al., 2003; Mann et al., 2008, 1999; McGirr & Turecki, 2008; Oquendo et al., 2000; Zouk, Tousignant, Seguin, Lesage, & Turecki, 2006). A few other studies did not show such a clear association between these constructs.

Giegling et al. (2009) found support for the role of impulsivity in suicidal behaviour, but was not conclusive on the role of anger and aggression in suicide (n = 111). They tested aggression and self-aggression as predictors of a suicide attempt. They also compared aggression and impulsivity in the impulsiveness and the violence of the actual suicide attempt. They concluded that impulsive traits were associated with self-aggression, but not with aggression. However, these findings are not directly comparable to the results of the current study, as the methodologies were different.

Hawton, Kingsbury, Steinhardt, James, and Fagg (1999) found higher trait anger scores, but the impulsivity measures showed no difference between adolescents with repeated or non-repeated self-harm behaviours. Patients diagnosed with BPD who presented with a suicide attempt, differed from BPD patients with no suicide attempt with regards to life-time aggression, but life-time impulsivity showed no difference between the two groups (n = 44) (Oquendo et al., 2000). Critchfield et al. (2004) found impulsive aggression correlated with aggression measures only, in a BPD sample with suicidal behaviour, whereas the relationship between impulsive aggression and impulsivity did not reach statistical significance.

It is interesting to note that a significant effect was found for the use of antidepressant medication in adolescent suicide attempters (n = 40) and a non-suicidal control group (Bridge et al., 2015). Suicide attempters showed higher aggression in the Point Subtraction Aggression Paradigm (PSAP), but only if they were not using antidepressant medication at the time of the study (p = 0.049). Impulsive aggression as measured by the Delay Discounting Task (DDQ) was significantly correlated to a family history of suicide in this study. This study did not examine the effects of psychotropic medication on aggression and impulsivity, although the information was collected. This could lead to some bias in the study and might be an interesting avenue for further studies.

Taken together with the significantly higher scores in impulsivity and aggression, this study found a positive association between impulsivity, aggression and suicidal behaviour. This relationship has been the focus of many international studies, especially in populations with psychiatric illness, including BPD, mood disorders, and psychosis (Bridge et al., 2015; Giegling et al., 2009; Grunebaum et al., 2006; Hawton et al., 1999; Horesh et al., 1997; Malone et al., 2003; Mann et al., 2008, 1999; McGirr & Turecki, 2008; Oquendo et al., 2000; Zouk et al., 2006). Most of these concurred with the findings of the current study, which tested these parameters in a South African population for the first time.

5.2.2 Impulsive aggression and suicidal behaviour: clarification of terms

Even though all of the previously mentioned studies found some positive association with impulsivity, aggression or impulsive aggression with suicidal behaviour, there were some difficulties in making direct comparisons with the current study. Some clarification of the terms was discussed in Chapter 2, section 2.5.3. One of the problems of finding conclusive evidence for the role of aggression and impulsivity in suicidal behaviour, is the interchangeable way studies refer to impulsivity and aggression and impulsive aggression.

Coccaro, Kavvoussi, Berman, and Lish (1998) pointed out that this lack of conceptual differentiation of the terms used to describe the relevant key behaviours, lead to confusion when comparisons are made between different variables. This problem was experienced during the subsequent discussion of the results and in finding supporting evidence to strengthen the argument. Aggression, impulsivity and impulsive aggression were defined in Chapter 2, section 2.5. A short overview will be provided here to elucidate the discussion point.

Trait impulsivity was defined as acting without thinking or forethought, and with no consideration of the possible consequences (Cardinal, 2006; Evenden, 1999; Moeller et al., 2001). Aggression was defined as the intention of harm (Baron & Richardson, 1994) with two classifications of aggression that have become widely accepted, namely proactive (premeditated) and reactive (impulsive) aggression (Coccaro et al., 1998; Moeller et al., 2001; New et al., 2002). Reactive or impulsive aggression has been described as follows (see Chapter 2, section 2.5.3): a single trait-like dimension (Coccaro et al., 1989; Siever & Davis, 1991); a subconstruct of impulsive behaviours (Seroczynski et al., 1999); a subconstruct of aggressive behaviours (Barratt et al., 1994; Barratt & Slaughter, 1998); or the combination of two separate traits (Depue & Lezenwerger, 2001). The use of the term impulsive aggression in the current literature necessitated the inclusion of the RPQ in the current study to evaluate the role of reactive aggression or impulsive aggression as a sub-construct of aggression.

Several studies have found that impulsive aggression was very salient in suicidal behaviour (Brodsky et al., 2001; Mann et al., 1999; McGirr & Turecki, 2007; Melhem et al., 2007; Placidi et al., 2001; Turecki, 2005). This led to the question if reactive aggression would show a higher correlation with total trait impulsivity in suicidal attempters than in the general population and if this difference would be significant. As can be seen by the ANCOVA plots in Figure 4.2, the largest difference in correlations between the suicidal and control group, was with regards to trait impulsivity (Ave_B) and reactive aggression (R). As

previously discussed, reactive aggression also showed the highest correlations with physical and total trait aggression.

The other question that arose was if reactive aggression would differ from proactive aggression with regards to suicidal behaviour. The statistical analysis found the median proactive reactive difference was 0.59 at a 95% confidence interval with $p < 0.0001$ (see appendix G). Therefore, reactive aggression was significantly higher than proactive aggression and a positive relationship between reactive aggression and suicidal behaviour can be inferred from that. The results indicated that although reactive and proactive aggression were related, as shown by the correlations between these constructs, these subtypes were distinguishable, as also found by Hecht and Latzman (2015). Therefore, there is a strong possibility that reactive aggression, as a sub-construct of aggression, might be used as the term impulsive aggression as a single trait-like dimension when referring to suicidal behaviour. However, further studies will be required to investigate this claim. As for now, research studies should not use the term impulsive aggression loosely. The terms should be defined properly and investigated with the appropriate measuring instrument so confusion between terms is minimised and studies can show homogeneity with regards to methodology and results.

In conclusion, the quantitative analysis for the current study found a strong positive association between impulsivity, aggression, reactive aggression and suicidal behaviour in a South African population. Scores on impulsivity measures could act as predictors for aggression scores. There is also a strong possibility to use the term impulsive aggression as a single trait-like construct when referring to suicidal behaviour.

5.3 Molecular analysis

The association between impulsivity, aggression and suicidal behaviour may be mediated by deficient serotonergic activity in the PFC. As reviewed in Chapter 2, section 2.7, the serotonergic system has been implicated in the diathesis underlying suicidal behaviour. Strong evidence from the literature also pointed to impaired serotonergic functioning underlying impulsive and aggressive behaviours (Dumais, Lesage, Lalovic, et al., 2005; Kamali et al., 2001; Mann et al., 1996; Zanarini et al., 2004). Following this assumption, certain genetic variants within the serotonergic system were identified through the current literature that may have a possible relationship with an endophenotype for suicidal behaviour. The question arose whether *HTR1A* (rs6295), *HTR1B* (rs6296), *HTR2A* (rs6311), and *SLC6A4* (*HTTLPR*) could be associated with suicidal behaviour. The main aims of the molecular part of the study were, therefore, to compare the allelic and genotypic variation in the suicide and control group, and to compare these genotypes to the quantitative phenotypes of impulsivity and aggression.

The cohort (n = 50) gave informed consent for the molecular study (Appendix D). Suicide attempters were informed of the study by the psychiatrist or psychologist, and if they gave informed consent to participate; they had the option of either, completing the questionnaires and providing a saliva sample during their consultation, or they could contact the researcher to provide these on a different day. Although the sensitive nature of DNA collection was explained to the psychologists and psychiatrists, they were nevertheless busy, and at times did not store the saliva samples under the correct conditions, nor did they always inform the researcher immediately that samples were available for collection. As a result, saliva may have degraded in these sub-optimal conditions. Upon review, the samples which were difficult to amplify corresponded to the samples which were stored in these sub-optimal conditions.

Where the sample could be amplified for several of the genes studied, but not for all, it was likely that the DNA was degraded, and only smaller target regions were still intact. Amplification was repeated on DNA samples where possible, but once the sample was depleted, no further troubleshooting could take place. Ideally, primers should be redesigned to flank the target region more tightly, such that amplification could still take place using a degraded template. Due to ethical reasons pertaining to the construct under investigation, no contact information was collected from participants; therefore, saliva could not be recollected from the specific individuals. These individuals were not excluded from the study; however, the missing genotypes were not included in the statistical analysis.

There are new ways to collect DNA samples which can possibly be explored in future studies. One such example is the GeneFix™ Saliva DNA collection kit from Oragen. This device uses a DNA stabilisation buffer. It can maintain the DNA yield for more than 60 months at room temperature if the initial DNA yield exceeds 180 ng/µl. This would minimise the problem of losing possible samples due to the difficulties experienced in this study. This may also allow for the inclusion of a wider geographical area as the transport for collection of the samples will only have to be scheduled for once a certain number of participants is reached. Even though difficulties were experienced, it still acted as valuable experience for future research into this construct.

5.3.1 Genotype and allele frequencies

In order to determine if suicidal behaviour was associated with any of the selected SNPs, the genotypic and allelic frequencies were analysed for the suicide attempters and control group. Each possible allelic variant and genotype was observed in the total cohort (Table 4.3). *HTR2A* rs6311 showed a significant difference in genotype frequencies between the suicide attempters and the control group. However, no significant differences were observed for the frequencies of *HTR1A* rs6295, *HTR1B* rs6296 and *SLC6A4 HTTLPR*. The total South African cohort was compared to different population groups and comparisons were

also drawn between the suicide and control groups with regards to genotype frequencies (Figure 4.5). Genotypic and allelic frequencies corresponded for the most part with those provided by the NCBI website (NCBI, n.d.), with the exception of *HTR1B*.

5.3.1.1 HTR1A receptor gene

The allele frequencies of *HTR1A* for the total cohort of the present study were 0.53 for the C and 0.47 for the G allele, which is comparable to the global allele frequencies reported by the NCBI from 1000Genomes (2015): 0.45 for C and 0.55 for G. Heterozygosity was most common for this SNP, with a CG frequency of 0.47 and homozygosity at 0.22 for CC and 0.31 for GG, globally (NCBI, n.d.). Other studies done on populations also found similar results in Japanese (Kishi et al., 2009), German and Italian (Serretti, Mandelli, et al., 2007), and Hungarian populations (Benko et al., 2010).

The genotype (χ^2 = 0.651; df = 2; p = 0.7673) and allele (χ^2 = 0,546; df = 2; p = 0.4813) frequencies showed no significant difference between the suicide attempters and control group (Table 4.3). These results mirror the results found in a German and Italian cohort (Serretti, Mandelli, et al., 2007). Suicidal behaviour was investigated in both populations (n = 854) between suicide attempters, suicide completers and healthy subjects. The study did not find any association between rs6295 and suicidal behaviour in either population. *HTR1A* rs6295 did not show the possibility to differentiate between any of their groups: controls versus suicide attempters (p = 0.91); controls versus suicide completers (p = 0.95); or completers versus attempters (p = 0.89). They concluded that *HTR1A* had no association with suicidal behaviour, although they found evidence of a marginal association between aggression and impulsivity with regards to rs6295 and suicidal behaviour, which will be discussed in a later section.

In a meta-analysis between patients with suicidal behaviour (n = 957) and controls (n = 957), the pooled OR was calculated at 1.09 with p(Z) = 0.80, which indicated non-significant association of the G allele of rs2695 with suicidal behaviour (Angles et al., 2012). An updated meta-analysis was performed, including a case-control study in a Mexican population with suicide attempters (n = 152) and healthy controls (n = 264), and once again no association was found (González-Castro, Tovilla-Zárate, Juárez-Rojop, Garcia, Genis, et al., 2013). A few other studies also reported no associations for this SNP (Benko et al., 2010; Videtic et al., 2009; Wrzosek et al., 2012; Yoon & Kim, 2009). In contrast to this, there have been some positive reports with regards to the G allele of rs2695 and suicidal behaviour (Lemonde et al., 2003; Samadi Rad et al., 2012).

5.3.1.2 HTR1B receptor gene

For *HTR1B* (rs6296), this study found the allele frequencies for C and G for the total cohort as 0.20 and 0.80, respectively. This shows a deviation from the global population allele frequencies reported by the NCBI, with C at 0.66 and G at 0.43 (Figure 4.5). The deviation from the current study may be due to the small size of the specific population or due to the geographic location, however, several other studies in a variety of populations found similar allelic frequencies as the current study. The current findings were similar to those observed in another South African population in the same geographic location, as determined in a Master's study at the University of the Free State (n = 33) (Odendaal, 2013). In an American Caucasian population (n = 359) the C allele frequency was 0.23 and G was 0.78, with genotypic frequencies of 0.57 for GG, 0.38 for GC, and 0.04 for CC (Conner et al., 2010). A Canadian population (n = 668) had frequencies of 0.24 for C, 0.76 for G, GG at 0.57, GC at 0.37, and CC at 0.06 (Zouk et al., 2007). A further Finnish (Hakulinen et al., 2013), American (Conner et al., 2010), and Han Chinese population (Gao, Zhu, Wei, Li, & Lai, 2011) found frequencies similar to this. According to these studies and the current study, homozygosity for the G allele was most common.

In the comparison between the suicide attempters and control group, no significant differences were observed for the genotype (χ^2 = 1.761; p = 0.6505) and allele (χ^2 = 0.003; p = 1.000) frequencies. Therefore, it was concluded that there was no significant association between *HTR1B* rs6296 and suicidal behaviour in the current South African population. Huang et al. (2003) performed a study in a mixed population group in the USA (n = 490), and also found no significant difference between the allelic (χ^2 = 0.03; p = 0.866) and genotypic (χ^2 = 0.10; p = 0.951) frequencies for the patients with a suicide attempt and the healthy controls. They concluded that there was no association with rs6296 and suicide attempts, however, there was some relationship with this SNP in the patients with major depression and a history of suicide attempts (χ^2 = 5.44; p = 0.065). No association was found between this SNP and completed suicide in a Japanese population (Nishiguchi et al., 2001) or in a French-Canadian population (Zouk et al., 2007). In contrast to this, an association was found between rs6296 and suicidal behaviour, but in a cohort of psychiatric patients with or without a suicide attempt (n = 159). They found the G allele contributed to the risk of a suicide attempt (p = 0.028).

5.3.1.3 HTR2A receptor gene

The allele frequencies for the *HTR2A* rs6311 were 0.57 for A and 0.43 for G in the total cohort. This slightly deviated from the frequencies reported by die NCBI for the global population (NCBI). They reported the G allele to be more common with a frequency of 0.56 and A at 0.44. Most populations showed an almost equal distribution of the A and G alleles. The genotypes frequencies followed the same trend as the populations as reported by the NCBI (see figure 4.5). This is also true for a Spanish (Saiz et al., 2008), Turkish (Boke et al., 2007), Swedish (Kling et al., 2008) and an Indian population (Guhathakurta et al., 2009).

The allele and genotype frequencies showed significant differences at a 95% confidence interval level, between the suicide attempters and control group. The genotype frequencies for AA, AG and GG were 0.43, 0.57, and 0.0 for the suicide group and 0.16, 0.56,

and 0.28 for the control group, respectively (χ^2 = 11.48; df = 2; p = 0.0066). The suicide group did not present with any homozygosity for the G allele. The allele frequencies between the two groups showed a significant difference of p = 0.0053 at a 95% confidence interval level (χ^2 = 8.042; df = 1) (see Figure 4.4). The healthy control group's allelic frequency was consistent with those of several other populations and the NCBI, as mentioned in the previous paragraph.

Due to the lack of the G homozygosity in the suicide group and the deviation in genotype comparisons observed in other suicidal groups, it was difficult to draw a conclusion from the results obtained for *HTR2A* rs6311 for the current study. However, it seems that the A allele of rs6311 conferred a risk for suicidal behaviour. The absence of the A allele resulting in the GG genotype was only observed in the control group. The presence of the GG homozygous genotype could, therefore, be protective against suicidal behaviour. The possibility that the over-representation of the A allele in the suicide group might be due to some other common factor, cannot be ruled out and further analysis is needed in further studies to confirm this result. The findings of this study indicated a positive association between *HTR2A* rs6311 and suicidal behaviour.

The rs6311 SNP in the *HTR2A* gene has not been as intensively investigated compared to the rs6313 SNP. However, some positive associations were found for the rs6311 polymorphism and suicidal behaviour, which are comparable to the current study. A significant association was reported with the A allele of *HTR2A* rs6311 and suicidal behaviour (p = 0.001) in a meta-analysis of 73 studies (Li et al., 2006). In a Spanish population of suicide attempters with different psychiatric disorders (n = 193) and healthy controls (n = 420), the genotype frequencies deviated slightly from the current study. The allele frequencies in the suicide attempters were 0.49 for the A allele and 0.51 for the G allele and the control group had frequencies of 0.45 (A) and 0.55 (G). Initially they found no significant difference between the groups, but after comparing the frequencies between impulsive and non-impulsive suicide attempters and the control group, they found a

significant higher frequency of the A allele in the non-impulsive suicide attempter (χ^2 = 11.92; corrected p = 0.021). Despite this, the study concluded that they found no association with suicidal behaviour according to the pooled data (Saiz et al., 2008).

5.3.1.4 SLC6A4 transporter gene

For the VNTR in the upstream region of the *SLC6A4* gene, *HTTLPR*, the total cohort presented almost equally for the LL, LS, and SS genotypes, with the SS genotype frequency being slightly lower. Only some of the suicide attempters presented with the rare extra-long (X) allele. Results from different populations found heterozygosity to be favoured, including two Caucasian (Beevers, Wells, Ellis, & McGeary, 2010; Heils et al., 1996), African (Kinnear et al., 2000), Columbian (Ospina-Duque et al., 2000) German (Bondy, Erfurth, De Jonge, Kru, & Meyer, 2000), and a Korean population (Janssen, Zwinderman, Olivier, & Waldinger, 2014). Most populations did not show a particular preference for either long or short allele. The X allele observed in the current population was not present in the above mentioned studies, but it has been reported in populations of African Americans (Gelernter et al., 1997; Vijayendran et al., 2012).

No statistical difference was found between the allelic and genotypic frequencies for the suicide and control group. However, three suicide attempters (12%) in the current study presented with the X allele. Due to the small number of individuals with this variant, it had to be excluded from statistical analysis, which required at least five observations. The X allele is not very common (Gelernter et al., 1997; Vijayendran et al., 2012) and research on this allele is scarce. Research on the X allele with regards to suicidal behaviour could not be found. From the results of this study, as the X allele was only observed in the suicide attempters, the X allele may confer a risk for suicidal behaviour and it should be investigated in further studies. A larger cohort will create less bias in these results.

Once again, there was a number of conflicting results with regards to the association between *HTTLPR* and suicidal behaviour. Joiner, Johnson, and Soderstrom (2002) found that the chance of having the *SS* genotype increased when the subject had a family history of suicide. The S allele was found to be more common in suicide victims, but the findings were not statistically significant (Mann et al., 2000). A longitudinal study comprising of 103 suicide attempters indicated that the S allele conferred an increased risk for a subsequent suicide attempt, and an association between the SS genotype and suicide attempts was found ($p = 0.01$) (Courtet et al., 2004).

In a recent review study of 119 case-control studies, the data of 31 case-control studies, with 6 324 cases and 10 285 controls, pertaining to *SLC6A4* and suicidal behaviour was analysed (Clayden, Zaruk, Meyre, Thabane, & Samaan, 2012). An association between the S allele and suicidal behaviour with a pooled OR of 1.06, $p = 0.4$ at a 95% confidence interval, was found. The suicide attempts were analysed separately and a statistical significant association with pooled OR of 1.13 and $p = 0.001$ was found with the S allele. The pooled data for completed suicide did not reach statistical significance.

The rs25531 SNP occurring in the long allele rendering it phenotypically equivalent to the short allele has not been investigated, as the main aim was just to determine the different influence of the L or S allele, in order to draw a comparison with most studies regarding *HTTLPR* and suicidal behaviour. It has, however, been noted that the transition from an A to a G causes the under expressed long allele (Hu et al., 2005; Wendland, Martin, Kruse, Lesch, & Murphy, 2006). This can be an avenue for future studies.

As can be deduced from the above discussion, discrepancies existed for all of the studied SNPs regarding suicidal behaviour in the literature. These discrepancies can be contextualised in the following way: firstly, inclusion criteria of the diagnoses in the populations of patients differ between different studies. The current study included only

suicide attempters with or without a comorbid psychological disorder. Secondly, the genetic heterogeneity in the different populations might have an effect on the results. Different South African races were included. Thirdly, the sample sizes and geographic locations differed between different studies. Suicide attempters (n = 25) in the Free State region were included in the current analysis.

The following section will describe the influence of the selected SNPs with regards to impulsivity and aggression between the suicide and control group. The above mentioned factors should be kept in mind to contextualise the results inferred in the next section.

5.3.2 The association between impulsivity and aggression in suicidal behaviour with serotonergic genetic variants

Due to the number of studies that found a relationship between suicidal behaviour and impulsive and/or aggressive behaviour, this possible association was addressed in section 5.2.1. It was concluded that, in the current study, a clear association existed between these personality traits and suicidal behaviour. However, when testing the association of selected genes thought to predispose to suicidal behaviour, the only significant association was between *HTR2A* rs6311 and suicide attempts. The A allele seemed to confer a risk for suicidal behaviour. *HTR1A* rs6295, *HTR1B* rs6296 and *SLC6A4 HTTLPR* did not show any significant associations. The presence of the X allele for *HTTLPR* was found only in the suicide attempter group and should be researched further in a larger cohort. As discussed in the previous section, not finding a clear association is common in most genetic association studies. In order to overcome the difficulties in finding the underlying molecular genetic mechanism for suicidal behaviour, it was suggested to use an endophenotype such as impulsive and aggressive behaviours. If a relationship could be found for the genes underlying these behaviours in the suicide group, it presented a more direct pathway to identify predisposing genetic factors for suicidal behaviour. As a result, there were some interesting findings regarding the association of the genotype variations

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with impulsivity and aggression in the suicide group. The subsequent section will explore this assumption in more detail.

HTR1A rs6295 is a functional polymorphism in the promoter region which regulates the expression of the gene. This SNP may decrease the transcriptional activity of the gene and has been implicated in suicidal behaviour (Lemonde et al., 2003). A significant association was found between impulsivity and this genetic variant. Participants (n = 725) with homozygous GG genotypes scored significantly higher on the impulsivity measures when analysing the differences between the genotypes (Eysenck Impulsiveness, Ventureness and Empathy Scale – Impulsiveness subscale: $p = 0.008$; BIS-11: $p = 0.014$) (Benko et al., 2010). However, no association between *HTR1A* and aggression-related phenotypes was found, except for the rs6295 G allele and anger in suicidal behaviour in females (Serretti et al., 2009; Serretti, Mandelli, et al., 2007).

In this study, statistical analysis revealed a few significant associations between *HTR1A* rs6295 and impulsivity and aggression as determined by self-report measures (Appendix L). Significant differences were found between the CC and GG genotypes in the suicide group for attentional impulsiveness ($p = 0.0245$), total impulsiveness ($p = 0.0240$), and aggression measures, including hostility ($p = 0.0172$), anger ($p = 0.045$), and total aggression measured by the BPAQ ($p = 0.0472$). No significant differences were found between the genotypes for the control group. When analysing the trends between the genotypes and questionnaire scores presented in Table 4.5, the presence of the G allele led to an increase in the mean scores of impulsivity and aggression in the suicide group. This led to the conclusion that the G allele might play a predisposing role for impulsive and aggressive behaviours in subjects also presenting with suicidal behaviour. However, this was not significant, so further analysis is needed in South African population groups.

HTR1B is an autoreceptor located on pre- and postsynaptic neurons (Maura et al., 1993) with rs2696 in the coding region of this gene (Sidenberg et al., 1993). An association

between the G allele of *HTR1B* rs2696 and a higher rate of suicide was found in Caucasians (χ^2 = 10.7; p < 0.001), however, no association was found with self-report measures of impulsive aggression as measured by BDHI in that study (n = 145) (New et al., 2001). Self-report anger and hostility were examined with regards to *HTR1B* polymorphisms (n = 361). A trend for rs6295 for the hostility ratings in the male group, but not the female group, was found. Evidence was found for the role of *HTR1B* SNPs in impulsive aggressive behaviours in suicide completers, however, this association was not found specifically for rs6296 (Zouk et al., 2007).

HTR1B rs6296 showed no significant differences between the genotypes for either group in this study. For rs6296 the homozygous GG genotype showed a lower score for impulsivity and aggression than the heterozygous CG genotype in both the suicide and control groups, but the absence of the CC genotype complicated drawing a conclusion on the trend found for this SNP.

HTR2A encodes the 5-HT2A receptor and SNP rs6311 has been found to have an effect on the promoter activity of the gene. This might affect the transcription of the gene. The inadequate functioning of the promoter has been implicated in a number of psychiatric disorders (Abdolmaleky et al., 2011; Myers et al., 2007).

Studies investigating the genetic influence of *HTR2A* rs6311 on impulsivity and aggression found results mainly in favour of the predisposing role of the G allele in impulsive and aggressive traits. With regards to these personality traits and suicidal behaviour, the results were mixed. One of the first association studies that found a positive association with rs3611 and personality traits was done by Preuss, Koller, Bahlman, Soyka, and Bondy (2000). They found an association with impulsivity as measured by the BIS in alcoholics. Several subsequent studies have partially confirmed these findings. Giegling, Hartmann, Moller, and Rujesco (2006) investigated the association of this SNP with anger- and aggression-related personality traits (n = 566). The GG homozygous individuals presented

with more anger-related traits and accordingly, the G allele was associated with aggression and specifically with lower aggression inhibition. The G allele also conferred a higher risk for suicidal behaviour. A link between impulsivity and *HTR2A* SNPs (n = 460) was hypothesised. The rs6311 SNP showed a trend for association of the G allele with cooperativeness as measured by the Temperament character inventory (TCI) (p = 0.07) (Serretti, Calati, et al., 2007). Nomura et al. (2006) found conflicting results, with the A allele involved in higher impulsivity as measured by a behavioural task in 71 healthy volunteers. The A allele was also implicated in a group of nonimpulsive suicide attempters as compared to impulsive attempters and a control group (Saiz et al., 2008).

In the current study, for *HTR2A* rs6311*,* no suicide attempters presented with the GG genotype, therefore the only comparison that could be drawn was that of AA and AG and its influence on impulsive and aggressive behaviours. The homozygous A genotype was more prevalent in the group presenting with suicidal behaviour when comparing frequencies between the two groups (Table 4.3). With the interpretation of the trends for the genotypes with regards to impulsivity and aggression in the suicide group, the group homozygous for the A allele scored higher that the heterozygous AG genotype subjects for aggression, but not for impulsivity (Table 4.5). No conclusion can be drawn based on these results, as the scores did not show a clear difference and the GG genotype was absent. However, in the control group, when just comparing impulsivity and aggression with no regards to suicidal behaviour, the G allele showed a similar direction of scores with the absence of the G allele scoring the lowest, to the presence of one G allele, to two G alleles with the highest scores for impulsivity as well as aggression. This seems contradictory, as the A allele was found mostly in suicide attempters, with the G allele acting as a possible protective factor. With regard to the trends observed in the traits, the presence of the G allele seemed to increase aggression and impulsivity scores.

SLC6A4 is involved in the reuptake of serotonin into the presynaptic neuron and contains the *HTTLPR* VNTR (Lesch et al., 1996). The low-expressing S allele of this VNTR was associated with Neuroticism, as measured by the NEO Personality inventory – Revised,

which is a trait related to anxiety, hostility and depression (n = 505). It was also related to decrease Agreeableness, which range from cooperativeness to aggressiveness (Lesch & Merschdorf, 2000). These findings were replicated (n = 399) and pointed towards a strong link of the S allele with hostile and aggressive behaviours (Greenberg et al., 2000). A longitudinal study comprising 103 suicide attempters indicated that the *S* allele conferred an increased risk for a subsequent suicide attempt, and an association between the SS genotype and suicide attempts was found. The SS genotype also corresponded with a marked increase in impulsivity scores of the BIS-11 (Courtet et al., 2004). Paaver et al*.* (2007) also reported higher mean scores as measured by the BIS-11 in subjects carrying the S allele. Several other studies have supported the association of the S allele and mood disturbances including impulsivity, aggression, hostility and anger (Courtet et al., 2001; Gerra et al., 2005; Lesch & Merschdorf, 2000; Silva et al., 2010). The S allele has shown to lead to decreased protein expression of the serotonin transporter in the brain and platelets (Lesch et al., 1996).

The current results for *SLC6A4 HTTLPR* indicated significant differences between the homozygous SS and LL subjects with regards to anger (p = 0.0481) and motor impulsiveness (p = 0.0498), with a few other scales almost reaching significance (Appendix L). The SS genotype seems to play a role in both aggression and impulsivity, regardless of the presence of suicidal behaviour or not as seen by the similar directions of the scores for the questionnaires presented in Table 4.5.

Impulsive and aggressive behaviours functions on a continuum with dispositions ranging from normal to pathological. Therefore, it was difficult to obtain consistent findings with regards to these behaviours. The analysis of the genetic contribution towards these behaviours presented with conceptual and methodological difficulties (Lesch & Merschdorf, 2000). The evidence from this and other studies converged on the idea that impulsive and aggressive factors influence suicidal behaviour and that this might be associated with reduced serotonergic activity. This in turn was possibly underlain by functional genetic variants. Even though these variants might not play a primary causal role in the

presentation of suicidal behaviour as influenced by impulsive and aggressive behaviours, their involvement in mood regulation and adaptive responses to the environment make them prime targets for personalised pharmocogenetic interventions. This may reduce the effect of impulsivity and aggression as an endophenotype and in turn target the associated suicidal behaviour. This study provided evidence for the use of impulsive and aggressive behaviours as an endophenotype for suicidal behaviour.

Chapter 6 Conclusion

This study was conducted on 25 suicide attempters and matched controls in the Free State Province, South Africa. The main aim was to determine if impulsivity and aggression were associated with suicidal behaviour, and if these behaviours were due to genetic variables in the serotonergic system in a South African cohort. Impulsivity and aggression were chosen, as these personality traits were identified in the current literature as some of the strongest associations with suicidal behaviour, independent of comorbid psychiatric disorders. It was also suggested as a possible endophenotype by several researchers (Courtet et al., 2011; Mann et al., 2009; Turecki, 2005). It might contribute towards a better understanding of the predisposition towards suicidal behaviour. This, in turn, could facilitate early identification of individuals at risk and improve prevention of the detrimental outcomes of suicidal behaviour.

Impulsive and aggressive behaviours have been consistently implicated in suicidal behaviour in various populations. This study was the first to confirm these findings in a South African population. Significant correlations were observed between impulsivity and aggression, and these were associated with suicidal behaviour. The strongest associations included hostility and reactive aggression.

Suicidal behaviour is characterised by behavioural dysregulation, with impulsive and aggressive behaviours being particularly prominent. Impulsivity is often comorbid with aggression and together they create the tendency to act in an impulsive, aggressive and destructive way on emotions such as anger or on suicidal ideation. These individuals show high hostility and act without considering the long-term consequences of their behaviour. This happens mostly when they experience a stressor in their lives (Mann et al., 2009; Turecki, 2005). They tend to struggle to cope with stressors, as they are particularly sensitive to emotional and environmental stimuli. Negative life events early in life may trigger maladaptive behaviour, which provide a basis for the abnormal expression of

impulsivity and aggressive behaviours which could turn into suicidal behaviour (Turecki, 2005).

Since impulsivity and aggression is heritable, it forms one of the pathways to suicidal behaviour (Brent & Mann, 2005; Mann et al., 1999). Parental impulsive aggression may lead to family instability and abuse. These act to increase the risk for psychopathology and even suicidal behaviour in children. If the child then has the genetic predisposition towards suicidal behaviour, the child may have a greater chance to act on suicidal thoughts (Brent & Mann, 2005).

In order to study the chosen constructs, it was important to correctly define these behaviours. Difficulties in defining such complex behaviours as impulsivity, aggression and suicidal behaviour were experienced by several researchers in previous studies. In the current study difficulty was experienced in defining impulsive aggression. In order to address the discrepancies found in the literature with regards to defining this trait, the RPQ was included as a measurement. Impulsive reaction was defined as reactive aggression which forms a subconstruct of aggression. A significant association was found between reactive aggression and total trait aggression in the suicide attempters. Therefore, this study concurred with several studies that the type of aggression experienced with regards to suicidal behaviour was reactive or impulsive in nature (Critchfield et al., 2004; Mann et al., 2009). This strengthens the argument to use impulsive aggression as a single trait-like dimension with regards to suicidal behaviour.

This study supported the use of impulsivity and aggression as an endophenotype for suicidal behaviour, due to the association found with suicidal behaviour. The use of the endophenotype may provide a quantitative way to test individuals at risk for suicidal behaviour and may have a wider impact (Courtet et al., 2011). These personality traits as an endophenotype may prove better predictors of suicidal behaviour than specific personality disorders (Blumenthal, 1990). Some individuals escape diagnosis, as these traits may not

present with enough psychosocial dysfunction in the individuals. They may not receive the necessary interventions, even though they are at risk for suicidal behaviour. The use of these endophenotypes could also lead to a more direct pathway to determine the underlying biological mechanism of suicidal behaviour. The serotonergic system has been identified as a possibility of such a mechanism.

All of the genes studied here have been implicated in the dysregulation of the serotonergic system and either suicidal or impulsive and aggressive behaviours or a combination of those in the existing literature. However, the findings for the specific variants, *HTR1A* rs6295, *HTR1B* rs2696, *HTR2A* rs6311, and *SLC6A4 HTTLPR*, have been inconsistent in the literature. It was important to study the possible association of these SNPs in a South African population. No significant associations were observed between the genotype frequencies of the suicide attempters and control group for *HTR1A*, *HTR1B*, and *SCL6A4*. The suicide attempters and control group showed a significant difference for the *HTR2A* genotypes, but as the cohort was small, this association could have occurred by chance, and should therefore be interpreted with caution. The effects of genetic variation on disorders are indirect and multi-factorial and it is very difficult to determine predisposing genes. Genes code for proteins consisting of amino acid sequences, and the downstream effects may act on several risk factors rather than on the observable phenotype of the disease itself (Plomin & Rutter, 1998). The phenotype is also influenced by gene and environment interaction.

Some interesting trends were observed and it may be possible that some alleles confer a risk for impulsivity or aggression. *HTR1A* rs6295 presented with significant differences between the GG and CC genotypes in the suicide attempters for some impulsivity and aggression subscales. The GG genotype seemed to confer the highest risk for the presence of these behaviours in the suicide attempters. The G allele of *HTR2A* rs6311 might play a role in the increase in impulsive and aggressive traits. The trends also suggested that individuals who presented with the S allele for *SLC6A4* had higher impulsivity and aggression. Several studies supported this finding (Courtet et al., 2001; Gerra et al.,

2005; Lesch & Merschdorf, 2000; Silva et al., 2010). It is essential to identify the possible risk factors for suicidal behaviour as the genetic effects can act through a susceptibility to these risk factors. From the results of this study that investigated the association of impulsivity and aggression on suicidal behaviour, it is clear that these traits are risk factors of suicidal behaviour. The possibility of the G allele of *HTR1A* rs2695 and the S allele of *SLC6A4 HTTLPR*, conferring a risk to suicidal behaviour, was also confirmed in this study. Discrepancies existed for the findings for *HTR2A* rs6311. An interesting observation of only suicide attempters presenting with the X allele of *SLC6A4 HTTLPR*, should be explored in future studies in a larger cohort. The genetic contribution of impulsivity, aggression and impulsive aggression should be studied in more depth.

These findings should not be overestimated. Neither the genes, nor the personality traits are causal factors of suicidal behaviour. Psychological and biological traits are complex and the environment also plays a role in the manifestation of the behaviour. The influence of all possible contributing factors on suicidal behaviour falls outside the scope of this study. The underlying genetic mechanisms provide a reaction spectrum for the behaviour to take place. Prior suicide attempts, negative life events, and coping mechanisms are just some of the factors that will influence the expression of suicidal behaviour within that spectrum.

Only suicide attempters were included in this study to account for a more homogenous phenotype group. It has been suggested that suicide attempters and completers should be considered as two distinct phenotypes (Clayden et al., 2012). All suicide attempts were corroborated by registered psychologists and psychiatrists to diminish the chance of the inclusion of parasuicides. However, there are also certain limitations. Even though only suicide attempters were included in the study, they were not assessed for any comorbid disorders. A large number of studies analysed suicidal behaviour in psychiatric patients with a comorbid disorder and there is a lack of studies with no comorbid disorders such as bipolar disorder, schizophrenia or depression (Clayden et al., 2012).

A possible bias in the study is that possible future suicidal behaviour in the control group cannot be ruled out. If the total cohort was larger, the likelihood of this would be smaller. To control for the possibility of a future suicide attempt in the control group, a question with regards to serious suicidal ideation was included in the test battery of this group. If the participant indicated strong thoughts towards attempting suicide, the participant was excluded from the study. Even though controls were excluded from the study if they had previous serious suicide ideation, the study could not control for future suicidal ideation. For reasons discussed, the cohort was small with only 25 participants and possible future replication studies should use a larger cohort.

The study group was matched with regards to gender, age and race. Due to the small cohort, some genotypes could not be analysed and this could have led to false positives and skewed the results of the effects of certain genotypes on behaviour. All the genes for both groups however, were in HWE. The small study might not have sufficient power to detect associations. Bonferroni correction for multiple testing was not applied, as it gives very conservative estimates and might have resulted in other statistical errors in such a small sample size. The best solution to determine true associations is an expansion study with a larger cohort.

Future directions should include the *MAO-A* gene as a possible influence on suicidal behaviour. It has already been associated with aggression in several studies and is the metabolising step in the serotonergic pathway (Bondy, Buettner, & Zill, 2006; Courtet, Jollant, Castelnau, Buresi, & Malafosse, 2005; Eisenberger, Way, Taylor, Welch, & Lieberman, 2007). The dopaminergic system in conjunction with the serotonergic system should also be considered. Serotonin is a neuromodulater, and as such, acts on several other neurotransmitter systems. Evidence has been found that serotonin and dopamine interact at the neurophysiological level (Daw, Kakade, & Dayan, 2002; Kapur & Remington, 1996). Serotonergic hypofunction has been implicated in the predisposition to impulsive, aggressive and suicidal behaviours as already discussed in Chapter 2. Dopaminergic hyperfunction may contribute in an additive way to this serotonergic deficit (Seo et al.,

2008). This interaction should be investigated in a South African population, as there are no studies pertaining to this interaction with regards to suicidal behaviour in South Africa.

Despite the limitations of the study, the findings support other studies with regards to impulsivity and aggression as an endophenotype for suicidal behaviour. There is a paucity of molecular genetic research with regards to suicidal behaviour in South Africa. Even though there are so many complex factors contributing towards suicidal behaviour, this study has taken the steps to identify possible genetic variants that may underlie an endophenotype for suicidal behaviour in a South African population. To the best of the author's knowledge, this study is the first to demonstrate the relationship between impulsivity, aggression and suicidal behaviour in South Africans. In addition, it shows a possible contribution of serotonergic gene variants underlying these behaviours in a South African sample of suicide attempters. Considering the limitations of this study, these results add a valuable component to the body of literature on suicidal behaviour and contribute towards behavioural genetic research in South Africa. This will ultimately lead to a better understanding of suicidal behaviour and possible interventions for South Africans who are at risk of attempting or completing suicide.

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Appendix A

M.Sc Project by S.Louw

EVER TRIED TO COMMIT
SUICIDE?

Your **DNA** might be responsible.

Take part in a research study done at the University of the Free State, Department of Genetics.

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
NATURAL AND AGRICULTURAL SCIENCES
NATUUR- EN LANDBOUWETENSKAPPE

Tel: 084 529 0975
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About the Research

The main aims of the research are:

- to determine if there are certain genes that might be responsible for suicidal behaviour, and
- to determine if impulsive-aggressive behaviours can influence suicidal behavior.

Research may contribute to a better understanding of suicidal behaviour and lead to better prevention strategies.

What is expected of you?

- Must have had a previous suicide attempt; be at least 18 years old; male or female; can be on medication.
- If you meet these criteria, contact the researcher.
- You will be asked to complete a 20-minute questionnaire and provide a saliva sample if possible.
- Everything is handled confidentially.

Departement Genetika (116) / Department of Genetics (116)
Fakulteit Natuur- en Landbouwetenskappe
Faculty of Natural and Agricultural Sciences

"Satisfaction of one's curiosity is one of the greatest sources of happiness in life."

- Linus Pauling

About the Researcher

- Completing a M.Sc degree in Behavioural Genetics under the guidance of Prof. Spies
- Qualifications: B.Sc Behavioural Genetics (UFS), B.Sc Honours Psychology (UFS), PGCE (UFS)
- Interested in science and human behaviour
- Loves reading, movies and socializing with friends

CONTACT DETAILS:
TEL: 084 529 0975
E-MAIL: susanlouw@hotmail.com

Appendix B

Faculty of Natural and Agricultural Sciences

01-Sep-2014

Dear Miss Susan Louw

Ethics Clearance: Correlation between the genetic polymorphisms for depression, aggression and impulsivity on suicidal behaviour

Study Leader/Supervisor: Spies, Johannes

Principal Investigator: Miss Susan Louw

Department: Genetics (Bloemfontein Campus)

This letter confirms that a research proposal with tracking number: **UFS-HSD2014/0024** and title: **'Correlation between the genetic polymorphisms for depression, aggression and impulsivity on suicidal behaviour'** was given ethics clearance by the Ethical Committee.

Please ensure that the Ethical Committee is notified should any substantive change(s) be made, for whatever reason, during the research process. This includes changes in investigators. Please also ensure that a brief report is submitted to the Ethical Committee on completion of the research. The purpose of this report is to indicate whether or not the research was conducted successfully, if any aspects could not be completed, or if any problems arose that the Ethical Committee should be aware of.

Note:
1. This clearance is valid from the date on this letter to the time of completion of data collection.
2. Progress reports should be submitted annually unless otherwise specified.

Yours Sincerely

Prof. Neil Heideman
Chairperson: Ethical Committee
Faculty of Natural and Agricultural Sciences

Prof. NJL Heideman: Dekaan/Dean
Natuur- & Landbouwetenskappe
Natural & Agricultural Sciences
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Information leaflet and consent form for psychiatrists   **March 2014**

Dear Psychiatrist/Psychologist

You are hereby kindly requested to take part in a study for the completion of a Master's Degree in Behavioural Genetics with the title: **The association of specific polymorphisms of aggression, impulsivity and depression on suicidal behaviour.**

About the study

The main aim of the study is to find out if it is possible to link certain genes to suicidal behavior and other personality factors such as aggression and impulsive behaviours. The focus will be on polymorphisms of certain genes within the serotonergic system, which have been implicated in suicidal behaviour in various research studies. If a link can be found between specific genes and suicidal behaviour, or between certain environmental factors and suicide, this information can be used to develop therapeutic programs or medication that might help to manage the behaviour.

Participation

In order to complete this study, it is necessary to obtain DNA in the form of a saliva sample from individuals who tried to commit suicide. The suicide attempter will further need to complete a questionnaire on behavioural traits of aggression, impulsivity and depression. It is requested that a professional psychiatrist/psychologist help with the identification of suicide attempters, the handing out of a questionnaire and obtaining a saliva sample.

The inclusion criteria for the research participant include the following: has to be literate in English and between the ages of 18 and 100. The patient **must** have a previous **suicide attempt**, even if it is not recent. The patient may be on medication. The participant will remain anonymous, but will be asked to provide a few personal details which will be treated with the strictest confidence and in accordance with the highest ethical standards. The completion of this questionnaire should take no longer than 30 minutes. Participating in the study is completely voluntary and there will be no negative effects on the participant. The

participant will not receive any compensation or feedback. The DNA sample may be used in further research done by the Department of Genetics of the University of the Free State.

It is asked that the participating psychiatrist/psychologist provide the possible research participant with a short motivation of why the study is conducted as well as a packet containing an information leaflet, a consent form, a questionnaire and a vial. All information is provided in the leaflet for the participant. The general consent form, questionnaire and vial will each contain a unique subject number. It is of extreme importance that these codes should match with each other. The research participant needs to sign a general consent form and then complete the questionnaire in full. All necessary instructions are provided in written format. The participant will be asked to provide about 1.5 ml of saliva in a vial and the saliva sample will then be stored in a container provided by the student. The participant should be the **only** person to touch the vial to prevent possible DNA contamination and replace it in the sachet provided. Once the psychiatrist has obtained a signed general consent form, a completed questionnaire and saliva sample from the suicide attempter, he/she will let the student know and it will be collected the same/next afternoon. The date of saliva sampling must please be filled in by the psychiatrist/psychologist on the general consent form as well as the questionnaire. It is possible for the participant to complete the forms at a later date, but it is preferable that it is completed the same time as the saliva sampling.

Contact information

This study will be undertaken by a Master's student at the University of the Free State, under the guidance of Professor Spies (Academic Department Head: Genetics) and Miss Heathfield (Lecturer: Forensic Genetics).

Please feel free to contact Susan Louw (cell nr: **084 529 0975**) or Professor Spies (work nr: 051 401 2261) with any questions you may have regarding this study. You may contact the Secretariat of the Ethics Committee of the Faculty of Natural and Agricultural Sciences, UFS at telephone number (051) 401 2322 if you have questions regarding the Ethics of this study.

Your cooperation is sincerely appreciated as this study cannot be completed without your help.

Researcher: Susan Louw   Supervisor: Prof J.J. Spies

(Cell nr: 084 529 0975)   (Work nr: 051 401 2261)

UNIVERSITY OF THE FREE STATE UNIVERSITEIT VAN DIE VRYSTAAT YUNIVESITHI YA FREISTATA	UFS·UV NATURAL AND AGRICULTURAL SCIENCES NATUUR- EN LANDBOUWETENSKAPPE	**Departement Genetika (116) / Department of Genetics (116) Fakulteit Natuur- en Landbouwetenskappe Faculty of Natural and Agricultural Sciences** Posbus / P.O. Box 339, Bloemfontein 9300, South Africa E-pos / E-mail: Genetics@ufs.ac.za ☎ +27-(0)51-401-2595🖷 +27-(0)86-518-7317

Information leaflet and consent form for participants   **March 2014**

Dear participant

You are hereby kindly requested to take part in a study for the completion of a Master's Degree in Behavioural Genetics with the title:

 The association of specific polymorphisms for aggression, impulsivity and depression on suicidal behaviour.

About the study

The main aim of the study is to find out if it is possible to link certain genes to suicidal behavior and other personality factors such as depression, aggression and impulsive behaviours. Our DNA carries a unique genetic code that we inherit and is partly responsible for who and what we are.   It might be possible that it plays a role in suicidal behaviour and this study will contribute to research to determine if this might be true.

The findings of this study will not have any impact on the behavioural trait in yourself, but may eventually benefit others to manage their behavioural traits. If a link can be found between certain genes and suicidal behaviour, or between certain environmental factors and suicide, this information can be used to develop therapeutic programs or medication that might help to manage the behaviour.   Your participation will act as a control to determine if the selected genes are truly unique to suicidal behaviour.

Participation

To take part in this study, you will be required to complete a questionnaire, as well as provide a DNA sample in the form of a saliva sample. Genes are found in every part of your body and therefore will be present in the saliva which will be obtained from you. You will **NOT** be hurt physically or emotionally if you take part in this study. However, you are free withdraw from the study at any time, and by doing so, there will be **NO** negative effect on you in any way. Participating in this study is completely voluntary and will not cost you any money. Similarly, you will also not receive any money or any other compensation if you

participate in this study. The DNA sample taken from you might also be used in further research done by the Department of Genetics of the University of the Free State.

You will be asked to provide a few personal details, but you can be assured that this will be treated with the strictest confidence and in accordance with the highest ethical standards. Furthermore, the information collected from you will only be used for research at the University of the Free State, and will **NOT** be available to any other person or company. <u>Your DNA will **NOT** be used for **ANY OTHER REASON** other than research.</u> The results of this study may be published in a scientific journal, but no personal information will accompany the results. All in all, participating in this study will not require more than 30 minutes of your time.

<u>Contact information</u>

This study will be undertaken by a Master's student at the University of the Free State, under the guidance of Professor Spies (Academic Department Head: Genetics) and Miss Heathfield (Lecturer: Forensic Genetics).

Please feel free to contact Susan Louw (cell nr: 084 529 0975) or Professor Spies (work nr: 051 401 2261) with any questions you may have regarding this study. You may contact the Secretariat of the Ethics Committee of the Faculty of Natural and Agricultural Sciences, UFS at telephone number (051) 401 2322 if you have questions about your rights as a research participant.

Thank you for your cooperation.

_____   _____

Researcher: Susan Louw   Supervisor: Prof J.J. Spies

(Cell nr: 084 529 0975)   (Work nr: 051 401 2261)

SUBJECT PARTICIPATION NUMBER: _____ **(Office use only)**

General consent

You have been asked to participate in the research study titled: **The association of specific polymorphisms for aggression, impulsivity and depression on suicidal behaviour.**

Your participation in this research is completely voluntary, and there will be no negative consequences for you should you decide to withdraw from the study. If you agree to participate, you will be required to sign this document.

Consent form for genetics research

For the above mentioned study your permission is requested to use some of your DNA for laboratory tests. The tests will involve an analysis of the genetic composition of a saliva sample taken from you. This is done in order to gain a better understanding of the genetic underpinnings of certain behavioural traits, for example suicidal behaviour. Your DNA might also be used in further research done by the Department of Genetics of the University of the Free State.

You can be ensured that your genetic information will be stored in a completely secure environment, to which only researchers of the Department of Genetics will have access. This includes securely locked freezers and locked filing cabinets. All information will be treated with the **strictest confidence**. Your genetic material and information will be disposed of should you decide to withdraw from the study. In addition, we will not provide any feedback to you regarding your results. You do not have to agree to take part in this research, and you are free to withdraw from the research at any time.

The research study, including the above information has been described to me in a language that I understand. I understand what my involvement in the study means and I voluntarily agree to participate.

Office use only

SALIVA SAMPLE OBTAINED ON _____ **(DATE)**

SALIVA SAMPLE NUMBER: _____

_____   _____

Participant Signature   **Date**

APPENDIX E

SUBJECT PARTICIPATION NUMBER: _____ (Office use only)

QUESTIONNAIRE

You have been asked to participate in a research study. Please note that by completing this questionnaire you are voluntarily participating in this research study. You will remain anonymous and your data will be treated confidentially at all times. You may withdraw from this study at any given moment during the completion of the questionnaire. Kindly note that the results of the study may be published.

SECTION A: PERSONAL INFORMATION			ID Number:		
INSTRUCTIONS: Please mark the applicable response with an X					
1. **Gender:**	Male			Female	
2. **Age:**					
3. **Highest education level achieved:**					
4. **Home language:**					
5. **Race (For statistical purposes only):**	White	Black	Colored	Indian	Other
6. **Are you currently taking any medication?**	Yes		No	Please specify _____ _____ _____	
7. **Have you ever had any plans to try and commit suicide?**	Yes		No	If yes, have you ever tried? Yes / No	

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SECTION B: THE BARRAT IMPULSIVENESS SCALE VERSION 11 (BIS-11)

INSTRUCTIONS:

People differ in the ways they act and think in different situations. This is a test to measure some of the ways in which you act and think. Read each statement and put an X on the appropriate circle on the right side of this page. Do not spend too much time on any statement. Answer quickly and honestly.

1	2	3	4
Rarely/Never	**Occasionally**	**Often**	**Almost Always/Always**

	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/Always
1. I plan tasks carefully.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
2. I do things without thinking.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
3. I make-up my mind quickly.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
4. I am happy-go-lucky.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
5. I don't "pay attention."	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
6. I have "racing" thoughts.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
7. I plan trips well ahead of time.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
8. I am self-controlled.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
9. I concentrate easily.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
10. I save regularly.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
11. I "squirm" at plays or lectures.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
12. I am a careful thinker.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
13. I plan for job security.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
14. I say things without thinking.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
15. I like to think about complex problems.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always

16. I change jobs.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
17. I act "on impulse."	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
18. I get easily bored when solving thought problems.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
19. I act on the spur of the moment.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
20. I am a steady thinker.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
21. I change residences.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
22. I buy things on impulse.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
23. I can only think about one thing at a time.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
24. I change hobbies.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
25. I spend or charge more than I earn.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
26. I often have irrelevant thoughts when thinking.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
27. I am more interested in the present than the future.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
28. I am restless at the theater or lectures.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
29. I like puzzles.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always
30. I am future-orientated.	1 Rarely/Never	2 Occasionally	3 Often	4 Almost Always/ Always

SECTION C: THE REACTIVE PROACTIVE AGGRESSION QUESTIONNAIRE (RPQ)

INSTRUCTIONS:
There are times when most of us feel angry, or have done things we should not have done. Do not spend a lot of time thinking about the items – just give your first response.
Rate each of the items below by making use of the following response categories:

0 = never
1 = sometimes (less than once a week)
2 = often (more than once a week)

How often have you……

1.	Yelled at others when they have irritated you or made you cross.	0 Never	1 Sometimes	2 Often
2.	Had fights with others to show who is the leader.	0 Never	1 Sometimes	2 Often
3.	Reacted angrily when pushed or irritated by others.	0 Never	1 Sometimes	2 Often
4.	Taken things from other people with the aim of hurting them.	0 Never	1 Sometimes	2 Often
5.	Gotten angry when frustrated.	0 Never	1 Sometimes	2 Often
6.	Vandalized something for fun.	0 Never	1 Sometimes	2 Often
7.	Had temper outbursts or lost your temper.	0 Never	1 Sometimes	2 Often
8.	Damaged things because you felt mad.	0 Never	1 Sometimes	2 Often
9.	Had a fight to be cool.	0 Never	1 Sometimes	2 Often
10.	Hurt others to win a game.	0 Never	1 Sometimes	2 Often
11.	Become angry or mad when you don't get your way.	0 Never	1 Sometimes	2 Often
12.	Used physical force to get others to do what you want.	0 Never	1 Sometimes	2 Often
13.	Gotten angry or mad when you lost a game.	0 Never	1 Sometimes	2 Often
14.	Gotten angry when others threatened you.	0 Never	1 Sometimes	2 Often
15.	Used force to obtain money or things from others.	0 Never	1 Sometimes	2 Often
16.	Felt better after hitting or yelling at someone.	0 Never	1 Sometimes	2 Often
17.	Threatened and bullied someone.	0 Never	1 Sometimes	2 Often

18.	Hit others to defend yourself.	0 Never	1 Sometimes	2 Often
19.	Gotten others to gang up on someone else.	0 Never	1 Sometimes	2 Often
20.	Carried a weapon to use in a fight.	0 Never	1 Sometimes	2 Often
21.	Gotten angry or mad or hit others when teased or made fun of.	0 Never	1 Sometimes	2 Often
22.	Yelled at others so they would do things for you.	0 Never	1 Sometimes	2 Often

SECTION D: BUSS-PERRY AGGRESSION QUESTIONNAIRE

INSTRUCTIONS:

Please rate each of the following items in terms of how characteristic they are of you. Use the following scale for answering these items. Do not spend a lot of time thinking about the items – just give your first response.

1	2	3	4	5	6	7
Extremely characteristic of me					Extremely uncharacteristic of me	

	RATING 1 - 7
1. Once in a while I can't control the urge to strike another person.	
2. Given enough provocation, I may hit another person.	
3. If somebody hits me, I hit back.	
4. I get into fights a little more than the average person.	
5. If I have to resort to violence to protect my rights, I will.	
6. There are people who pushed me so far that we came to blows	
7. I can think of no good reason for ever hitting a person.	
8. I have threatened people I know.	
9. I have become so mad that I have broken things.	
10. I tell my friends openly when I disagree with them.	
11. I often find myself disagreeing with people	
12. When people annoy me, I may tell them what I think of them.	
13. I can't help getting into arguments when people disagree with me.	
14. My friends say that I'm somewhat argumentative.	
15. I flare up quickly but get over it quickly.	
16. When frustrated, I let my irritation show.	
17. I sometimes feel like a powder keg ready to explode.	
18. I am an even-tempered person.	
19. Some of my friends think I'm a hothead.	
20. Sometimes I fly off the handle for no good reason.	

21.	I have trouble controlling my temper.	
22.	I am sometimes eaten up with jealousy.	
23.	At times I feel I have gotten a raw deal out of life.	
24.	Other people always seem to get the breaks.	
25.	I wonder why sometimes I feel so bitter about things.	
26.	I know that "friends" talk about me behind my back.	
27.	I am suspicious of overly friendly strangers.	
28.	I sometimes feel that people are laughing at me behind me back.	
29.	When people are especially nice, I wonder what they want.	

SECTION E: PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

INSTRUCTIONS:
Over the past *2 weeks,* how often have you been bothered by any of the following problems? Read each statement and put an X on the appropriate number on the right side of this page. Do not spend a lot of time thinking about the items – just give your first response.

0 = Not at all
1 = Several days
2 = More than half the day
3 = Nearly every day

	How often have you……				
1.	Little interest or pleasure in doing things.	0 Never	1 Several days	2 More than half the day	3 Nearly every day
2.	Feeling down, depressed, or hopeless.	0 Never	1 Several days	2 More than half the day	3 Nearly every day
3.	Trouble falling or staying asleep, or sleeping too much.	0 Never	1 Several days	2 More than half the day	3 Nearly every day
4.	Feeling tired or having little energy.	0 Never	1 Several days	2 More than half the day	3 Nearly every day
5.	Poor appetite or overeating.	0 Never	1 Several days	2 More than half the day	3 Nearly every day
6.	Feeling bad about yourself – or that you are a failure or have let yourself or your family down.	0 Never	1 Several days	2 More than half the day	3 Nearly every day

7.	Trouble concentrating on things, such as reading the newspaper or watching television.	0 Never	1 Several days	2 More than half the day	3 Nearly every day
8.	Moving or speaking so slowly that other people could have noticed. Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual.	0 Never	1 Several days	2 More than half the day	3 Nearly every day
9.	Thoughts that you would be better off dead or of hurting yourself in some way.	0 Never	1 Several days	2 More than half the day	3 Nearly every day
10.	If you checked off *any* problems, how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?	Not difficult at all _____ Somewhat difficult _____ Very difficult _____ Extremely difficult _____			

Office use only

SALIVA SAMPLE OBTAINED ON _____ (DATE)

SALIVA SAMPLE NUMBER: _____

Thank you for your time and cooperation. It is really appreciated.

Researcher: Susan Louw

(Cell nr: 084 529 0975)

Appendix F

Table A1: **Typical cycling conditions for the PCR of each gene region amplified according to optimised in-house protocols, followed on the G-Storm GSi Thermal Cycler (Gene Technologies Ltd.).**

Step	Temperature	Duration	Cycles
HTR1A			
Initial denaturation	94°C	5 min	1
Denaturation	94°C	30 s	
Annealing	59.5°C	40 s	35
Extension	72°C	50 s	
Final extension	72°C	10 min	1
HTR1B			
Initial denaturation	94°C	5 min	1
Denaturation	94°C	20 s	
Annealing	61°C	45 s	31
Extension	72°C	30 s	
Final extension	72°C	10 min	1
HTR2A			
Initial denaturation	94°C	5 min	1
Denaturation	94°C	20 s	
Annealing	60°C	60 s	30
Extension	72°C	30 s	
Final extension	72°C	10 min	1
SLC6A4			
Heat lid	112°C		
Initial denaturation	98°C	7 min	1
Denaturation	95°C	30 s	
Annealing	63°C	30 s	3
Extension	72°C	30 s	
Denaturation	95°C	30 s	
Annealing	61°C	30 s	25
Extension	72°C	60 s	
Final extension	72°C	10 min	1

Appendix G

Comparisons of proactive and reactive domains for the RPQ.

The difference P – R was calculated for each subject. Thereafter, the two groups of respondents, suicide attempters and control group, were compared with regard to the P – R difference using the non-parametric Wilcoxon test. Since the two groups did not differ significantly regarding the P – R difference (P=0.48), a point estimate and distribution-free 95% confidence interval for the median P – R difference was calculated for the pooled data from the 2 groups; similarly, a P-value associated with the hypothesis of zero median difference was calculated using the signed rank test.   The median P – R difference was 0.59, 95% confidence interval 0.54 to 064. Thus the P – R difference is significantly different from zero (P<0.0001; signed rank test).

```
                    The NPAR1WAY Procedure

         Wilcoxon Scores (Rank Sums) for Variable D_RP
                  Classified by Variable group

                         Sum of      Expected       Std Dev          Mean
   group        N        Scores      Under H0       Under H0        Score
   ------------------------------------------------------------------------
   SUI          25        674.0        637.50      51.398801       26.960
   CON          25        601.0        637.50      51.398801       24.040

                  Average scores were used for ties.


                    Wilcoxon Two-Sample Test

             Statistic (S)             674.0000

             Normal Approximation
             Z                           0.7004
             One-Sided Pr >   Z          0.2418
             Two-Sided Pr >  |Z|         0.4837

             t Approximation
             One-Sided Pr >   Z          0.2435
             Two-Sided Pr >  |Z|         0.4870

         Z includes a continuity correction of 0.5.


           Monte Carlo Estimates for the Exact Test

             One-Sided Pr >= S
             Estimate                    0.2423
             99% Lower Conf Limit        0.2412
             99% Upper Conf Limit        0.2434

             Two-Sided Pr >= |S - Mean|
             Estimate                    0.4840
             99% Lower Conf Limit        0.4828
             99% Upper Conf Limit        0.4853

             Number of Samples          1000000
             Initial Seed             363195000


                    Kruskal-Wallis Test

             Chi-Square                  0.5043
             DF                               1
             Pr > Chi-Square             0.4776
```

```
                         The NPAR1WAY Procedure

                         Hodges-Lehmann Estimation

                         Location Shift      0.0909

                                       Interval          Asymptotic
               95% Confidence Limits    Midpoint      Standard Error

                  -0.0909      0.2727      0.0909            0.0928


                         The UNIVARIATE Procedure
                              Variable:  D_RP

                                  Moments

        N                           50    Sum Weights                   50
        Mean                0.57818182    Sum Observations      28.9090909
        Std Deviation       0.37718722    Variance               0.1422702
        Skewness            -0.0361133    Kurtosis              0.90564315
        Uncorrected SS      23.6859504    Corrected SS          6.97123967
        Coeff Variation      65.236783    Std Error Mean        0.05334233


                         Basic Statistical Measures

               Location                      Variability

        Mean        0.578182      Std Deviation            0.37719
        Median      0.590909      Variance                 0.14227
        Mode        0.545455      Range                    2.00000
                                  Interquartile Range      0.54545

     Note: The mode displayed is the smallest of 2 modes with a count of 6.


                      Tests for Location: Mu0=0

            Test             -Statistic-      -----p Value------

            Student's t     t   10.83908     Pr > |t|     <.0001
            Sign            M         22     Pr >= |M|    <.0001
            Signed Rank     S      569.5     Pr >= |S|    <.0001


                         Quantiles (Definition 5)

                                           95% Confidence Limits
               Quantile        Estimate      Assuming Normality

               100% Max       1.6363636
               99%            1.6363636     1.28299       1.70210
               95%            1.0000000     1.05879       1.39154
               90%            1.0000000     0.93659       1.22854
               75% Q3         0.8181818     0.72480       0.96316
               50% Median     0.5909091     0.47099       0.68538


                         Quantiles (Definition 5)

                  95% Confidence Limits    -------Order Statistics-------
        Quantile      Distribution Free     LCL Rank  UCL Rank    Coverage

        100% Max
        99%           1.363636   1.6363636      49        50        30.56
        95%           1.000000   1.6363636      45        50        88.53
        90%           0.909091   1.6363636      41        50        97.03
        75% Q3        0.636364   1.0000000      32        44        95.19
        50% Median    0.545455   0.6363636      19        33        95.11
```

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Appendix H

Figure A.1 Molecular weight marker (O'RangeRuler DNA Ladder, Fermentas, Thermo Scientific)
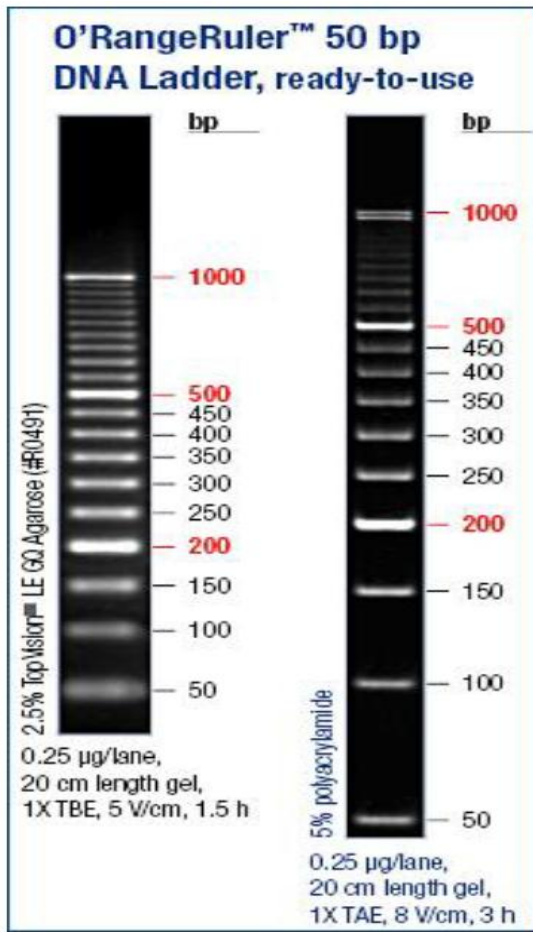
Appendix I

Representation of gels.
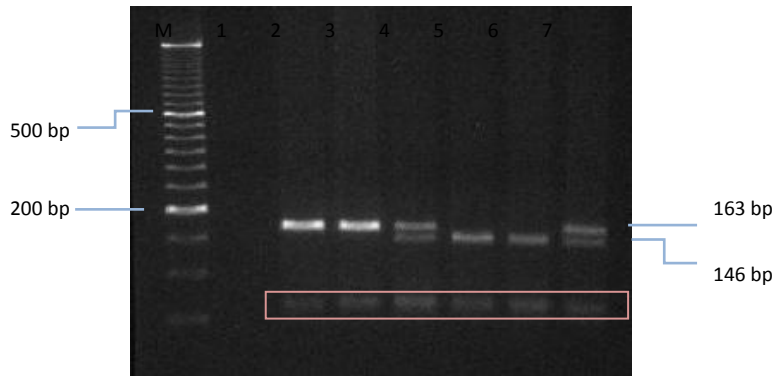
Figure A.2 *HTR1A* **DNA fragments that underwent** *BseGI* **digestion.** M: 50 bp MWM; 1: Negative water control; 2 - 7: Digested PCR product. Lanes 2, 3: 163 bp size (CC); Lanes 4,7: 163 and 146 bp sizes (GC); Lanes 5, 6: 146 bp size, 17 bp size not clearly distinguishable (GG); primer dimers visible at the bottom as indicated by the box**.**
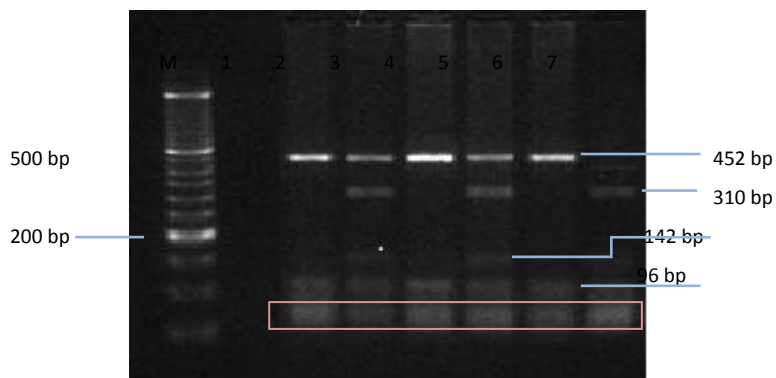
Figure A.3 *HTR1B* **DNA fragments that underwent** *HincII* **digestion.** M: 50 bp MWM; 1: Negative water control; 2 - 7: Digested PCR product; Lanes 2, 4, 6: 452 and 96 bp sizes (GG); Lanes 3, 5: 452, 310, 142, and 96 bp sizes (GC); Lanes 7: 310, 142, and 96 bp sizes (CC); primer dimers visible at the bottom as indicated by the box**.**
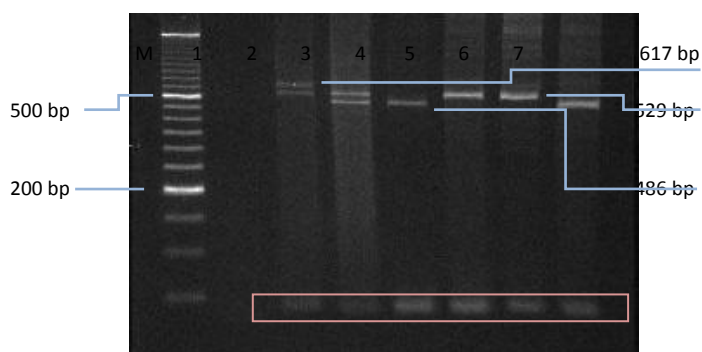
Figure A.4 *SLC6A4* **VNTR.** M: 50 bp MWM; 1: Negative water control; 2 - 7: *HTTLPR*; Lanes 2: 617 and 529 bp sizes (XL); Lanes 3: 529 and 486 bp sizes (SL); Lanes 4, 7: 486 bp (SS); Lane 5, 6: 529 bp size (LL); primer dimers visible at the bottom as indicated by the box**.**

Appendix J

Table A.2 The genotypes for the suicide attempters and control group for *HTR1A*, *HTR1B*, *HTR2A* and *SLC6A4*.

	Suicide Attempters					Control Group			
	HTR1A	*HTR1B*	*HTR2A*	*SLC6A4*		*HTR1A*	*HTR1B*	*HTR2A*	*SLC6A4*
S1	CG	CG	AG	XL	C1	GG	GG	GG	LL
S2	CG	GG	AA	LL	C2	CG	GG	GG	LS
S3	CG	GG	AG	SS	C3	CG	GG	AG	LS
S4	CG	CC	AA	LL	C4	CG	GG	AA	LS
S5	CG	GG	AA	LS	C5	CC	GG	AG	SS
S6	CG	GG	AG	SS	C6	CG	GG	AG	LL
S7	CG	CG	AA	LS	C7	CG	GG	AA	LS
S8	CG	GG	AG	LS	C8	CG	GG	AG	LS
S9	CG	CG	AA	LL	C9	CG	CG	GG	LS
S10	CG	GG	AA	LL	C10	CG	GG	GG	LL
S11	CC	GG	-	LS	C11	CG	CG	AG	LL
S12	CC	GG	AG	LS	C12	CG	GG	AG	LL
S13	CC	GG	AG	LL	C13	CG	CG	GG	LL
S14	GG	GG	AA	LS	C14	CC	CG	AA	LL
S15	CG	CG	AG	XX	C15	CC	CG	AA	SS
S16	CG	-	-	-	C16	CG	GG	AG	LL
S17	CG	GG	AA	-	C17	GG	GG	AG	SS
S18	CC	GG	AG	LS	C18	GG	GG	AG	SS
S19	GG	-	-	SS	C19	CG	GG	AG	LS
S20	GG	CG	AG	SS	C20	-	GG	AG	SS
S21	GG	-	-	SS	C21	CC	CG	AG	LS
S22	CG	CG	AG	LL	C22	CC	CG	AG	LL
S23	CG	CG	AG	LS	C23	CG	CG	GG	LL
S24	CG	GG	AG	LS	C24	CC	CG	AG	LL
S25	CG	GG	AA	XS	C25	CG	CG	GG	SS

Appendix K

Hardy-Weinberg Equilibrium

Table A.3 Hardy-Weinberg equilibrium for each genetic variant with degrees of freedom (df) and representative chi-square value.

| Polymorphism | Suicide Attempters | | | Control Group | | |
	df	N	Chi-square	df	N	Chi-square
rs6295	1	25	0.1296	1	24	0.0711
rs6296	1	22	0.0015	1	25	0.0625
rs6311	1	21	0.1589	1	25	0.0186
HTTLPR	3	23	0.1797	1	25	0.1110

Appendix L

Table A.3 SUICIDE ATTEMPTERS - Mean response and standard deviation for each scale of the questionnaires stratified by genotype with genotype comparisons between scores.

		Non-planning	Motor impulsivity	Attentional impulsivity	BIS-11	Proactive Aggression	Reactive aggression	RPQ	Physical aggression	Verbal aggression	Anger	Hostility	BPAQ
rs6295 (n=25)													
Genotypes	GG (n = 4)	2.80 ±0.71	2.73 ±0.31	2.97 ±0.47	2.82 ±0.50	0.77 ±0.52	1.27 ±0.68	1.02 ±0.60	2.97 ±0.97	2.60 ±1.43	2.54 ±0.46	1.84 ±0.57	2.49 ±0.79
	GC (n = 17)	2.54 ±0.57	2.21 ±0.50	2.70 ±0.43	2.46 ±0.40	0.34 ±0.34	0.98 ±0.40	0.66 ±0.32	4.52 ±1.36	4.15 ±1.93	3.65 ±1.55	3.13 ±1.15	3.86 ±1.25
	CC (n = 4)	2.11 ±0.25	2.16 ±0.41	2.19 ±0.58	2.15 ±0.10	0.32 ±0.40	0.82 ±0.72	0.57 ±0.40	4.39 ±1.90	4.40 ±1.74	4.75 ±1.68	4.19 ±2.19	4.42 ±1.87
Statistical analysis	GG vs GC	0.4212	0.0618	0.2987	0.1158	0.0513	0.2990	0.0986	0.0598	0.1432	0.1886	0.0870	0.0707
	GG vs CC	0.0994	0.1010	**0.0243***	**0.0240***	0.1053	0.2098	0.1055	0.1681	0.1805	**0.0451***	**0.0172***	**0.0472***
	CC vs GC	0.1848	0.8355	0.0565	0.1620	0.9303	0.5677	0.6762	0.8654	0.8114	0.1918	0.1515	0.4458
rs6296 (n=21)													
Genotypes	GG (n = 14)	2.40 ±0.51	2.19 ±0.49	2.62 ±0.54	2.38 ±0.37	0.34 ±0.37	0.81 ±0.42	0.57 ±0.30	4.47 ±1.42	3.99 ±1.80	3.95 ±1.54	3.24 ±1.35	3.92 ±1.32
	GC (n = 7)	2.73 ±0.72	2.52 ±0.44	2.64 ±0.57	2.63 ±0.53	0.44 ±0.50	1.22 ±0.62	0.83 ±0.55	3.98 ±1.95	3.34 ±2.17	2.98 ±1.64	2.96 ±1.82	3.35 ±1.77
Statistical analysis	GG vs GC	0.2336	0.1498	0.9171	0.2187	0.5966	0.0874	0.1745	0.5216	0.4800	0.1980	0.6974	0.4133
rs3611 (n=21)													
Genotypes	GA (n = 12)	2.58 ±0.66	2.37 ±0.52	2.64 ±0.54	2.52 ±0.46	0.37 ±0.44	0.92 ±0.60	0.64 ±0.46	4.72 ±1.77	4.77 ±1.81	4.20 ±1.81	3.69 ±1.69	4.32 ±1.64
	AA (n = 9)	2.35 ±0.55	2.12 ±0.52	2.69 ±0.51	2.36 ±0.42	0.42 ±0.38	1.07 ±0.43	0.75 ±0.34	3.78 ±1.14	2.91 ±1.84	3.03±1.26	2.60 ±0.96	3.12 ±0.94
Statistical analysis	GA vs AA	0.4237	0.2912	0.8026	0.5354	0.7754	0.5205	0.5751	0.1799	**0.0328***	0.1133	0.0998	0.0662
HTTLPR (n=20)													
Genotypes	LL (n = 7)	2.40 ±0.61	2.16 ±0.58	2.52 ±0.57	2.34 ±0.44	0.48 ±0.31	1.12 ±0.33	0.80 ±0.19	3.95 ±1.45	3.77 ±2.14	3.63 ±1.65	3.43 ±1.41	4.70 ±1.37
	LS (n = 9)	2.44 ±0.42	2.34 ±0.43	2.56 ±0.50	2.44 ±0.28	0.30 ±0.41	0.80 ±0.63	0.55 ±0.45	4.47 ±1.63	3.89 ±2.13	4.10 ±2.03	3.18 ±1.79	3.92 ±1.74
	SS (n = 4)	2.75 ±0.78	2.52 ±0.64	3.00 ±0.43	2.73 ±0.63	0.77 ±0.52	1.32 ±0.60	1.05 ±0.56	3.56 ±1.59	3.55 ±1.91	3.00 ±1.14	2.34 ±1.30	3.09 ±1.44
Statistical analysis	LL vs LS	0.8853	0.4905	0.8863	0.6579	0.3932	0.2544	0.2371	0.5194	0.9127	0.6099	0.7594	0.7798
	LL vs SS	0.3420	0.2832	0.1539	0.6579	0.2622	0.5573	0.3410	0.6897	0.8681	0.5751	0.2890	0.5398
	LS vs SS	0.3824	0.5795	0.1695	0.2507	0.0686	0.1250	0.0561	0.3430	0.7911	0.3165	0.3908	0.3854

Table A.4 CONTROL GROUP - Mean response and standard deviation for each scale of the questionnaires stratified by genotype with genotype comparisons between scores.

		Non-planning	Motor impulsivity	Attentional impulsivity	BIS-11	Proactive Aggression	Reactive aggression	RPQ	Physical aggression	Verbal aggression	Anger	Hostility	BPAQ
						rs6295 (n=24)							
	GG (n = 3)	1.97 ±0.45	2.06 ±0.55	2.13 ±0.54	2.04 ±0.45	0.03 ±0.05	0.58 ±0.10	0.30 ±0.07	5.11 ±1.02	5.13 ±1.01	5.33 ±0.86	5.38 ±0.78	5.24 ±0.87
Genotypes	GC (n = 15)	2.10 ±0.54	1.95 ±0.47	2.11 ±0.68	2.04 ±0.45	0.13 ±0.18	0.60 ±0.35	0.36 ±0.24	5.51 ±1.14	4.16 ±1.39	4.16 ±1.39	4.89 ±1.45	4.96 ±1.05
	CC (n = 6	2.21 ±0.47	1.97 ±0.48	2.27 ±0.90	2.14 ±0.55	0.18 ±0.22	0.97 ±0.54	0.58 ±0.36	5.43 ±0.89	4.80 ±1.40	3.93 ±1.74	4.33 ±1.58	4.66 ±1.14
	GG vs GC	0.7010	0.7078	0.9716	1.000	0.4147	0.9231	0.7231	0.5625	0.2706	0.6099	0.5999	0.6799
Statistical analysis	GG vs CC	0.5144	0.7911	0.7804	0.7833	0.2579	0.1705	0.1632	0.6829	0.7323	0.1438	0.3163	0.4428
	CC vs GC	0.6494	0.9176	0.6497	0.6876	0.5466	0.0647	0.1148	0.8712	0.3410	0.1374	0.4295	0.5567
						rs6296 (n=25)							
Genotypes	GG (n = 15)	2.04 ±0.50	1.93 ±0.47	2.22 ±0.62	2.04 ±43	0.07 ±0.11	0.59 ±0.28	0.33 ±0.17	5.61 ±0.90	4.63 ±1.27	4.99 ±0.96	4.88 ±1.23	5.09 ±0.76
	GC (n = 10)	2.18 ±0.51	1.99 ±0.45	1.99 ±0.82	2.06 ±0.53	0.20 ±0.23	0.83 ±0.52	0.51 ±0.36	5.27 ±1.22	4.22 ±1.46	4.29 ±1.68	4.79 ±1.66	4.72 ±1.32
Statistical analysis	GG vs GC	0.4832	0.7396	0.2875	0.9359	0.0824	0.1487	0.0964	0.4189	0.4661	0.1945	0.8809	0.3789
						rs3611 (n=25)							
	GG (n = 7)	2.42 ±0.47	2.23 ±0.39	2.04 ±0.64	2.25 ±0.46	0.19 ±0.24	0.68 ±0.36	0.44 ±0.29	5.25 ±1.42	3.89 ±1.67	4.78 ±1.48	4.86 ±1.56	4.79 ±1.45
Genotypes	GA (n = 14)	1.99 ±0.41	1.77 ±0.40	2.19 ±0.66	1.96 ±0.39	0.11 ±0.17	0.71 ±0.38	0.41 ±0.26	5.42 ±1.09	4.46 ±0.95	4.58 ±1.06	4.81 ±1.20	4.88 ±0.72
	AA (n = 4)	1.89 ±0.67	2.11 ±0.52	2.06 ±1.06	2.02 ±0.69	0.05 ±0.05	0.61 ±0.63	0.33 ±0.34	6.06 ±0.61	5.50 ±1.59	5.04 ±2.09	4.91 ±2.06	5.40 ±1.19
	GG vs GA	0.0647	**0.0240***	0.6559	0.1938	0.3219	0.8685	0.8426	0.7319	0.3444	0.7610	0.9475	0.8509
Statistical analysis	GG vs AA	0.0855	0.6499	0.9536	0.4322	0.1991	0.8164	0.5528	0.2308	0.0562	0.7630	0.9573	0.3625
	GA vs AA	0.6908	0.1554	0.7642	0.8358	0.5311	0.6955	0.6204	0.2922	0.1640	0.5619	0.9101	0.3922
						HTTLPR (n=25)							
	LL (n = 11)	2.05 ±0.45	1.79 ±0.34	1.82 ±0.58	1.89 ±0.36	0.15 ±0.23	0.74 ±0.47	0.44 ±0.33	5.39 ±0.85	4.44 ±1.54	5.03 ±1.23	5.08 ±1.37	5.05 ±1.01
Genotypes	LS (n = 8)	2.06 ±0.57	1.97 ±0.46	2.28 ±0.74	2.08 ±0.46	0.16 ±0.16	0.64 ±0.32	0.40 ±0.22	5.92 ±0.96	4.45 ±1.19	5.02 ±1.12	5.23 ±1.14	5.26 ±0.84
	SS (n = 6)	2.23 ±0.54	2.24 ±0.55	2.48 ±0.70	2.30 ±0.56	0.03 ±0.05	0.65 ±0.41	0.34 ±0.22	5.04 ±1.34	4.53 ±1.35	3.71 ±1.39	3.88 ±1.47	4.31 ±1.11
	LL vs LS	0.9760	0.3799	0.1438	0.3639	0.9023	0.6142	0.7345	0.2794	0.9833	0.9888	0.8038	0.6576
Statistical analysis	LL vs SS	0.5001	**0.0498***	0.0631	0.0848	0.2060	0.6954	0.4812	0.4954	0.8919	**0.0481***	0.0871	0.1508
	LS vs SS	0.5429	0.2509	0.5873	0.3788	0.1966	0.9470	0.7091	0.1226	0.9126	0.0635	0.0707	0.0880

Table A.5 TOTAL COHORT - Mean response and standard deviation for each scale of the questionnaires stratified by genotype with genotype comparisons between scores.

		Non-planning	Motor impulsivity	Attentional impulsivity	BIS-11	Proactive Aggression	Reactive aggression	RPQ	Physical aggression	Verbal aggression	Anger	Hostility	BPAQ
						rs6295 (n=49)							
Genotypes	GG (n = 7)	2.44 ±0.72	2.44 ±0.53	2.61 ±0.64	2.49 ±0.60	0.45 ±0.54	0.97 ±0.61	0.71 ±0.57	3.89 ±1.46	3.69 ±1.79	3.73 ±1.61	3.36 ±1.98	3.67 ±1.65
	GC (n = 32)	2.33 ±0.59	2.09 ±0.50	2.42 ±0.63	2.27 ±0.47	0.24 ±0.30	0.80 ±0.24	0.52 ±0.32	4.99 ±1.34	4.16 ±1.67	4.24 ±1.51	3.95 ±1.56	4.38 ±1.27
	CC (n = 10	2.17 ±0.38	2.05 ±0.44	2.24 ±0.75	2.14 ±0.42	0.24 ±0.29	0.91 ±0.54	0.57 ±0.36	5.01 ±1.39	4.64 ±1.46	4.26 ±1.68	4.28 ±1.73	4.56 ±1.38
Statistical analysis	GG vs GC	0.6520	0.0912	0.5020	0.2793	0.1321	0.5857	0.2131	0.0606	0.5239	0.4435	0.3925	0.2145
	GG vs CC	0.3489	0.1085	0.2588	0.1541	0.1961	0.8820	0.4403	0.1025	0.2499	0.4991	0.2662	0.1854
	CC vs GC	0.4484	0.8118	0.4419	0.4813	0.9852	0.4165	0.6945	0.9600	0.3980	0.9711	0.5939	0.7064
						rs6296 (n=46)							
Genotypes	GG (n = 29)	2.21 ±0.53	2.05 ±0.49	2.41 ±0.61	2.21 ±43	0.20 ±0.30	0.70 ±0.36	0.45 ±0.27	5.06 ±1.29	4.32 ±1.56	4.49 ±1.35	4.09 ±1.52	4.53 ±1.20
	GC (n = 17)	2.41 ±0.64	2.21 ±0.51	2.26 ±0.78	2.29 ±0.59	0.30 ±0.37	0.99 ±0.58	0.64 ±0.46	4.74 ±1.63	3.86 ±1.78	3.75 ±1.75	4.04 ±1.91	4.15 ±1.62
Statistical analysis	GG vs GC	0.2661	0.3127	0.4645	0.5589	0.3291	**0.0242***	0.0716	0.4633	0.3905	0.1155	0.9232	0.3801
						rs3611 (n=46)							
Genotypes	GG (n = 7)	2.42 ±0.47	2.23 ±0.39	2.04 ±0.64	2.25 ±0.46	0.19 ±0.24	0.68 ±0.36	0.44 ±0.29	5.25 ±1.42	3.89 ±1.67	4.78 ±1.48	4.86 ±1.56	4.79 ±1.45
	GA (n = 26)	2.26 ±0.61	2.05 ±0.55	2.39 ±0.64	2.22 ±0.50	0.23 ±0.34	0.80 ±0.49	0.52 ±0.38	5.10 ±1.39	4.60 ±1.39	4.41 ±1.43	4.29 ±1.53	4.62 ±1.24
	AA (n = 13)	2.21 ±0.60	2.12 ±0.50	2.50 ±0.74	2.25 ±0.51	0.31 ±0.36	0.93 ±0.52	0.62 ±0.38	4.48 ±1.47	3.71 ±2.11	3.65 ±1.75	3.31 ±1.70	3.82 ±1.46
Statistical analysis	GG vs GA	0.5433	0.3944	0.2156	0.8900	0.8022	0.5549	0.6001	0.7974	0.3601	0.5754	0.4076	0.7669
	GG vs AA	0.4592	0.6360	0.1466	0.9790	0.4763	0.2633	0.2906	0.2491	0.7894	0.1246	0.0428	0.1266
	GA vs AA	0.7940	0.6762	0.6444	0.8339	0.5082	0.4191	0.4198	0.2044	0.1342	0.1531	0.0738	0.0847
						HTTLPR (n=45)							
Genotypes	LL (n = 18)	2.19 ±0.53	1.93 ±0.47	2.09 ±0.66	2.07 ±0.44	0.28 ±0.30	0.88 ±0.45	0.58 ±0.33	4.83 ±1.30	4.18 ±1.76	4.48 ±1.53	4.44 ±1.58	4.53 ±1.31
	LS (n = 17)	2.26 ±0.52	2.17 ±0.47	2.43 ±0.62	2.27 ±0.41	0.24 ±0.32	0.72 ±0.50	0.48 ±0.36	5.51 ±1.51	4.15 ±1.72	4.53 ±1.68	4.15 ±1.81	4.55 ±1.51
	SS (n = 10)	2.44 ±0.66	2.35 ±0.57	2.69 ±0.64	2.47 ±0.60	0.33 ±0.49	0.92 ±0.58	0.62 ±0.51	4.44 ±1.56	4.14 ±1.57	3.43 ±1.28	3.26 ±1.55	3.82 ±1.33
Statistical analysis	LL vs LS	0.6919	0.1647	0.1291	0.2039	0.7262	0.4224	0.4390	0.5184	0.8856	0.9312	0.6085	0.9582
	LL vs SS	0.2625	**0.0349***	**0.0291***	**0.0328***	0.7265	0.8785	0.7848	0.4970	0.8599	0.0897	0.0806	0.2067
	LS vs SS	0.4366	0.3436	0.3135	0.2824	0.5208	0.4018	0.3552	0.2252	0.9584	0.0802	0.1895	0.1959